

# Effect of differential fertilisation treatments on maize hybrid quality and performance under environmental stress condition in Hungary

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## Abstract

The correct choice of genotypes, able to overcome environmental stress conditions and genotype-adapted nutrient supply are very important, as they largely determine the efficiency of cultivation. Our aim was to quantify the effect of fertilization and of meteorological stress factors on the yield of genotypes and protein content of maize grain. Our studies were conducted in Hungary in the long-term field trial of the University of Debrecen in years with favourable (2016) and unfavourable water supply (2017) by using 5 different genotype hybrids, with ten NPK treatments (T) and without fertilization as control. In the group treated with the combination of increasing N+equally increasing PK, decrease in yield during stress was lower than in the case of combinations with increasing N+constant PK treatment. The effect of weather-stress factors could be decreased with hybrid specific nutrient supply. The hybrids Renfor, Fornad, and Sushi reacted with the lowest yield reduction to the T10 treatment combination as a result of the negative weather conditions, while the Armagnac preferred T7 and Loupiac T9 treatments. Armagnac reached its highest protein content – irrespective of the production year – with T5 ( $P<0.05$ ) combination, while in the case of Loupiac, the favourable value was ensured by T4 treatment combination ( $P<0.05$ ). In the case of Fornad, Renfor and Sushi, highest protein content was determined by higher treatment combinations in 2016, while in 2017, lower combinations showed the same results as higher ones.

## Introduction

The world's global population was 7.6 billion people in 2017 and it is expected to grow to 9.8 billion by 2050 (UN, 2017). The increase of population and the growing need for food will require increasing yields. So far, this goal could be achieved by using modern technologies and involving increasing amounts of agricultural lands into production (Schnepf et al, 2001). However, it is not possible to further increase agricultural lands to a noticeable extent.

Furthermore, climate change poses a global challenge for agriculture. Crop production, more specifically, maize production is one of the most exposed sectors to climate change (Huntingford et al, 2005). Dry and wet periods, extremely high or low temperature (Porter and Semenov, 2005; Fedoroff. et al, 2010; Mir et al, 2012) are becoming increasingly frequent even within a year or growing season. These impacts significantly affect yield quality and quantity even in the case of nearly identical production technologies (Hegyi et al,

2008; Schlenker and Roberts, 2009; Ványiné Széles et al, 2012; Meng et al, 2016). The solution is increasing the successfulness of sustainable agriculture-based maize production by means of genetic advancement and production technology (Hallauer and Carena, 2009; Zhang et al, 2015).

Genotypes have new characteristics in order to be more stable and suitable for food production, which is expected to be one of the main adaptation strategies to climate change (Tollenaar and Lee, 2002; Ramirez-Villegas et al, 2015).

Choosing the proper maturity hybrid suitable for the given production site has become increasingly important (Delic et al, 2009; Wang et al, 2016).

Yield capacity is in positive correlation with hybrid maturity. Hybrids with longer maturity have longer assimilation periods and higher yield capacity (Hegyi et al, 2007; Nagy, 2007; Djurović et al, 2014). As a result of breeding, especially increasing the stress tolerance, there are short maturity hybrids, whose yield capacity is

close to that of longer maturity hybrids (Tollenaar and Wu, 1999; Vulchinkov et al, 2013).

Hungary is situated at the border of the continental climate and the temperate zone, where, based on the 20-year-average, mid-ripening hybrids were the most successful, but their yield surplus was only 0.5 t ha<sup>-1</sup> higher than that of early ripening hybrids. The yield of late ripening hybrids was nearly the same as that of early ripening hybrids (Nagy, 2008).

The yield potential of maize is very high (25 t ha<sup>-1</sup> – absolute dry yield), the highest among all cereals, but it can only be achieved under artificial environmental circumstances (Cassman et al, 2003). In practice, the yield potential of maize produced under various agroecological conditions may decrease to a smaller or greater extent as a result of environmental stress factors. The lack or abundance of fundamental environmental conditions (light, temperature, water, nutrients) leads to stress which blocks the growth and development of maize, thereby limiting production (Duvick and Cassman, 1999; Evans and Fischer, 1999; Lobell et al, 2009; Bruce et al, 2002; Videnović et al, 2013).

In addition to a proper choice of hybrids, there are several agrotechnical factors, of which nutrient replenishment is the most effective way of fully exploiting yield potential and increasing yield (Pepó et al, 2006; Grassini et al, 2011).

Yield can be increased using nitrogen, which is the most important nutrient for maize (Sangoi et al, 2001; Pikul et al, 2005; Tilman et al, 2011). However, harmonious nutrient supply and maintaining proper NPK balance are indispensable during the vegetation period (Kovacevic et al, 2006; Zang et al, 2007; Alley et al, 2009). For this reason, the proper choice of hybrids is an absolute necessity, as different hybrids have different nutrient conversion characteristics.

The protein content of maize mainly depends on genetic characteristics (Hegyí and Berzy, 2009; Izsáki, 2009), however, good quality became a less important aspect in the course of developing maize hybrid in order to increase productivity and production safety. This phenomenon led to the gradual decrease of the nutrition value of hybrids, mostly due to the decrease of protein and oil content (Uribe-larrea et al, 2004; Zhang et al, 2008). Protein synthesis can be affected with N fertilisation (Luit et al, 1999), while yield quantity and quality can also be increased (Hasaneen et al, 2009).

Protein content is greatly affected by climatic factors, such as heat units, as well as rainfall in June, July and August and rainfall distribution (Asghari and Hanson, 1984).

The inclusion of new hybrids in the common production system constantly calls for involving new genotypes in scientific experiments. For this reason, this study aims at exploring how maize hybrids of different genotypes and fertilisation (as one of the most significant technological elements) can mitigate or affect the harmful impact of climatic factors on yield and the protein content of the maize grain.

## Materials and Methods

### Experimental site and maize hybrids

The experimental trials and evaluations presented in this study were performed at the Experiment Site of the University of Debrecen in Hungary (47° 33' N, 21° 26' E, 111 m asl), on calcareous chernozem soil in a small plot long-term polyfactorial field experiment with four replications and a strip plot design in 2016 and 2017, under natural precipitation supply. Maize hybrids belonging to different FAO classes (Armagnac, FAO 490; Fornad, FAO 420; Loupiac, FAO 380; Renfor, FAO 320 and Sushi, FAO 340) were tested in experimental trials.

### Soil characteristics

Based on the soil analysis results of 2012, the average pH<sub>KCl</sub> value of the soil was 6.6 (slightly acidic), which is optimal from the aspect of crops' nutrient uptake. The Arany plasticity index is 39 in the upper (20 cm) layer of the soil, while the amount of water-soluble salts (anions and cations) is 0.04%, which represents low salt content. Carbonic chalk content is around 0% in the upper 80 cm layer of the soil, but it is 12% (i.e. moderately chalky) from 100 cm down. The organic matter content is 2.3% in the upper 20 cm layer of the soil, but it does not exceed 1.0% at a depth of 120 cm. The potassium level of the soil is adequate, while P level is average.

### Fertilization treatments

In addition to the non-fertilised (control) treatment, ten treatments were used in the long-term field experiment. Treatments 1-5 involved NPK doses of a constant proportion of 1 N : 0.75 P<sub>2</sub>O<sub>5</sub> : 0.88 K<sub>2</sub>O, with the basic N dose being 30 kg N ha<sup>-1</sup> and 1, 2, 3, 4 and 5 times this basic dose. Treatments 6-10 involved identical proportions of 184 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> and 216 kg ha<sup>-1</sup> K<sub>2</sub>O in addition to increasing N doses (Table 1).

### Experimental details

Plant density was 73 thousand plants per ha. The previous crop was maize. Maize was sown on 19/04/2016 and 25/04/2017 and it was harvested on

**Table 1 - Fertilizer treatments applied in the experiment, 2016 and 2017**

Treatment	Fertilizer dose kg ha <sup>-1</sup>			
	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	
<b>Control</b>	0	0	0	
T1	30	23	27	
increasing N+equally	T2	60	46	54
increasing PK treatment	T3	90	69	81
combination	T4	120	92	108
	T5	150	115	135
	T6	60	184	216
increasing N+constant proportion	T7	120	184	216
PK treatment combination	T8	180	184	216
	T9	240	184	216
	T10	300	184	216

14/10/2016 and 12/10/2017. The harvested grain yield was corrected to a moisture content of 14%.

### Environment traits

Potential evapotranspiration (PET) was calculated based on the method of Szász (1973).

$$PET = \beta [0.0095(T-21)^2(1-R)^{2/3}f(v)] \quad (1)$$

where PET= potential evapotranspiration [mm day<sup>-1</sup>], T= daily mean temperature [°C], R= relative humidity, f(v): the effect function wind speed, β: factor of expressing the oasis effect. The oasis effect is the ratio of environment and evaporating water.

### Statistical analysis

The correlation between the dependent variable (yield, protein content) and the production factor (fertiliser, genotype) was evaluated using a general linear model (GLM). Duncan test was applied for the determination of the significant difference from the control. Evaluation was performed using SPSS for Windows 21.0.

## Results and discussion

### Evaluation of meteorological data

Meteorological data measured at the trial area were compared to the mean value of the period 1985-2015.

During the vegetation period of 2016, every month – except for April – had a precipitation surplus, a total of 450 mm precipitation has fallen, which exceeded the mean value of 30 years by 110 mm (Figure 1). The PET value was 140 mm higher than the amount of precipitation. The number of heat days in the growing season (when daytime warming was higher than 30 °C) was 35. Altogether, the mean temperature of the

**Table 2 - Variance analysis results of yield and grain protein content of maize hybrids for fertilizer treatments of five hybrids in each year**

Factor	Source of variation	2016	2017
yield of maize hybrids	hybrids (A)	***	***
	fertiliser (B)	***	***
	hybrids x fertiliser (A x B)	ns	***
grain protein content	hybrids (A)	***	***
	fertiliser (B)	***	***
	hybrids x fertiliser (AxB)	ns	***

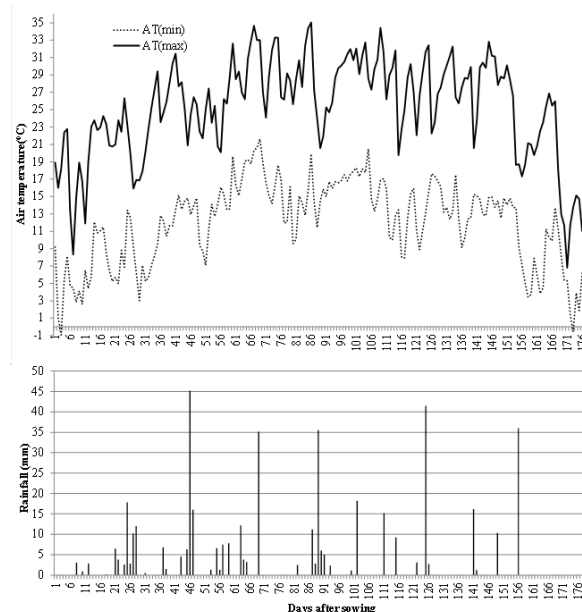
ns-not significant, \*\*\*significant at P=0.001

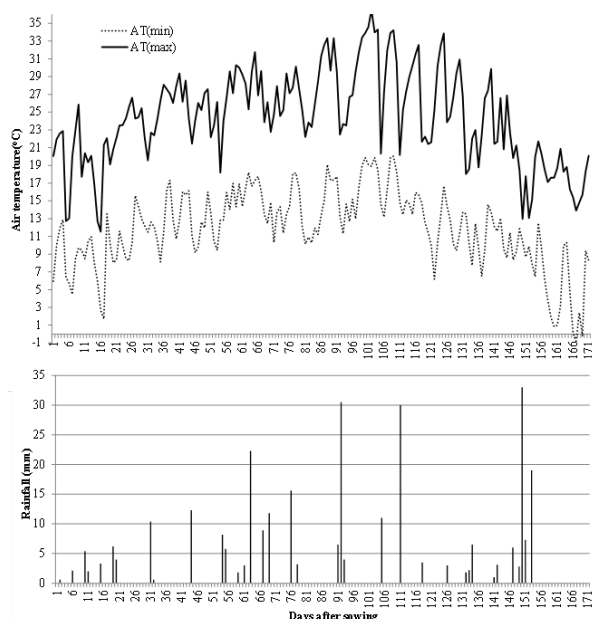
growing season was 16.5 °C, which was different from the average by only a few decimals (+0.3 °C).

2017 growing season ended with 349 mm precipitation. Three months (April, July, September) were rainier than the average, while the other three months (May, June, August) were below the average (Figure 2). The value of potential evapotranspiration (+325 mm) was significantly higher than the amount of precipitation. The temperature in April, May, July and September is a few decimals lower than the average, but June and August were 1.7-1.8 °C warmer than the average. The number of days with maximum temperature above 30 °C was 26.

### The effect of fertilisation and genotype on the maize hybrid yield

The analysis of variance (Table 2) confirmed the effect of genotype (P<0.001) and fertilisation (P<0.001)

**Fig. 1 - Maximum and minimum air temperature and the amount of rainfall during the growing season of maize, 2016 (April-October)**

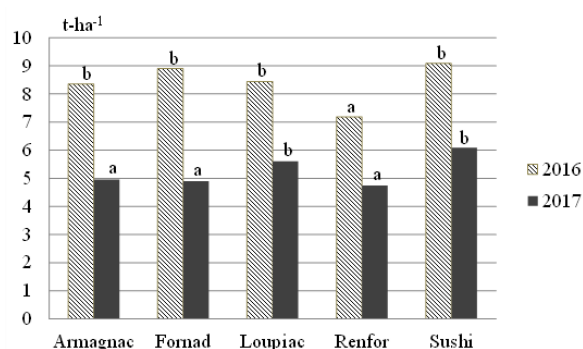


**Fig. 2 - Maximum and minimum air temperature and the amount of rainfall during the growing season of maize, 2017 (April-October)**

on yield both in 2016 and 2017. The Mean Square value shows the priority of the effect of fertilisation in both years. The effect of genotype x fertilisation in 2017 was significant ( $P < 0.001$ ), which means that these two factors were able to increase yield in a positive interaction, but this correlation was not significant in 2016 (Table 2).

#### **Non - fertilised control effect on yield of different maize hybrids**

In the non-fertilised treatments, yield ranged between 7.181 and 9.088 t ha<sup>-1</sup> in 2016 and between 4.951 and 6.088 t ha<sup>-1</sup> in 2017. In 2016, the hybrids Sushi and Fornad performed outstanding nutrient conversion ability (9.088 t ha<sup>-1</sup> and 8.904 t ha<sup>-1</sup>, respectively). Compared to Sushi, a 8-9% yield decrease was shown in the case of Loupiac and Armagnac. Based on the Duncan's test, these four hybrids can be considered identical, while the most significant decrease (27%) ( $P < 0.05$ ) could be shown in the case of Renford (Figure 3). In 2017, Sushi and Loupiac showed favourable nutrient conversion ability (6.088 t ha<sup>-1</sup> and 5.599 t ha<sup>-1</sup>, respectively). There was no significant correlation between the two hybrids. Compared to Sushi, the greatest yield decreases were observed in the case of Armagnac (23%) and Fornad (24%), while the most significant ( $P < 0.05$ ) decrease (28%) was shown in the case of Renford. Based on the Duncan's test, Armagnac, Fornad and Renfor can be considered identical (Figure 3).



**Fig. 3 -Yield (t - ha<sup>-1</sup>) of different maize hybrids, grown in non fertilized condition during 2016 and 2017. Legend: Columns indicated with different letters show significant differences from each other based on the Duncan's test at a probability level of  $P \leq 0.05$**

#### **Effect of fertilization treatments on yield of different maize hybrids**

The five maize hybrids of different genetic background tested in the experiment responded to fertiliser treatments differently in both years (Table 3), as described in detail below.

##### **Armagnac**

The significance analysis of fertiliser treatments performed for each genotype showed that Armagnac responded to the lowest fertiliser dose (T1) with 27% yield increase ( $P < 0.05$ ) in 2017. The next fertiliser level (T2) resulted in a further 25% increase in yield ( $P < 0.05$ ). There was no further significant difference among the treatments. In 2017, Armagnac did not respond to the lowest fertiliser dose (T1) with significant yield increase. The T2 treatment resulted in a significant yield increase (25%) in comparison with the F1 fertiliser treatment. Additionally, the T7 treatment was the one to increase yield by 35% in comparison with the T6 treatment. The most successful significant ( $P < 0.05$ ) treatment combination was that of T7 (Table 3).

##### **Fornad**

In 2016, the highest statistically verified yield (50%,  $P < 0.05$ ) for Fornad hybrid was T5. In 2017 there were significant differences between T3 and T5 (15%) and T6 and T7 (25%). treatments. The highest significant yield was obtained as a result of the T8 treatment combination, while further increasing fertiliser doses decreased yield, although not significantly (Table 3).

##### **Loupiac**

In the case of the Loupiac hybrid in 2016, as a result of the T1 treatment ( $P < 0.05$ ), yield increased by 19%. A significant difference was observed between the T1 and the T2 treatments (29%,  $P < 0.05$ ), as well as between the T1 and the T5 treatments (41%,  $P < 0.05$ ).

**Table 3 - The effect of fertiliser treatment combinations and climatic factors on the yield of maize hybrids (t ha<sup>-1</sup>) (2016 and 2017)**

Hybrids	Year	NPK treatment combination, kg ha <sup>-1</sup>										
		Non-fertilised	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Armagnac (FAO 490)	2016	8.354±0.40a A***	10.651±0.64b A***	13.346±0.95d A**	13.611±0.76d A***	15.142±0.92d A**	15.319±0.80d A**	11.187±0.71bc A**	13.119±0.66cd A*	13.679±0.53d A*	14.562±0.40d A***	14.068±0.96d A*
	2017	4.591±0.41a B	6.005±0.20ab B	7.508±0.53c B	7.106±0.48bc B	9.200±0.69d B	9.625±0.75de B	8.337±0.24cd B	11.250±0.61f B	11.514±0.21f B	10.865±0.34ef B	11.517±0.34f B
Formad (FAO 420)	2016	8.904±0.39a A***	10.155±0.30ab A***	12.208±0.34cd A***	12.521±0.29cd A**	12.664±0.71cd A	13.355±0.84d A*	11.171±0.61bc A**	12.195±0.72cd A*	12.347±0.39cd A	12.880±0.28cd A	12.632±0.29cd A
	2017	4.900±0.18a B	6.171±0.31b B	6.978±0.61bc B	9.542±0.54d B	10.366±0.80de A	10.994±0.38ef B	8.142±0.50c B	10.162±0.34de B	12.800±0.30g A	12.138±0.38fg A	12.047±0.33fg A
Loupjac (FAO 380)	2016	8.441±0.42a A***	10.080±0.33b A***	13.051±0.33de A***	13.074±0.33de A***	12.859±0.68de A*	14.190±0.31e A**	11.017±0.64bc A	12.305±0.40cd A**	12.580±0.30d A**	12.034±0.37cd A*	12.954±0.33de A**
	2017	5.599±0.27a B	5.935±0.46a B	8.203±0.17bc B	7.840±0.64b B	10.455±0.52de B	9.986±0.87de B	9.349±0.27bcd A	10.174±0.34de B	10.537±0.35de B	10.642±0.18de B	10.924±0.32e B
Renfor (FAO 320)	2016	7.181±0.34a A***	10.438±0.27bc A***	10.832±0.48bc A***	11.302±0.54cd A**	11.744±0.27cde A**	12.530±0.33de A***	9.699±0.38b A***	11.234±0.39c A***	13.014±0.19e A***	12.979±0.49e A***	12.557±0.52de A**
	2017	4.745±0.22a B	4.960±0.40a B	5.120±0.63a B	6.668±0.75b B	8.389±0.56cd B	8.369±0.32cd B	6.875±0.13b B	7.631±0.25bc B	8.466±0.24cd B	8.463±0.42cd B	9.588±0.245d B
Sushi (FAO 340)	2016	9.088±0.29a A**	11.028±0.71b A**	12.318±0.79bcd A*	12.761±0.23cd A***	13.474±0.28cd A*	13.866±0.50d A**	12.137±0.45bc A***	12.969±0.41cd A*	13.720±0.42cd A*	13.562±0.42cd A	12.736±0.57cd A
	2017	6.088±0.43a B	6.629±0.37a B	8.627±0.69b B	9.652±0.318b B	11.011±0.655c B	11.688±0.245cd B	9.326±0.099b B	11.170±0.30c B	12.035±0.23cd B	12.575±0.48d A	12.699±0.29d A

Values followed by different lowercase letters within a row are significantly different from different fertiliser treatments under the same water condition within a year ( $P < 0.05$ ).

Values followed by different capital letters within a column are significantly different from different water supply under the same fertilizer treatment within a year ( $P < 0.05$ ).

Statistically, the T2 treatment was successful. In 2017, the yield of the non-fertilised treatment of Loupiac significantly differed from all fertiliser levels, with the exception of the T1 treatment. A significant difference was observed between the T1 and the T2 treatments (38%,  $P < 0.05$ ), as well as the T2 and the T4 treatments (27%,  $P < 0.05$ ). The highest yield could be obtained with the T10 treatment, while the T4 treatment was the most successful from the statistical aspect and the difference between the two treatments was only 4% (Table 3).

### **Renfor**

In 2016, of the examined hybrids, Renfor provided the best response to the low fertiliser dose (T1), resulting in a yield surplus of 3.257 t ha<sup>-1</sup>, i.e. a 45% increase ( $P < 0.05$ ). The T7 treatment had an outstandingly positive effect ( $P < 0.05$ ). In 2017, the T1 and T2 treatments increased the yield of Renfor in comparison with the non-fertilised treatment, but the observed increases of 5% and 8% were not significant in accordance with the Duncan's test. As a result of the T3 treatment, yield increased by 30% ( $P < 0.05$ ) in comparison with the T2 treatment. A further increase of 14% ( $P < 0.05$ ) in yield was observed as a result of the T4 treatment, but the T6 treatment decreased yield by 18% ( $P < 0.05$ ). The greatest significant difference (34%  $P < 0.05$ ) resulted from the T6 treatment in comparison with the T2 treatment. In addition, the T4 and the T7 treatments resulted in similar outcomes. The difference between the most successful significant treatment of T4 and the highest yield was 15%, which was not significant (Table 3).

### **Sushi**

In 2016, the T1 treatment had a positive (21%,  $P < 0.05$ ) effect on the yield of Sushi in comparison with the non-fertilised treatment. There was no difference between the yields resulting from the T1, the T6 and the T2 treatments at the significance level of 5%. Further treatments applied in the experiment had similar results, the T5 treatment increased the yield of the hybrid the most effectively ( $P < 0.05$ ). In 2017, the T11 treatment did not have any significant positive (9%) effect on the yield of Sushi in comparison with the non-fertilised treatment. The next fertiliser level (T2) increased the yield of the hybrid by 30%. The T5 treatment resulted in further significant yield increase as compared to the T3 treatment. There was a significant decrease (20%) as a result of the T6 treatment as compared to the T5 treatment. From the statistical aspect, the most successful treatment was shown to be that of T4 (Table 3).

### **Effect of fertilization treatments on average yield increase for maize hybrids**

In 2016, the average yield increase resulting from fertilisation was 4.124 t ha<sup>-1</sup>, averaged over the different hybrids involved in the experiment. This yield increase was shown to be the most significant in the case of Armagnac and Renfor. In addition, Loupiac and Sushi also performed well. As a result of fertilization, the Fornad hybrid achieved the lowest average yield increase. In 2017, the average yield increase resulting from fertilisation was 4.005 t ha<sup>-1</sup>, averaged over the examined hybrids. In this year, this yield surplus was shown to be the most significant in the case of Sushi. Fornad, Lupiac and Armagnac also performed well. The average yield increasing effect of fertilisation was the lowest in the case of Renfor (Table 4).

### **The effect of fertilisation and genotype on the maize hybrid grain protein content**

The performed analysis of variance confirmed the effect of genotype ( $P < 0.001$ ) and fertilisation ( $P < 0.001$ ) on the protein content of the maize grain both in 2016 and 2017. The MS value shows the priority of the fertiliser effect in both years, which means that, in accordance with the findings of Luit et al. (1999), Hasaneen et al. (2009) and Da Silva et al. (2005), protein content is genetically determined (Idikut et al, 2009; Randjelovic et al, 2011), but it can be affected with fertilisation (Ványiné Széles and Nagy, 2012).

The correlation between genotype and fertilisation was significant ( $P < 0.001$ ) in 2017, but not in 2016 (Table 2).

### **Effect of Non-fertilised control and fertilization treatments effect on protein content of different maize hybrids**

#### **Armagnac**

The protein content of the non-fertilised treatment of Armagnac significantly differed from all treatment combinations in a more favourable crop year (2016), with the exception of the T1 treatment. Significant difference was observed between the T6 and the T7 treatments (12.8%,  $P < 0.05$ ), the T2 and the T3 treatments (7.2%,  $P < 0.05$ ), as well as between the T5 and the T6 treatments (-11.6%,  $P < 0.05$ ), due to the notable decrease of the nitrogen dose. The highest protein content resulted from the T10 treatment, but the most successful and statistically significant ( $P < 0.05$ ) treatment combination was that of T5. Under unfavourable climatic circumstances (2017), the T4 treatment was the first to cause a significant change in the protein content of Armagnac (11.3%,  $P < 0.05$ ). A significant decrease was shown as a result of the T6

**Table 4 - Effect of fertilization treatments on average yield increase for maize hybrids (2016 and 2017)**

Year	Hybrids	NPK treatment combination, kg ha <sup>1</sup>										Average
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	
2016	Armagnac (FAO 490)	2.297	4.992	5.257	6.788	6.965	2.833	4.765	5.325	6.208	5.714	<b>5.114</b>
	Fornad (FAO 420)	1.251	3.304	3,617	3.760	4.451	2.267	3.291	3.443	3.976	3.728	<b>3.309</b>
	Loupiac (FAO 380)	1.639	4.610	4.633	4.418	5.749	2.576	3.864	4.139	3.593	4.513	<b>3.973</b>
	Renfor (FAO 320)	3.257	3.651	4.121	4.563	5.349	2.518	4.053	5.833	5.798	5.376	<b>4.452</b>
	Sushi (FAO 340)	1.940	3.230	3.673	4.386	4.778	3.049	3.881	4.632	4.474	3.648	<b>3.769</b>
2017	Armagnac (FAO 490)	1.054	2.557	2.155	4.249	4.674	3.386	6.299	6.563	5.914	6.566	<b>3.947</b>
	Fornad (FAO 420)	1.271	2.078	4.642	5.466	6.094	3.242	5.262	7.920	7.238	7.147	<b>4.527</b>
	Loupiac (FAO 380)	0.336	2.604	2.241	4.855	4.386	3.750	4.575	4.938	5.043	5.325	<b>4.108</b>
	Renfor(FAO 320)	0.215	0.375	1.923	3.644	3.624	2.131	2.887	3.721	3.718	4.843	<b>2.256</b>
	Sushi (FAO 340)	0.541	2.539	3.565	4.923	5.600	3.238	5.083	5.947	6.487	6.612	<b>5.185</b>

treatment in comparison with the T5 treatment. The extent of decrease was 13.4% ( $P < 0.05$ ). The highest protein content was reached by applying the T9 treatment combination. However, the T5 treatment was shown to be significant (Table 5).

### **Fornad**

As regards the hybrid Fornad, the first treatment combination to result in significant increase (17.6%,  $P < 0.05$ ) in comparison with the non-fertilised protein content was that of T4 in 2016. Duncan's test did not show any difference between the T4, T5 and the T6 treatments. In addition, all further treatments constituted one group. The highest protein content resulted from the T9 treatment, but the T7 treatment can also be considered successful based on the performed analysis. Similarly to 2016, it was the T4 treatment which increased (18.1%,  $P < 0.05$ ) the protein content of Fornad in 2017. The T6 treatment reduced the protein content (by 8.7%,  $P < 0.05$ ) in comparison with the T5 treatment. The highest and statistically significant treatment combination was that of T9 (Table 5).

### **Loupiac**

As regards the hybrid Loupiac, the first treatment combination to significantly increase the protein content in comparison with the non-fertilised treatment (9.4%,  $P < 0.05$ ) was that of T3. Based on the Duncan's test, all further treatments constituted one group. The highest protein content resulted from the T10 treatment, while the T4 treatment was statistically significant. No significant difference could be observed between the non-fertilised, the T1, T2 and the T6 treatments in the dry year of 2017. The T6 treatment resulted in a significant reduction of protein content (31.6%,  $P < 0.05$ ) in comparison with the T5 treatment. Similarly to 2016, the successful and significant treatment combination was that of T4, although the highest protein content was obtained as a result of the T10 treatment, due to the high amount of nitrogen fertiliser (Table 5).

### **Renfor**

The protein content of the hybrid Renfor in the non-fertilised treatment significantly differed from all fertiliser levels, with the exception of the T1 treatment. Significant differences were observed between the T1 and the T2 treatments (11.7%,  $P < 0.05$ ), the T5 and the T6 treatments (-12.4%,  $P < 0.05$ ), as well as the T6 and the T7 treatments (14.6%,  $P < 0.05$ ). The treatment combination of T8 was shown to be successful. In 2017, the protein content of the hybrid Renford was similar both in the case of the non-fertilised and the T6 treatment. Compared to the non-fertilised treatment,

the T1 treatment slightly increased protein content (6.8%,  $P < 0.05$ ). The subsequent treatment of T2 resulted in a further increase of 11.7% ( $P < 0.05$ ). There was a significant difference between the T2 and the T5 treatment (7.2%,  $P < 0.05$ ). The protein content greatly decreased as a result of the T6 treatment in comparison with the T5 treatment (-17.1%,  $P < 0.05$ ). The biggest and also most successful treatment was that of T10 (Table 5).

### **Sushi**

In 2016, the T1 and T2 treatments did not result in a significant increase of protein content of Sushi. The subsequent treatment of T3 resulted in a 12% increase ( $P < 0.05$ ) in comparison with the non-fertilised treatment. A further increase of 8.8% ( $P < 0.05$ ) could be detected between the T3 and the T4 treatment. Based on the Duncan's test, all other treatments were classified into one group. The highest protein content was provided by the T10 treatment, but the T5 treatment was significant from the statistical aspect. In 2017, the protein content of Sushi was the same in the case of the non-fertilised, the T1, T2 and the T6 treatments. There was no significant difference between the T4, T5, T7 and the T8 treatments either. The protein content resulting from the T9 treatment was worth emphasising (Table 5).

### **Differential effect of fertilization treatments on protein content increase in maize hybrids**

Fertilization, in the average of hybrids, increased protein content by an average of  $1.5 \text{ g} \times 100 \text{ g}^{-1}$  in 2016 and  $1.2 \text{ g} \times 100 \text{ g}^{-1}$  in 2017. In 2016, the average protein content increasing effect of fertilization was the highest in the Armagnac hybrid, while in the other examined hybrids the protein content surplus was the same. In 2017, the surplus protein content proved to be more significant in the case of the Sushi and Fornad hybrids. The average protein content increasing effect of fertilization was the lowest in the case of the Armagnac hybrid (Table 6).

Based on the examination of each NPK treatment combination, it can be observed that in 2016, the protein content of Loupiac significantly exceeded ( $P < 0.05$ ) that of Fornad on all fertiliser levels, with the exception of the T9 treatment. The greatest difference was observed in the case of the T6 treatment (16.3%). The hybrids Armagnac, Renfor and Sushi did not show any significant difference in the increasing N+constant proportion PK treatment combinations, while there was significant difference between the protein content of Armagnac and Renfor and Sushi in the case of the increasing N+same proportion PK treatment combination.



**Table 5 - The effect of fertiliser treatment combinations and climatic factors on the protein content of maize grain (g x 100 g<sup>-1</sup>) (2016 and 2017)**

Hybrids	Year	NPK treatment combination. kg ha <sup>-1</sup>										
		Non-fertilised	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Armagnac (FAO 490)	2016	7.5±0.077a A	7.8±0.259ab A	8.3±0.086bc A	8.9±0.212de A	9.4±0.149ef A	9.6±0.081fg A	8.6±0.188cd A	9.7±0.348fg A	9.6±0.119fg A	9.6±0.064fg A	10.0±0.228g A
	2017	8.0±0.136a B**	8.4±0.119ab A	8.4±0.225ab A	8.2±0.259a A	8.9±0.267bc A	9.3±0.263cd A	8.2±0.246a A	9.0±0.158c A	9.3±0.213cd A	9.8±0.150d A	9.7±0.110d A
Formad (FAO 420)	2016	7.4±0.181ab A	7.2±0.236a A	8.0±0.236bc A	8.2±0.284bc A	8.7±0.206cd A	9.0±0.275de A	8.6±0.492cd A	9.7±0.217ef A	9.6±0.132ef A	10.2±0.187f A	10.0±0.225f A
	2017	7.2±0.135a A	7.1±0.193a A	7.2±0.135a B*	7.4±0.149a A	8.5±0.330bc A	8.8±0.158c A	8.1±0.196b A	8.7±0.064c B**	9.0±0.165c B*	10.1±0.295d A	9.8±0.155d A
Loupiac (FAO 380)	2016	8.5±0.226a A	8.2±0.249a A	8.9±0.132ab A	9.3±0.250bc A	10.0±0.165cd A	10.0±0.137d A	10.0±0.409cd A	10.6±0.125d A	10.6±0.217d A	10.4±0.047d A	10.8±0.352d A
	2017	8.1±0.382ab A	8.3±0.332ab A	8.4±0.210ab A	8.7±0.110bc A	9.6±0.165cd A	10.0±0.259d A	7.6±0.302a B**	9.9±0.344d A	9.9±0.248d A	10.2±0.197d A	10.4±0.217d A
Renfor (FAO 320)	2016	8.1±0.183ab A	7.7±0.143a A	8.6±0.255bc A	8.9±0.141c A	9.6±0.132d A	10.0±0.131de A	8.9±0.385c A	10.2±0.202de A	10.3±0.137ef A	10.4±0.095ef A	10.8±0.131f A
	2017	7.3±0.120a B**	7.8±0.258b A	8.3±0.187cd A	8.0±0.154bc B**	8.7±0.135de B	8.9±0.212e B**	7.6±0.086ab B*	8.1±0.091bc B***	8.3±0.1322cd B***	8.7±0.110de B***	9.4±0.028f B***
Sushi (FAO 340)	2016	8.1±0.295a A	7.7±0.376a A	8.5±0.197ab A	9.1±0.110b A	9.9±0.178c A	10.2±0.122cd A	8.9±0.390b A	10.2±0.137cd A	10.2±0.160cd A	10.8±0.158d A	10.9±0.158d A
	2017	7.6±0.223a A	7.9±0.129ab A	8.1±0.062ab A	8.4±0.273bc A	9.5±0.173d A	9.4±0.188d B*	7.5±0.149a B*	9.0±0.193cd B**	9.6±0.232d A	10.2±0.205e A	10.2±0.040e B**

Values followed by different lowercase letters within a row are significantly different from different fertiliser treatments under the same water condition within a year ( $P < 0.05$ ).

Values followed by different capital letters within a column are significantly different from different water supply under the same fertilizer treatment within a year ( $P < 0.05$ ).

**Table 6 - Effect of fertilization treatments on average protein content increase for maize hybrids (2016 and 2017)**

Year	Hybrids	NPK treatment combination, kg ha <sup>-1</sup>										Average
		T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	
2016	Armagnac (FAO 490)	0.3	0.8	1.4	1.9	2.1	1.1	2.2	2.1		2.1	<b>1.7</b>
	Fornad (FAO 420)	-0.2	0.6	0.8	1.3	1.6	1.2	2.3	2.2	2.8	2.5	<b>1.5</b>
	Loupiac (FAO 380)	-0.3	-0.4	0.8	1.5	1.5	1.5	2.1	2.1	1.9	2.3	<b>1.4</b>
	Renfor (FAO 320)	0.4	0.5	0.8	1.5	2.0	0.8	2.1	2.2	2.3	2.7	<b>1.5</b>
	Sushi (FAO 340)	-0.4	0.4	1.0	1.8	2.1	0.8	2.1	2.1	2.7	2.8	<b>1.5</b>
2017	Armagnac (FAO 490)	0.4	0.4	0.2	0.9	1.3	0.2	1.0	1.3	1.8	1.7	<b>0.9</b>
	Fornad (FAO 420)	-0.1	0.0	0.2	1.3	1.6	0.9	1.5	1.8	2.9	2.6	<b>1.3</b>
	Loupiac (FAO 380)	0.2	0.3	0.6	1.5	1.9	-0.5	1.8	1.8	2.1	2.3	<b>1.2</b>
	Renfor (FAO 320)	0.5	1.0	0.7	1.4	1.6	0.3	0.8	1.0	1.4	2.1	<b>1.1</b>
	Sushi (FAO 340)	0.3	0.5	0.8	1.9	1.8	-0.1	1.4	2.0	2.6	2.6	<b>1.4</b>

Values followed by different lowercase letters within a row are significantly different from different fertiliser treatments under the same water condition within a year ( $P < 0.05$ ).

Values followed by different capital letters within a column are significantly different from different water supply under the same fertilizer treatment within a year ( $P < 0.05$ ).

In the dry year of 2017, Fornad and Loupiac – two hybrids with different protein content – showed significant difference ( $P < 0.05$ ) when compared to each other on all fertiliser levels, with the exception of the T6 treatment combination. There was no significant difference between the protein contents of Armagnac, Renfor and Sushi in the non-fertilised, the T1, T2, T3 and the T9 treatments, while this effect could not be detected in the other treatment combinations.

In accordance with the conclusions of Feng et al. (1993) and Singh et al. (2005), the protein content of the maize grain increased both in 2016 ( $p < 0.001$ ) and 2017 ( $p < 0.001$ ) in the case of all examined hybrids as a result of increasing fertiliser dose, which confirmed the findings of Tsai et al. (1992) and Raja (2003), i.e., fertiliser treatments containing N not only limit yield,

but they also affect maize grain quality.

### **The impact of climatic factors on the yield of maize hybrids**

The analysis of variance (ANOVA) performed for each genotype showed the significant impact of climatic factors ( $P < 0.001$ ) on yield in the case of all five examined hybrids and, based on the MS value, their influence was greater than that of the similarly significant fertilisation. The correlation between climatic factors x fertilisation was also present in the case of all examined hybrids ( $P < 0.001$ ).

Due to the 778 mm precipitation in the winter period and growing season of 2016, as well as the 44 mm difference between the precipitation sum and the PET value, higher yield could be obtained – averaged over

the different treatment combinations – in 2017, when the PET value significantly exceeded (+311 mm) the amount of precipitation. In 2016, the yield of Renfor (55.8%) and Armagnac (46.1%) showed the biggest difference in comparison with the 2017 results. The yield of Loupiac showed a 33.1% difference, while the same values were 25.7% and 23.5% in the case of Renfor and Sushi, respectively. These findings are in conformity with those of Adebayo and Menkir (2014), i.e., drought significantly reduces hybrid yields (-70%) and there is a difference between results in terms of the extent of deviation.

The various yields obtained as a result of the applied treatment combinations show significant differences due to the changes in climatic factors. Resulting from the unfavourable weather in 2017, the greatest decrease was observed as a consequence of the T1 treatment of three hybrids – Loupiac (-69.8%,  $P<0.001$ ), Renfor (-111.0%,  $P<0.001$ ) and Sushi (-66.4%,  $P<0.01$ ). As regards Armagnac (-91.5%,  $P<0.001$ ), the most significant decrease was caused by the T3 treatment, while the same phenomenon was caused by the non-fertilised treatment in the case of the hybrid Fornad (-81.7%,  $P<0.001$ ) (Table 3).

The obtained results confirm the findings of Fixen et al. (2005) and Roberts (2008), i.e., interactions may increase or decrease the uptake and utilisation of nutrients, thereby affecting yield which is also modified by genotype and climatic factors (Pepó and Karancsi, 2017).

In 2016, when the amount of precipitation was much higher in the growing season (450 mm), the average yield related to the increasing N + same proportion PK treatment combination group decreased in the case of Armagnac and Loupiac, while it increased for Fornad, Renfor and Sushi in comparison with the increasing N + constant proportion PK treatment combination. No significant difference was shown between the two treatment combination groups, except for Renfor ( $P<0.05$ ). In 2017, when there was 340 mm precipitation in the growing season and the PET value was high (674 mm), the increasing N + same proportion PK treatment combination group resulted in higher yields of Armagnac ( $P<0.001$ ), Fornad ( $P<0.001$ ) and Sushi ( $P<0.01$ ) in comparison with the increasing N + constant proportion PK treatment combination. The obtained results confirm the conclusions of Ma et al. (2006) and Zörb et al. (2014), i.e., crops with a better supply of PK have stronger resistance to climatic stress.

### The impact of climatic factors on the protein content of maize hybrids

In 2017, when the prevailing water supply circumstances were unfavourable, the protein content of Armagnac increased by  $0.5 \text{ g} \times 100 \text{ g}^{-1}$  ( $P<0.01$ ) as a result of the non-fertilised treatment in comparison with the more favourable protein content in 2016. In the rest of the applied treatment combinations, no difference was shown between the different years. As regards Fornad, the greatest significant difference resulted from the T7 treatment combination ( $1.0 \text{ g} \times 100 \text{ g}^{-1}$ ,  $P<0.01$ ), but the protein content increases caused by the T2 treatment ( $0.8 \text{ g} \times 100 \text{ g}^{-1}$ ,  $P<0.05$ ) and the T8 treatment ( $0.6 \text{ g} \times 100 \text{ g}^{-1}$ ,  $P<0.05$ ) were also significant. In the case of Loupiac, significant increase could only be detected in relation to the T6 treatment ( $2.4 \text{ g} \times 100 \text{ g}^{-1}$ ,  $P<0.01$ ). As regards Renfor, significant differences were found in the case of all treatment combinations, except for the T1 and T2 treatments. The biggest difference was found in the T7 ( $2.1 \text{ g} \times 100 \text{ g}^{-1}$ ,  $P<0.001$ ) and the T8 treatment ( $2.0 \text{ g} \times 100 \text{ g}^{-1}$ ,  $P<0.001$ ). In the case of Sushi, the significant effect of environmental factors could be detected in three treatment combinations T5 ( $P<0.05$ ), T6 ( $P<0.05$ ) and T7 ( $P<0.01$ ) (Table 5).

**Table 7 - Correlation of the yield and protein content of different genotype maize hybrids (2016 and 2017)**

yield*protein content lin. reg	Hybrids	2016. year	2017. year
Armagnac			
r		0.721	0.775
R <sup>2</sup>		0.519***	0.600***
		$y' = -5.551 + 2.051 \text{ prot.}$	$y' = -14.676 + 2.654 \text{ prot.}$
Fornad			
r		0.617	0.823
R <sup>2</sup>		0.380***	0.678***
		$y' = 3.364 + 0.960 \text{ prot.}$	$y' = -8.728 + 2.157 \text{ prot.}$
Loupiac			
r		0.593	0.601
R <sup>2</sup>		0.352***	0.361***
		$y' = 0.436 + 1.176 \text{ prot.}$	$y' = -1.785 + 1.160 \text{ prot.}$
Renfor			
r		0.711	0.674
R <sup>2</sup>		0.505***	0.454***
		$y' = -1.889 + 1.377 \text{ prot.}$	$y' = -8.062 + 1.835 \text{ prot.}$
Sushi			
r		0.750	0.817
R <sup>2</sup>		0.562***	0.668***
		$y' = 1.422 + 1.152 \text{ prot.}$	$y' = -7.373 + 1.964 \text{ prot.}$

\*\*\*significant at  $P=0.001$

### Correlation analysis

The correlation between the yield and grain protein content of hybrids with different genotypes was examined. Based on the performed statistical analysis, the correlation between the different variables can be described using a linear function, which was also confirmed by the F test at a significance level of 0.1%.

There was a close correlation between the protein content of the examined hybrids and their yield, except in the case of Loupiac, where average correlation was found (0.593\*\*\* in 2016 and 0.601\*\*\* in 2017). The correlation coefficient of Sushi was the highest in 2016 ( $r=0.750^{***}$ ), while that of Fornad was the highest in 2017 (0.823\*\*\*). The examined hybrids showed a significant correlation between yield and protein content in the year with favourable water supply conditions (2017) (Table 7).

### Conclusions

Our results confirm that great emphasis must be put on the different fertilizer response and nutrient utilization of maize hybrids, which is greatly influenced by climatic factors. It has been confirmed that the tolerance of hybrids to environmental stress factors can be increased by using increasing N + constant proportion PK treatment combination. The maximum and economically achievable yields were consistent with all genotypes except for the Armagnac hybrid in favourable production years, whereas in unfavourable production years vintage only the Fornad hybrid linked these two values. It was confirmed that the fertilizer treatments increased the protein content in both years; however, protein content was higher in years with better water supply than in the dry year. Overall, the results contribute to improving the utilization of NPK fertilizer in maize hybrids and to selecting a hybrid that is more suitable for the purpose of utilization, which leads to a more environmentally friendly and cost-effective production.

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