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Full Length Research Paper

Effect of nitrogen doses on the chlorophyll concentration, yield and protein content of different genotype maize hybrids in Hungary

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The chlorophyll (Chl) concentration of maize was compared to its grain yield and protein content in six different N treatments (0, 30, 60, 90, 120 and 150 kg N ha^{-1}) in a multifactorial field experiment with four replications and a split plot design in Eastern Hungary (47°33' N, 21°26' E, 111 m asl) in two years (2009 and 2010). Nitrogen fertilisation significantly ($p < 0.001$) increased Chl content in both years. In the dry year (2009), the Chl content did not increase as a result of N fertilisation above 120 kg N ha^{-1}, while the efficiency of higher N rates (150 kg N ha^{-1}) could be shown in the wet year (2010), varying per hybrid. The weather-induced abiotic stress effects on yield and protein content could be reduced by N fertilisation, but this required high input costs. In 2009, both hybrids were treated with the highest N fertiliser dose (150 kg N ha^{-1}) in order to reach the statistically highest yield (Mv 277 hybrid 6.76 t ha^{-1}; Kenéz hybrid 9.86 t ha^{-1}), and protein content (Mv 277 hybrid 10.2 g per 100 g dry matter; Kenéz hybrid 9.8 g per 100 g dry matter), while lower N fertiliser doses were enough in the favourable crop year of 2010. The correlation analysis results showed that weather parameters significantly influence the closeness of correlations, but are always positive. We concluded that the on-site measurement of leaf chlorophyll (SPAD) serves as a good indicator of the N demand of maize, as the difference between N-deficient and adequate N treatments is thereby more easily made. Therefore, the Chl content of maize leaf can be used effectively in developing recommendations for soil N replenishment.

Key words: Maize, fertilisation, chlorophyll concentration, protein content.

INTRODUCTION

Intensive crop nutrition is indispensable for yield increase. Nevertheless, determination of the optimal fertiliser dose is one of the most difficult crop production tasks. One has to consider the nutrient management and nutrient binding ability of the soil, whereas, the nutrient utilisation ability and the fertiliser reaction of the produced hybrid and the crop year effect also have to be taken into account (D' Haene et al., 2007). Of the three macro-elements (NPK), researchers considered nitrogen fertilisation to have the highest yield increasing effect in maize on most soils (Shaahan et al., 1999; Nagy, 2008). Nitrogen plays a key role in several physiological crop processes. As a result of increasing N doses, the photosynthetic activity, leaf area index (LAI) and leaf area density (LAD) increase (Dwyer and Anderson, 1995; Tóth et al., 2002, Víg et al., 2008).

Traditionally, the selection of maize hybrids was determined by productivity and yield stability. Nevertheless, in recent years, quality parameters have become more significant. In Hungary, 89% of grain maize was used as forage, but other alternative uses of maize are also gaining ground. These alternative purposes require the knowledge of the hybrids' inner content values and the effect of the various available agrotechnical factors on quality.

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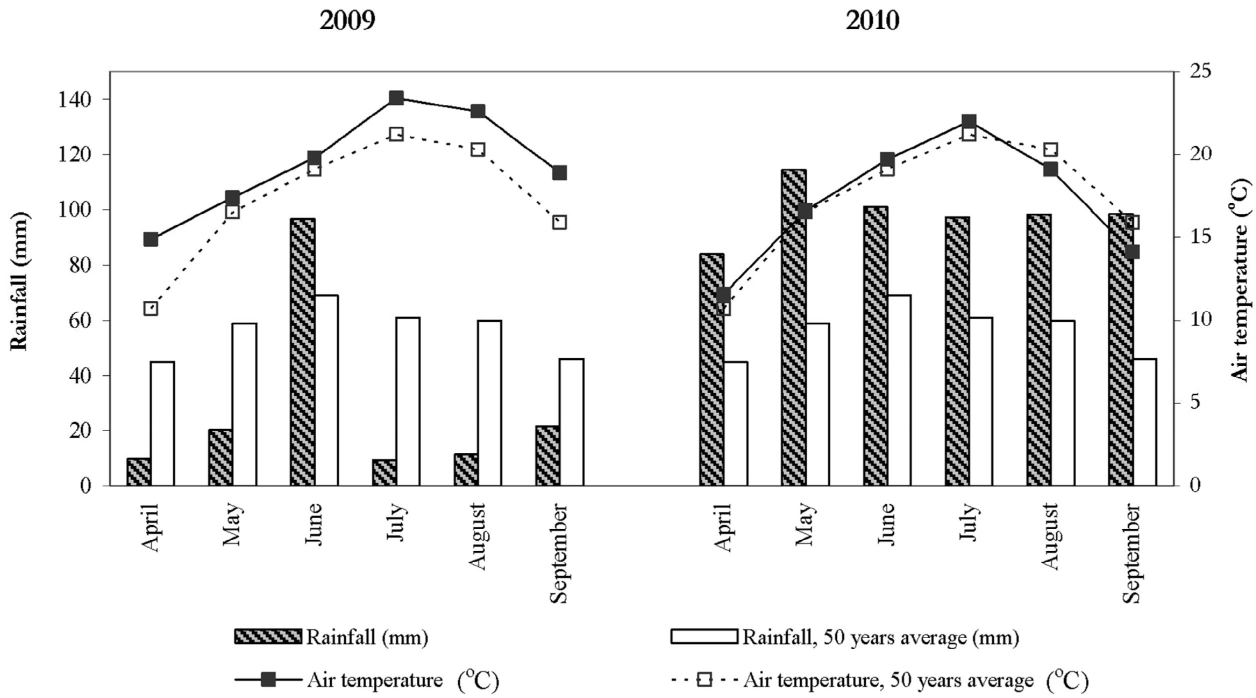

Figure 1. Monthly average of air temperature and rainfall over the 2009 to 2010 crop seasons.

The primary aim of our present study was to evaluate the effect of N fertilisation on the Chl content, grain yield and protein content of maize, as well as, the correlation between Chl content, grain yield and protein content in the growing seasons of 2009 and 2010. It was our secondary aim to examine whether the on-site measurement of leaf chlorophyll (SPAD) serves as a proper indicator for the N demand of maize.

MATERIALS AND METHODS

Production site description

The examinations were carried out at the Látókép Experimental Station of the Centre for Agricultural and Applied Economic Sciences of the University of Debrecen, in Eastern Hungary (47°33' N, 21°26' E, 111 m asl), in the growing seasons of 2009 and 2010. The experimental station was located in a moderately warm and dry production area on loess-based lowland mid-heavy pseudomyceliar chernozem (Mollisol-Calciustoll or Vermustoll/Pachic or Typic, silt loam, USDA '90 taxonomy) with deep humus layer. Based on the soil analysis data obtained in the spring of 2009, the average pH_{KCl} value of soil was 6.6 (slightly acidic), which is optimal from the point of crops' nutrient uptake. The Arany plasticity index in the upper (20 cm) layer of the soil was 39, the total amount of water-soluble salts (anions and cations) was 0.04%. The calcium-carbonate content in the upper 80 cm of the soil was around 0%, whereas, this figure was 12% from a depth of 100 cm. Organic matter content in the upper 20 cm layer of the soil was 2.3% and does not exceed 1% at a depth of 120 cm. The potassium supply of the soil was favourable, whereas its P supply was moderate.

The trifactoral field experiment (factor A is irrigation, factor B is fertilisation and factor C is hybrid) has four replications and a strip plot design. Here, block is equal to repetition. Fertilisation and hybrid treatments can be found in the main plots, both in irrigated and non-irrigated treatments. Therefore, the impacts of treatments placed across each other (fertiliser, hybrid) could be determined with the same accuracy. Plot size was 15 m^2. Weather was evaluated on the basis of the values measured and logged by the automatic weather station installed at the experimental site.

In the growing season of 2009, there was not enough rainfall (169 mm) for maize and even its distribution was unfavourable. Although, there was enough precipitation in June (97 mm), there were only 21 mm in July and August altogether (Figure 1). In 2009, the amount of rainfall was 171 mm less than the 50-year average (reference years: 1951 to 2000). As regards temperature, the situation was similarly unfavourable, since July – the period of maize flowering – was 2.2°C warmer than the 50-year average. In August, temperatures were also above the average (by 2.2°C). 2009 was a drought year; therefore, circumstances were not beneficial for maize production.

In 2010, the period between the harvesting of the previous crop and sowing was wet; the amount of rainfall was 328 mm, which was 85 mm more than the 50-year average. As a result of this phenomenon, soils were adequately supplied with water. In the growing season, the amount of precipitation exceeded the 50-year average of this period by 250 mm. The temperature during the growing season was sufficiently warm and even the number of sunny hours was higher than the 50-year average. Altogether, seen from the aspect of maize production, the weather in 2010 can be considered to have been wet (Figure 1).

Experimental layout

Six fertiliser treatments (0, 30, 60, 90, 120 and 150 kg N ha^{-1}) were used in the field experiment in both years. The entire amount of fertiliser (ammonium nitrate) was applied in the spring, 1 month before sowing. N, P or K fertiliser was applied in the examined

Table 1. Variance analysis results of the Chl content, yield and grain protein content of maize (2009 to 2010).

Factors	DF	2009			2010		
		Chlorophyll content	Protein g (100 g dry matter)$^{-1}$	Yield(t ha^{-1})	Chlorophyll content	Protein g (100 g dry matter)$^{-1}$	Yield(t ha^{-1})
		F values			*F values*		
Hybrids	1	51.346**	43.928**	388.272***	5.328^{ns}	0.422^{ns}	26.839*
Fertiliser	5	222.784***	53.609***	18.609***	21.680***	4.381*	13.474***
Hybrid*Fertiliser	5	1.316^{ns}	119.095***	2.845^{ns}	4.929***	187.788***	0.207^{ns}

ns, non -significant; *, P< 0.05 P < 0.01; ***, P < 0.001.

period. Sowing was carried out after winter ploughing and spring seedbed preparation on 15th April, 2009 and 23rd April, 2010, at 76 cm row spacing and 70 000 seeds per hectare. Short season maize hybrid Mv 277 SC (FAO, 310) and mid-season maize hybrid Kenéz (FAO, 410) was involved in the examinations. The preceding crop was maize. The harvested grain yield was determined at 14% moisture content. The measurements were carried out on the same plots in both years.

During the experiment, the relative chlorophyll concentration of maize leaves was measured using a SPAD-502 (Minolta, Japan) portable chlorophyll meter, based on the methods described by Yadava (1986) and Schepers et al. (1992). In both years, measurements were conducted in the reproductive (R1) phase. CMR (Chlorophyll Meter Readings) were obtained on the ear leaf of 20 plants per N treatment. In both years, samples were taken from both maize hybrids' yields in each treatment and the grain protein content was measured using a Foss Infratec^{TM} 1241 m, which is based on near-infrared-transmittance (NIT) technology.

Statistical analysis

A general linear model (GLM) was used to show the effect of treatments on the Chl content, yield and protein content of maize. In order to determine the treatment mean values, a significance level of 5% was set ($LSD_{5\%}$). During the multiple comparisons, Duncan's test was used to correct confidence intervals in order to avoid the accumulation of alpha errors. Quality parameters within the homogeneous group and yield did not differ from each other at the 5% significance level. Regression analysis was performed in order to determine the correlations between the chlorophyll content (Chl) of maize leaves measured in the growing season and the yield and protein content. The correlation between the variables can be described by a linear equation, which was also justified by an F test at the 0.1% significance level. This evaluation was carried out each year using SPSS for Windows 13.0.

RESULTS AND DISCUSSION

Chl content of maize leaves

The years 2009 and 2010 provided an opportunity to analyse the N fertiliser reaction of maize in connection with the Chl content of maize leaves, protein content and genotypes under different rainfall conditions. The Chl content of maize leaves significantly increased with the increase of N dose both in 2009 ($p < 0.001$) and 2010 ($p < 0.001$). The difference between the two hybrids was significant in 2009 ($p < 0.01$). There was significant ($p < 0.001$) interaction between hybrids × fertilisation in 2010 (Tables 1 and 2).

In both years, the lowest Chl content was measured on the non-fertilised plots. In 2009, the average Chl content of N-fertilised plots was 17.0% higher than that in 2010. The measured difference was significant ($p < 0.001$). In all N treatments, the Chl content of the Mv 277 hybrid in 2009 (56.7, $p < 0.01$) was higher than that of the Kenéz hybrid (51.7), whereas, there was no significant difference between the two hybrids in 2010.

Based on the Duncan's test at the 5% significance level, 120 kg N ha^{-1} were needed in order to attain the highest Chl content for both hybrids in 2009. In 2010, 150 kg N ha^{-1} were needed in the case of the Mv 277 hybrid and 60 kg N ha^{-1} were necessary in the case of the Kenéz hybrid (Figure 2). In the wet year (2010), the Chl content of hybrids was lower than in the dry year of 2009.

The extent of decrease was 11.1 ($p < 0.001$) in the case of the Mv 277 hybrid and 4.4 in the case of the Kenéz hybrid and was not significant.

Grain yield

Averaging the yield increments of both hybrids, it can be established that the yield increasing effect of fertilisation was 2.79 t ha^{-1} ($P < 0.001$) in 2009, whereas, this effect was significantly lower in 2010 (1.83 t ha^{-1}, $P < 0.001$). The yield of the Kenéz hybrid on the control plots was higher than that of the Mv 277 hybrid in both years, which means that the former utilised the natural nutrient stock of the soil better.

In 2009, the statistically highest yield of both

Table 2. Coefficient of variation of the Chl content, yield and grain protein content of maize, (2009 to 2010).

Hybrids	N Fertiliser	2009			2010		
		Chlorophyll content	Protein g (100 g dry matter)$^{-1}$	Yield (t ha^{-1})	Chlorophyll content	Protein g (100 g dry matter)$^{-1}$	Yield (t ha^{-1})
		---------CV%-------			---------CV%---------		
Mv 277	0	14.3	2.5	14.2	12.5	5.3	5.3
	30	10.6	3.5	7.1	13.5	5.4	7.2
	60	10.6	2.8	19.7	12.4	4.2	22.1
	90	8.1	4.7	12.9	11.2	1.9	7.5
	120	8.3	0.08	10.7	11.9	6.8	10.1
	150	12.7	1.1	12.1	13.8	3.5	3.7
Kenéz	0	16.9	0.6	11.8	11.5	1.4	18.9
	30	11.4	2.1	9.3	13.6	1.7	6.4
	60	12.4	4.8	11.2	10.8	2.9	21.9
	90	12.2	6.7	7.6	15.2	2.6	8.6
	120	11.7	3.0	10.9	11.5	1.5	11.3
	150	12.0	1.5	3.1	11.0	1.0	8.6

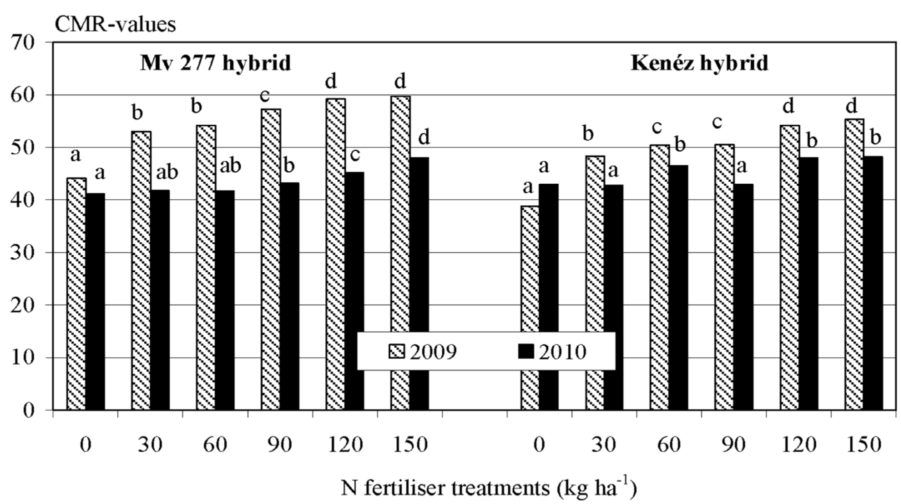

Figure 2. Effect of different N treatments on the Chl content of maize leaves in the R1 phase (2009 to 2010). Data marked with the same letter do not significantly differ from each other on the basis of the Duncan's test.

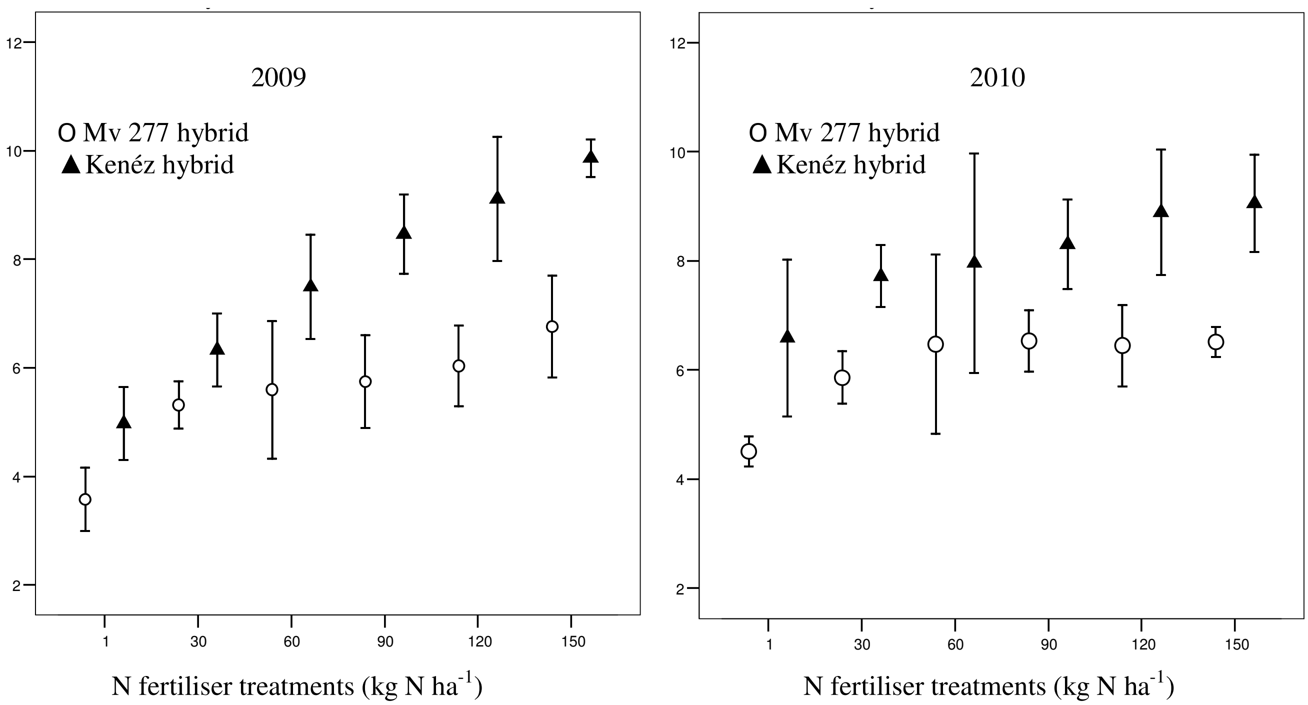

Figure 3. Maize yield as a result of fertiliser treatments (2009 to 2010). Values are mean ± SD expressed as t ha^{-1}.

hybrids was observed in the case of 150 kg N ha^{-1} N treatment, whereas, in 2010, 30 kg N ha^{-1} had to be applied in the case of the Mv 277 hybrid and the yield of the Kenéz hybrid was linearly increased up to 120 kg N ha^{-1}; however, the further increase of the N dose did not result in higher yield (Figure 3).

The results of the general linear model (GLM) show that the yield of the Kenéz hybrid was significantly higher compared to the Mv 277 hybrid, in both years. This difference is more significant in 2009 ($p < 0.001$) than in 2010 ($p < 0.05$). Nevertheless, the genotype × fertilisation interaction was not significant in either year (Table 1).

Protein content

Similar to the findings of Feng et al. (1993) and Singh et al. (2005), the grain protein content increased with increasing N doses both in 2009 ($p < 0.001$) and in 2010 ($p < 0.05$).

Based on the Duncan's test, it was established that 150 kg N ha^{-1} were necessary to reach the highest protein content in the case of both hybrids in 2009 (Mv 277 hybrid: 10.2 g per 100 g dry matter; Kenéz hybrid: 9.8 g per 100 g dry matter). In 2010, five homogeneous groups of the Mv 277 hybrid were set up and the highest protein content was measured in the case of 120 kg N ha^{-1} (10.6 g per 100 g dry matter). As concerning the Kenéz hybrid, the Duncan's test created four separated groups and the highest protein content was measured in the case of 90 kg N ha^{-1} (10.5 g per 100 g dry matter).

In 2009, the protein content of the Mv 277 hybrid was higher (9.5 g per 100 g dry matter; $p < 0.01$) in the average of N treatments than that of the Kenéz hybrid (8.8 g per 100 g dry matter). In 2010, there was no significant difference between the average protein content of hybrids across treatments.

The increase of protein content was similar (1.3 to 1.3 g per 100 g dry matter) in the case of both hybrids in 2009 in the average of fertiliser treatments. In 2010, fertilisation had a more expressed yield increasing effect in the case of the Kenéz hybrid (0.8 g per 100 g dry matter). In 2010, favourable precipitation supply resulted in the increase of grain protein content in the case of both hybrids (Mv 277 hybrid: 0.9 g per 100 g dry matter; Kenéz hybrid: 1.7 g per 100 g dry matter) and this increase was significant in all cases ($p < 0.01$). This phenomenon was also revealed by the fact that the grain protein content reduction of hybrids of different genotypes can be restored with proper nutrition.

Correlation analysis

The correlation analysis showed a positive ($p < 0.001$) correlation between Chl content and yield, which demonstrated that chlorophyll content plays an indispensable role in determining yield. The closeness of this correlation depended on environmental factors. In the dry year (2009), there was an average correlation in the case of both hybrids (Mv 277 hybrid: $r = 0.532$; Kenéz hybrid: $r = 0.539$), while a weaker correlation was observed in the

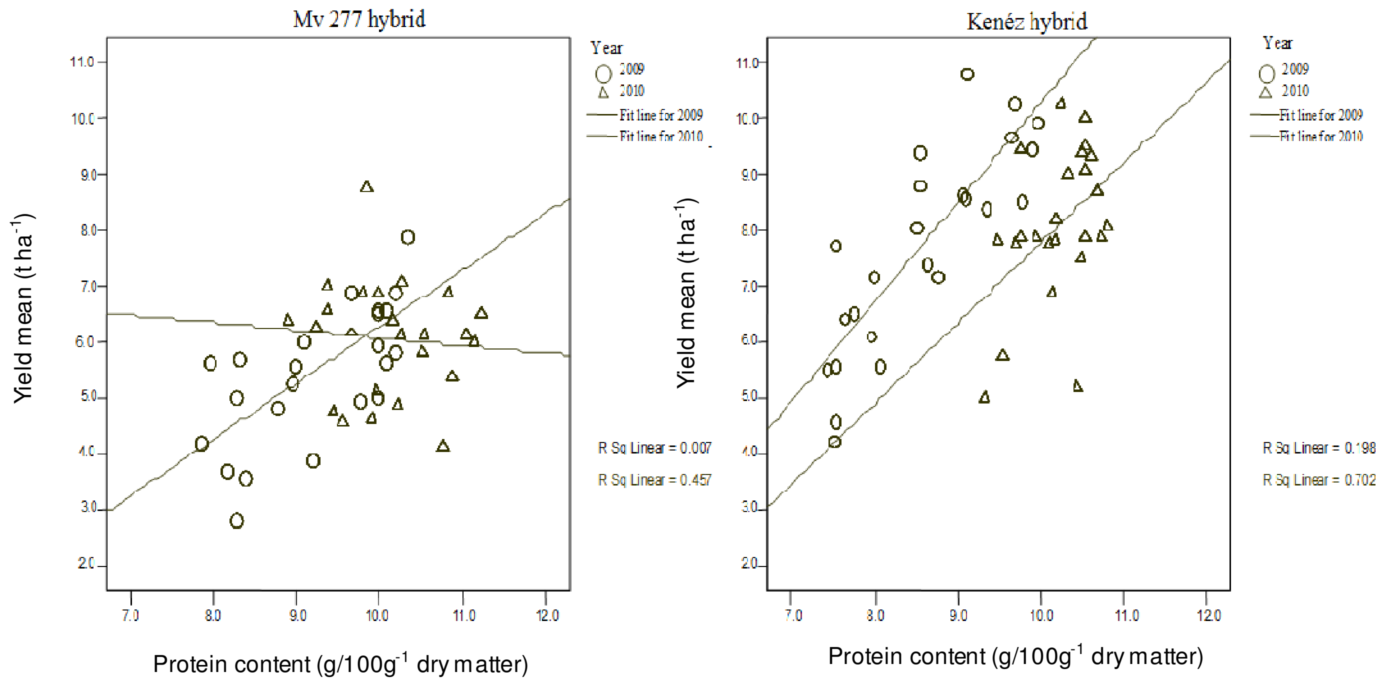

Figure 4. Correlation between the protein content and yield of maize hybrids, 2009–2010.

wet year (2010) (Mv 277 hybrid: r = 0.144; Kenéz hybrid: r = 0.219).

The correlation analysis supported the theory that there was a significant ($p < 0.001$) positive correlation between Chl content and the grain protein content. Therefore, the increase of chlorophyll content plays a key role in increasing the protein content. In 2009, Chl content had a 36.2% influence on the yield of the Mv 277 hybrid and a 33.6% influence on the yield of the Kenéz hybrid. Considering the coefficient of determination in the case of the Mv 277 hybrid, it was established that there was no correlation between Chl content and grain protein content in 2010. Nevertheless, there was a weak correlation (r = 0.213) between the two variables in the case of the Kenéz hybrid.

Several researchers (Bhatia and Rabson, 1987; Gallais et al., 2008) associated the negative correlation between grain yield and grain protein content with the dilution of nitrogen, whereas, Bertin and Gallais (2000) did not observe such results. The results of the regression analysis performed on each hybrid and in each year reinforced the findings of Bertin and Gallais (2000). In the dry year (2009), the grain protein content had a 45.7% positive influence on the yield of the Mv 277 hybrid and a 70.0% influence on the Kenéz hybrid. These results are significant ($p < 0.001$, $p < 0.001$). In 2010, there was a positive correlation between the two factors at a 0.1% significance level in the case of the Kenéz hybrid, whereas, no correlation was shown between the two variables in the case of the Mv 277 hybrid (Figure 4).

Conclusion

In both years, *N* fertilisation significantly improved Chl content, yield and grain protein content. There was a significant difference between the examined hybrids from the aspect of Chl content and protein content in the dry year (2009), but yield difference was significant in both years. The Chl content measured at the R1 growth phase provides a reliable forecast of yield and protein content, but the strength of this correlation differs for each hybrid and each crop year.

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