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Effects of nutrition and water supply on the yield and grain protein content of maize hybrids

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Abstract

The aim of this study was to examine the problems caused by unfavourable weather (dry and wet) at the R1 growth stage of maize (*Zea mays* L.), the treatment of these problems and the prevention of yield decrease, as well as the opportunities to increase quality using agrotechnical factors (fertilisation, irrigation). There was a further objective to determine whether Chl content of maize leaves at the R1 growth stage provided a reliable forecast on yield per hectare and grain protein content. This study was carried out in a moderately warm and dry production area on calcareous chernozem soil in Eastern Hungary (47°33' N, 21°26' E, altitude 111 m) in 2007 and 2008. Six different N fertilisers (0, 30, 60, 90, 120, 150 kg ha^{-1}) were applied in the field experiment, in irrigated and non-irrigated treatments, respectively. The obtained results show that there is a strong and significant positive correlation between Chl content and yield both in the non-irrigated treatment ($P<0.001$; $R=0.777$) and the irrigated treatment ($P<0.001$; $R=0.801$). A weak stochastic correlation ($R^2=26.2\%$) was found between the protein content and the Chl content in the irrigated treatment. A regression analysis showed a weak negative correlation ($P<0.001$; $R=0.345$) between yield and protein content in the non-irrigated treatment. In the irrigated treatment, a medium positive correlation was observed ($P<0.001$; $R=0.685$). The results of the yearly performed correlation analyses showed that while weather factors greatly affected the strength of correlations, they are still positive in all cases. The Chl content of maize leaves at the R1 growth stage provided a reliable forecast on yield per hectare and grain protein content at maturity. The observed correlation is always closer in the irrigated treatment than in the non-irrigated treatment.

Keywords: leaf chlorophyll content (Chl); nitrogen; irrigation; genotype; quality.
Abbreviations: chlorophyll meter reading (CMR); general linear model (GLM).

Introduction

Maize is one of the most important crops in Hungary and its sowing area is relatively constant (between 1.2–1.3 million ha). The total maize yield per year is between 4 and 9 million t (8.3 million t in 2004 and 4.0 million t in 2007) (Nagy, 2008). Yield reductions are primarily caused by drought, the average annual precipitation measuring 550–600 mm. Due to global warming, flooding and drought frequently occur in the same year. Currently, although nearly 500 thousand hectares of agricultural land require irrigation in Hungary, the majority of this area cannot be utilised, mainly due to economic reasons. On average, only 3–10% of Hungary's irrigated lands are used to produce maize. Even during drought periods, only a few per cent of the sowing area are irrigated. Even so, we should not neglect the role of irrigation. In order to avoid the yield fluctuations that depend on water supply, producers should irrigate much larger areas used to produce major crops, such as maize. Unfavourable water supply in the vegetative growth period delays the development of stem and leaf cells, causing shorter plants and less leaf area (Lauer, 2003; Sadras and Calviño, 2001). Drought during tasselling could result in 40–50% yield reduction (Claassen and Shaw, 1970). The water loss during tasselling and flowering reduces the grain number per row, whereas drought after pollination decreases single seed weight and causes a significant yield reduction (Shaw, 1977; Westgate and Boyer, 1986; Lauer, 2003). Temperature and water supply have a significant effect in the grain filling period (Jones et al., 1985; Tollenaar, 1989; Muchow, 1990; Ying, 2000). Drought during this period usually leads to smaller grains (Smith et al., 2004). As a result of unfavourable water supply, the speed and duration of dry matter accumulation decrease (Quattar et al., 1987; Andrade et al., 2005). Westgate and Garnt (1989) showed that even a short period of water shortage caused a significant decrease in the moisture content of grains. In order to increase yields, intensive plant nutrition is indispensable. Nevertheless, determining the optimal fertiliser dose is only one of the most difficult tasks. It is necessary to consider the nutrient management and nutrient-binding ability of the soil, as well as the nutrient utilisation ability and the fertiliser reaction of the produced hybrid. (Széll et al., 2005; D'Haene et al., 2007). Of the three macroelements (NPK), nitrogen is the main yield increasing fertiliser in most soils (Pala et. al., 1992; Shaahan et al., 1999; Nagy, 2008), and also plays a key role in several physiological processes of the crop. By increasing N fertilizer, the activity of photosynthesis improves and both the leaf area index (LAI) and leaf area duration (LAD) increase (Anderson et al., 1985; Dwyer and Anderson, 1995; Earl and Tollenaar, 1997; Tóth et al., 2002). For a long period, the selection of maize hybrids was mainly determined by productivity and yield stability. However, grain quality parameters have come to be more greatly preferred in recent years, making it necessary to know the effects of agrotechnical factors on grain quality. Our objectives were: a) to determine the effects of fertilisation, irrigation, genotype and their interaction on the Chl content, yield and grain protein content; b) to examine whether the Chl content of maize leaves obtained at the R1 growth stage provide a reliable prediction of yield and maize grain protein content.

Results

The results of the GLM showed that fertilisation had the greatest impact on the Chl content, yield and grain protein content in both years. The significance was P<0.001 in the case of all variables (Table 1). The effect of irrigation on the Chl content was not significantly different in 2007, whereas irrigation significantly (P<0.001) reduced Chl content in 2008 (Table 1). In both years, irrigation significantly affected yield (2007: P<0.001; 2008: P<0.01) and grain protein content (2007: P<0.05; 2008: P<0.01). There was a significant difference among the Chl contents of the different genotypes in both years. This difference was more significant (P<0.001) in 2007 than in 2008 (P<0.05) (Table 1). We found a significant difference (P<0.001) in the grain protein content of the different genotypes in 2007, but there was no significant difference in yield in either year (Table 1). The interaction between irrigation and fertilisation was significant in both years for Chl content, protein content and yield. The interaction between irrigation and hybrids was significant for Chl content in 2007 (P<0.01). The interaction between irrigation and hybrids was not significant for the grain protein content in either year. The interaction between irrigation and hybrids was not significant for yield in 2007, whereas it showed a significance of 5% in 2008. The two independent variables (hybrid, fertiliser) significantly influenced Chl content (P<0.01) and protein content (P<0.05) in 2008. There was no statistically significant interaction between hybrids × fertilisation for yield in either year (Table 1). Our research showed that the Chl content of the hybrids increased as a result of the increase in the fertiliser rate in both the irrigated and non-irrigated treatments, in both years (Table 2). Using the Duncan's test at the 5% significance level, we established that 90 kg N ha^{-1} (Debreceni 377) and 30 kg N ha^{-1} (Mv 277) were enough to reach the highest Chl content in the non-irrigated treatment in 2007 (47.7 and 49.1, respectively). In the irrigated treatment in 2007, 120 kg N ha^{-1} were needed in the cases of both hybrids in order to reach the highest level of Chl content (Debreceni 377:54.2; Mv 277:57.1). In the average of the fertiliser treatments, the Chl content of Mv 277 was higher both in the non-irrigated treatment (49.6; P<0.01) and the irrigated treatment (56.1; P<0.01) than the Chl content of the Debreceni 377 hybrid. Irrigation increased the Chl content of Debreceni 377 from 60 kg N ha^{-1} significantly (P<0.05), while in the case of the Mv 277 hybrid, a significant (P<0.05) increase was observed in the 120 kg N ha^{-1} treatment (Table 2). In 2008, the results of the Duncan's test showed that the highest Chl content in the non-irrigated treatment was obtained by using 60 kg N ha^{-1} in the case of the Debreceni 377 hybrid (55.5) and 90 kg N ha^{-1} in the case of Mv 277 (59.8) (Table 2). In the irrigated treatment, similarly to 2007, both hybrids needed 120 kg N ha^{-1} to reach the highest Chl content (Debreceni 377:52.7 and Mv 277: 55.8, respectively). Averaging the treatments, Debreceni 377 had the higher Chl content (53.9; P<0.05) in the non-irrigated treatment, whereas Mv 277 (48.8) had the higher value in the irrigated treatment. Nevertheless, this difference was not significant. Irrigation significantly decreased Chl content in the case of both hybrids - except for the 150 kg N ha^{-1} treatment of the Mv 277 hybrid - in all fertiliser treatments (Table 2). The quality and forage values of maize are basically determined by the grain protein, oil content and the compositions of amino and fatty acids. Although quality parameters are hereditary, these can be modified by ecological and agrotechnical factors (Izsáki, 2009). Conforming to the findings published by Feng et al. (1993) and Singh et al. (2005), the maize grain protein content increased with increasing nitrogen rates in both years and in the cases of both hybrids (Table 3). Independent of fertilisation and irrigation, its value was between 8.1–11.5 g (100 g dry matter)$^{-1}$. Using the Duncan's test, our study indicated that 120 kg N ha^{-1} were needed for both hybrids in the non-irrigated treatment in 2007, in order to reach the highest grain protein content (Debreceni 377: 11.5 g (100 g dry matter)$^{-1}$; Mv 277: 11.1 g (100 g dry matter)$^{-1}$). In the irrigated treatment, we could separate four homogeneous groups in the case of Debreceni 377 and there was the highest grain protein content when applying 150 kg N ha^{-1} (11.2 g (100 g dry matter)$^{-1}$). As for Mv 277, the Duncan's test created three distinguishable groups and the highest grain protein content was 120 kg N ha^{-1} (9.8 g (100 g dry matter)$^{-1}$). In the non-irrigated treatment, the grain protein content of Mv 277 was lower (P<0.05) than that of Debreceni 377 over the average of the six fertiliser treatments, while the average protein content was more significant (P<0.01) for Debreceni 377 in the irrigated treatment (Table 3). Irrigation significantly decreased the protein content of the Debreceni 377 hybrid in the 60 kg N ha^{-1}, 90 kg N ha^{-1} and 120 kg N ha^{-1} treatments and that of the Mv 277 hybrid in the 120 and 150 kg N ha^{-1} treatments. Weather significantly affects the raw protein content of grains (Szirtes et al., 1977) and this change closely correlates with the fluctuation of yield. Lilburn et al. (1991) reported lower protein content in wet years and higher protein content in dry years. The main effects are attributed to heat units, and the quantity and distribution of precipitation in June, July and August (Asghari and Hanson, 1984). Similarly in our study, irrigation significantly (P<0.05) decreased grain protein content in both of the hybrids we studied (Debreceni 377: 0.99 g (100 g dry matter)$^{-1}$; Mv 277: 0.82 g (100 g dry matter)$^{-1}$) (Table 3). In 2008, we again performed the Duncan's test and our results showed that the fertiliser dose necessary for the statistically highest grain protein content (Debreceni 377: 9.3 g (100 g dry matter)$^{-1}$; Mv 277: 9.2 g (100 g dry matter)$^{-1}$) was lower (60 kg N ha^{-1}) than that of 2007 for both hybrids. Moreover, even when the highest fertiliser rate was applied, the highest statistically significant grain protein content of 2007 could not be achieved. In the irrigated treatment, we could delineate three homogeneous groups from both hybrids. As for Debreceni 377, the highest protein content was reached when applying 60 kg N ha^{-1} (10.6 g (100 g dry matter)$^{-1}$), while we had to apply 90 kg N ha^{-1} in the case of the other hybrid to reach the highest value (10.9 g (100 g dry matter)$^{-1}$). In the non-irrigated treatment, the protein content of Mv 277 was lower (P<0.01) than that of Debreceni 377 over the average of the six fertiliser treatments. In the irrigated treatment, there was no significant difference between the hybrids. Under better water supply conditions (provided by a wet year or irrigation), a decrease in grain protein content can be expected, but this decline can be compensated with proper nutrient supply. There was no dilution effect (Győri and Sipos, 2005). Irrigation significantly increased the protein content of both hybrids, except for the non-fertilised and the 120 kg N ha^{-1} treatments. In the non-irrigated treatment, the yield of maize hybrids increased linearly up to 90 kg N ha^{-1} in the case of Debreceni 377 (5.8 t ha^{-1}), and 60 kg N ha^{-1} in the case of Mv 277 (5.3 t ha^{-1}) in 2 007. Further increasing the fertiliser doses caused

Table 1. Variance analysis results of Chl content of maize leaves, grain protein content and yield of maize hybrids for irrigation and fertiliser of two cultivars in each year.

Factor	Source of variation	2007	2008
Chl content	irrigation (A)	**NS**	***
	hybrids (B)	***	*
	fertiliser (C)	***	***
	irrigation × hybrids (A×B)	**	**NS**
	irrigation × fertiliser (A×C)	***	**
	hybrids × fertiliser (B×C)	NS	**
	irrigation × hybrids × fertiliser (A×B×C)	NS	NS
grain protein content	irrigation (A)	*	**
	hybrids (B)	***	NS
	fertiliser (C)	***	***
	irrigation × hybrids (A×B)	**NS**	**NS**
	irrigation × fertiliser (A×C)	*	**
	hybrids × fertiliser (B×C)	NS	*
	irrigation × hybrids × fertiliser (A×B×C)	NS	NS
yield of maize hybrids	irrigation (A)	***	**
	hybrids (B)	NS	NS
	fertiliser (C)	***	***
	irrigation × hybrids (A×B)	NS	*
	irrigation × fertiliser (A×C)	***	***
	hybrids × fertiliser (B×C)	NS	NS
	irrigation × hybrids × fertiliser (A×B×C)	NS	NS

NS—not significant, * significant at P=0.05, **significant at P=0.01, ***significant at P=0.001,

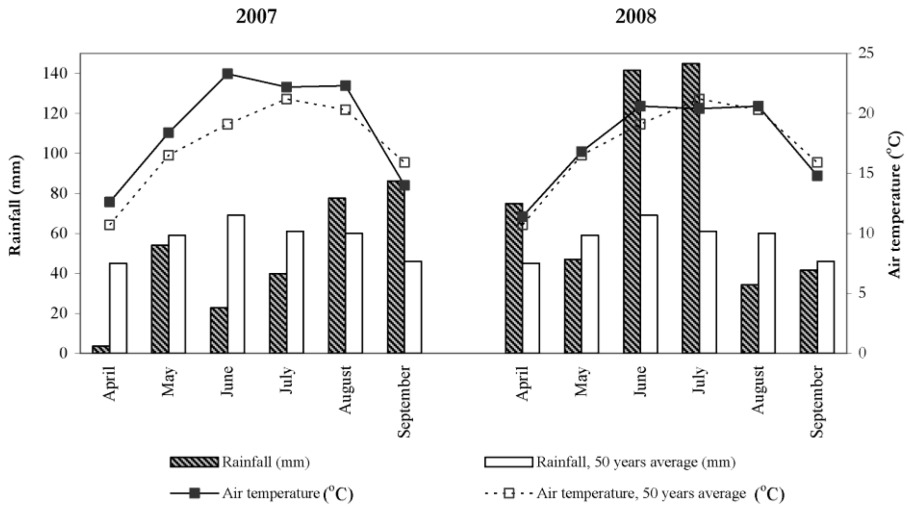

Fig 1. Monthly average of air temperature and rainfall over the 2007–2008 crop seasons

yield depressions in both hybrids. Several research results have shown that irrigation increases the efficiency of fertilisation (Otegui et al., 1995; Pandey et al., 2000; Farre and Faci, 2009). In irrigated treatment (i.e., where there is, as a result, a higher yield level), the positive correlation between irrigation and fertilisation makes higher fertiliser doses more economical than in the non-irrigated treatment (Yamada et al., 1972; Caliandro et al., 1983; Tsankova, 1981; Silega and Zakhariev, 1981). In the irrigated treatment, we created four statistically well separable groups for both hybrids, in order to indicate the significant differences between fertiliser treatments. 120 kg N ha^{-1} were enough for Debreceni 377 to reach the highest statistically significant yield, while this fertiliser dose was 90 kg N ha^{-1} in the case of Mv 277. In the average of the fertiliser treatments, there was no significant difference between the yields of the two hybrids in either irrigation treatments. Irrigation significantly increased yield in both the Debreceni 377 hybrid and the Mv 277 hybrid at each fertiliser level, except for the non-irrigated treatment (Table 4). In 2008, the Duncan's test showed that 60 kg N ha^{-1} were enough to reach the highest statistically significant yield in the non-irrigated treatment for both hybrids. The fertiliser dose necessary for the highest yield was 120 kg N ha^{-1} for Debreceni 377 and 60 kg N ha^{-1} for Mv 277 in the irrigated treatment (Table 4). Similarly to the yields obtained in 2007, no significant difference was observed in 2008 between the hybrids in either irrigation treatment in the average of the fertiliser treatments. Above the average of the fertiliser treatments, irrigation decreased the yield of both hybrids (Debreceni 377:-1.07 t ha^{-1}, Mv 277: -2.00 t ha^{-1}), significance levels: Debreceni 377: 5%, Mv 277: 1%.

Table 2. Effects of fertilizer treatments and water supply on Chl content of maize leaves (SPAD values) (2007 and 2008)

Cultivars	Water supply	2007						2008					
		Non-fertilised	30	60	90	120	150	Non-fertilised	30	60	90	120	150
Debreceni 377	Non-irrigated	39.94±2.23a A	43.08±0.82ab A	45.97±1.13bc A	47.74±0.26c A	46.74±1.53bc A	47.05±0.87bc A	45.44±0.82a A	51.43±1.78b A	55.52±1.25c A	56.75±0.36c A	56.88±0.46c A	57.49±0.64c A
	Irrigated	39.60±0.56a A	44.82±0.68b A	49.97±0.93c B	52.19±0.53d B	54.16±0.40e B	54.99±0.56e B	37.54±0.88a B	43.17±1.11b B	46.30±0.76bc B	48.48±0.80c B	52.71±1.40d B	54.65±2.17d B
Mv 277	Non-irrigated	43.24±1.49a A	49.07±1.30b A	50.73±1.51b A	49.15±2.01b A	52.50±1.60b A	52.80±1.96b A	45.82±1.66a A	50.56±2.89a A	56.35±1.46b A	59.81±1.40bc A	61.84±0.47c A	61.99±0.65c A
	Irrigated	41.25±0.33a A	48.01±0.44b A	49.79±1.25b A	54.14±1.50c A	57.05±0.60cd B	58.10±1.19d A	34.91±.122a B	40.55±0.82b B	50.17±1.90c B	52.51±2.64cd B	55.84±1.01de B	58.48±1.55e 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 ($P < 0.05$).

Table 3. Effects of fertilizer treatments and water supply on grain protein content (g (100 g dry matter)$^{-1}$) (2007 and 2008)

Cultivars	Water supply	2007						2008					
		Non-fertilised	30	60	90	120	150	Non-fertilised	30	60	90	120	150
Debreceni 377	Non-irrigated	9.26±0.28a A	9.75±0.23ab A	10.67±0.39bc A	10.65±0.40bc A	11.50±0.21c A	11.05±0.25c A	9.20±.025ab A	8.97±0.19a A	9.32±0.29abc A	9.19±0.60a A	9.97±0.11c A	9.92±0.35bc A
	Irrigated	8.50±0.14a A	9.15±0.13b A	9.27±0.23b B	9.10±0.26b B	10.20±0.17c B	11.22±0.18d A	9.12±0.24a A	9.92±0.16b B	10.62±0.16c B	10.60±0.12c B	10.52±0.19c A	11.00±0.17c B
Mv 277	Non-irrigated	8.50±0.25a A	8.70±0.23a A	10.15±0.29bc A	10.00±0.54b A	11.05±0.21cd A	11.20±0.23d A	8.27±0.17a A	8.20±0.15a A	9.20±0.45ab A	8.75±0.40a A	8.95±0.2.0a A	10.07±0.33b A
	Irrigated	8.12±0.14a A	8.17±0.28a A	9.15±0.37b A	9.10±0.24b A	9.77±0.41bc B	10.30±0.17c B	9.10±0.47a A	9.27±0.10ab B	10.07±0.17bc B	10.95±0.25cd B	9.90±0.38ab A	11.22±0.26d 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 ($P < 0.05$).

Correlation analysis

Based on the data of 384 plots, we examined the correlations between the Chl content and the grain protein content, the Chl content and yield, and the yield and the grain protein content in the average of the hybrids. The correlation between the variables can be described with a linear function, as was also shown by the F test to be at a 0.1% significance level. In 2007, both in the non-irrigated (R=0.334, P<0.05) and the irrigated treatments (R=0.664, P<0.001), we obtained a statistically significant positive correlation between the Chl content and the grain protein content. In 2008, the positive correlation between the two variables could be shown again. While the correlation between the Chl content and the grain protein content was weaker in the non-irrigated treatment, it was more significant (R^2=46%) in the irrigated treatment. Taking the value of the determination coefficient in the average of the two years into consideration, our data showed that there was no correlation between the Chl content and the grain protein content in the non-irrigated treatment. Nevertheless, there was a weak, positive stochastic correlation (R^2=26.2%) between the two variables in the irrigated treatment. There were positive correlations between the two variables in the case of the Debreceni 377 hybrid in 2007, both in the non-irrigated (P<0.01) and the irrigated (P<0.001) treatments, while there was a positive correlation only in the irrigated treatment (P<0.001) in 2008. Average correlation was found in the case of the Mv 277 hybrid in the non-irrigated treatment in 2007 and 2008 (P<0.01), while this correlation was closer in the irrigated treatment in both years (P<0.001) (Table 5).We also carried out an analysis of the correlation between the Chl content and yield. In 2007, there was a statistically significant (P<0.001) average positive correlation (R=0.587) between the variables (Chl content and yield) in the non-irrigated treatment, but this correlation became very strong (R=0.918) – in the average of the two hybrids – as a result of irrigation. As reflected by the findings of Montemurro et al. (2006), we showed that there is a close correlation between these variables and that this correlation is linear. In 2008, the correlation between the two variables in the average of the hybrids was strong (R=0.831) even in the non-irrigated treatment, becoming even stronger in the irrigated treatment (R=0.854). In the non-irrigated treatment, Chl content affected yield 69.1%, whereas this value was 73% in the irrigated treatment. Both in the non-irrigated and the irrigated treatments, we obtained a statistically significant (P<0.001) average positive correlation between the Chl content and yield in the average of years. This correlation was very close (R=0.777, R=0.801). In the case of the Debreceni 377 hybrid, there were close correlations (P<0.001) between the two variables, both in 2007 and 2008. As for the Mv 277 hybrid, there was average correlation in the non-irrigated treatment in 2007 (R=0.599) and in 2008 (R=0.587), while there was closer correlation in the irrigated treatments in both years (R=0.921, R=0.684) (Table 6). The negative correlation between the quantity and the protein content of grain yield was considered to be consequence of the dilution of nitrogen by several researchers (Bhatia and Rabson, 1987; Sander et al., 1987; Gallais et al., 2008), although Bertin and Gallais (2000) did not observe similar results. The yearly regression analysis results substantiated the findings of Bertin and Gallais (2000), since the grain protein content positively affected yield in the average of the hybrids in non-irrigated (R^2=14.3%) and irrigated treatments (R^2=52.7%) in 2007. The correlation was significant (P<0.01, P<0.001). In 2008, there was a positive correlation between the two factors at the 5% significance level in the non-irrigated treatment; however, this value improved (0.1%) in the irrigated treatment. The correlation was moderately close in this treatment (R=0.616). Nevertheless, in the non-irrigated treatment, the regression analysis showed a very weak negative correlation between the two variables (P<0.001) in the average of the hybrids and years. In the irrigated treatment, the positive moderately close correlation (R=0.685) remained in the average of the years. In the case of the Debreceni 377 hybrid, a close correlation was shown between the two factors only in the irrigated treatment in both years (R=0.785, P<0.001, R=0.697, P<0.001). The Mv 277 hybrid showed average correlations in the non-irrigated treatment both in 2007 (R=0.527, P<0.01) and 2008 (R=0.578, P<0.01), while there was a close correlation in 2007 (R=0.767, P<0.001) and an average correlation in 2008 (R=0.563, P<0.01) in the irrigated treatment (Table 7).

Discussion

It can be stated that the abiotic factors in 2007 and 2008 fundamentally affected the nutrient uptake, Chl content, yield and yield quality of maize. Munson and Nelson (1973) realised that leaf analysis is a valuable method for the diagnosis of the nutrient supply problems of plants and the Chl content of leaves is an important factor in photosynthesis and dry matter production (Ghosh et al., 2004). Contrary to Khadem et al. (2010) and in accordance with other researchers (Berzsenyi and Lap, 2003), the Chl content was 14.1% lower as a result of drought stress (2007) in comparison with the wet year (2008). In drought years, Chl content changes in relation to the drought sensitivity of the hybrid. There were significant differences between the examined hybrids concerning their Chl contents both under unfavourable (P<0.001) and favourable (P<0.05) water supply conditions (Table 1). The Chl contents of early ripening hybrids is higher than those of late ripening hybrids, while the Chl contents of different genotype hybrids belonging to the same FAO group differ from each other. The difference is more significant as a result of drought stress, as the Chl content of the Debreceni 377 hybrid was 6.4% (P<0.001) lower in 2007 than that of the Mv 277 hybrid. Since chlorophyll also contains nitrogen, change in chlorophyll content also shows the nitrogen uptake of a plant (Yadava, 1986; Kacar and Katkat 2007; Hawkins et al., 2009). As a result of nitrogen supply, dry matter accumulation becomes more intensive and the Chl content increases. Nitrogen fertilisation increased Chl content in the examined hybrids in both years (P<0.001), but drought stress (2007) produced, as a result, the circumstance that the presence of an optimal quantity of nutrients did not provide higher Chl content, as the water shortage made it impossible for plants to utilise nutrients to a necessary extent. Nitrogen uptake from the soil is related to the level of available water supply; therefore, the nitrogen and water utilisation efficiency of hybrids is often the same (Eghball and Maranville, 1991). In the case of unfavourable water supply, the relative water content of leaves decreases (Nautiyal et al., 2002), similarly to their photosynthetic activity and evapotranspiration; therefore, root growth and activity will also be reduced (Tarumingkeng and Coto, 2003). However, protein breakdown and amylase activity (starch breakdown) increase (Andrade et al., 2002, Bänzinger et al., 2002); therefore, if the amount of rainfall and the easily accessible water stock of the soil do not satisfy the water need of plants, this shortfall has to be replenished by irrigation (Petrasovits, 1970). In years with unfavourable water supply, the dryness of the soil and the low humidity of the air result in the fact that plants develop smaller leaves with thicker cuticles, which make the

Table 4. Effects of fertilizer treatments and water supply on maize hybrid yield mean +- 2 SE (t ha^{-1}) (2007 and 2008)

Cultivars	Water supply	2007						2008					
		Non-fertilised	30	60	90	120	150	Non-fertilised	30	60	90	120	150
Debreceni 377	Non-irrigated	2.98±0.08a A	3.61±0.12a A	4.92±0.31b A	5.82 ±0.26c A	4.63±0.34b A	3.69±0.18a A	8.36±0.26a A	9.57±0.88ab A	11.2±0.17bc A	11.02±0.38bc A	11.43±0.52c A	10.02±0.55bc A
	Irrigated	3.81±0.37a A	5.76±0.58b B	7.73±0.75c B	8.61±0.31c B	10.32±0.49d B	10.73±0.36d B	5.77±0.26a B	7.66±0.32b A	9.64±0.61c A	9.36±0.42c B	10.96±0.41d A	11.72±0.23d B
Mv 277	Non-irrigated	3.38±0.30a A	3.92±0.21ab A	5.21±0.21c A	5.12±0.57c A	4.77±0.51bc A	4.48±0.23abc A	8.04±0.15a A	10.09±0.79b A	11.20±0.62bc A	11.81±0.29c A	11.47±0.31bc A	11.98±0.22c A
	Irrigated	4.42±0.35a A	6.16±0.20b B	8.45±0.65c B	9.81±0.50cd B	10.28±0.35d B	10.83±0.66d B	5.44±0.53a B	7.39±0.36b B	9.27±0.51c A	9.80±0.59c B	10.21±0.75c A	10.50±0.36c 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 5. Correlation coefficients between Chl content, grain protein content and yield of maize hybrids.

Cultivars	Water supply	2007			2008		
		Chl - Protein	Chl - Yield	Protein - Yield	Chl - Protein	Chl - Yield	Protein - Yield
Debreceni 377	Non-irrigated	0.555^{**}	0.652^{***}	0.332^{NS}	0.381^{NS}	0.722^{***}	0.124^{NS}
	Irrigated	0.736^{***}	0.922^{***}	0.785^{***}	0.734^{***}	0.899^{***}	0.697^{***}
Mv 277	Non-irrigated	0.574^{**}	0.599^{**}	0.527^{**}	0.587^{**}	0.899^{***}	0.578^{**}
	Irrigated	0.747^{***}	0.921^{***}	0.767^{***}	0.684^{***}	0.886^{***}	0.563^{**}

Chl, protein, yield stands for Chl content of maize leaves, grain protein content and yield of maize hybrids respectively. Each correlation coefficient is calculated from pooled six different fertilizer treatments in the experiment. NS non significant; $^{**}P < 0.01$; $^{***}P < 0.001$.

leaf mass thicker, too (Caseley, 1989). For this reason, it was more difficult for the active ingredients to enter plants in 2007. Thus, the Chl content did not significantly change as a result of irrigation. In more favourable crop years (2008), irrigation reduced Chl content by 9.8% ($P<0.001$). The protein content of maize is primarily determined by its genetic characteristics (Hegyi and Berzy, 2009; Izsáki, 2009), which only ecological (Szász, 1977) and agrotechnical factors (Győri, 2010) can modify. In the rainy year (2008), the protein content of maize was lower (by 10.8%) than in the dry year, as reflected in the results of Gyenesné-Hegyi et al. (2001). A difference in the maize protein content was only found in the year with unfavourable water supply (2007) ($P<0.001$). The protein content of the Debreceni 377 hybrid was higher than that of the Mv 277 hybrid. Nitrogen affects protein synthesis (Luit el al., 1999), promotes green mass development, increases yield and improves yield quality (Hasaneen et al., 2009). N fertilisation increased grain protein content in both years ($P<0.001$) (Table 1) and maize protein content decreased with the increase of the average yield. Abiotic factors influence maize development and growth, determining the productivity of maize. In 2007, the highest yield loss was caused by the water shortage and drought in the vegetative period and during the flowering of maize. According to Quattar et al. (1987), there was a yield decrease due to the drought in the grain-filling period of maize. Tolleneaar and Lee, (2002) reported that drought stress during grain-filling dramatically decreases yield. There was no significant difference in yield between the hybrids in either year. Based on the obtained data, it was concluded that there were no significant differences between the examined hybrids concerning their heat and drought tolerance. Other researchers (Brar et al., 2001; Fallah et al., 2007; Khadem et al. 2010) also argue that fertilisation significantly influenced grain yield (Table 1) and that this significantly decreased as a result of drought stress (2007), since the insufficient nutrient supply during the period of increased nutrient need (flowering) caused a latent shortage of the given nutrient, thereby making it impossible to obtain an optimal yield. It is a general view that the use of fertilisers is most beneficial under irrigated conditions, when water supply is the main barrier to the nutrient uptake of plants. In the case of adequate nutrient supply, plants which are limited in their growth due to water stress would have higher mineral nutrient contents than those of the same productivity which are not exposed to water stress (Michael, 1981). The research results also show that there can be a yield decrease as a result of irrigation in certain years (2008), which draws attention to the fact that the main goal of producers is to provide the most favourable interaction of factors, i.e. the harmony of nutrients and water supply, since the effect of irrigation can be exploited only if adequate quantities of nutrients are applied. In the non-irrigated treatments, the Chl content measured at the R1 growth phase provides average (2007) and close prediction (2008), while it provides close prediction in both years in the irrigated treatments. According to Wang et al. (2008) and Pandey and Singh (2010), close prediction makes it easier to select high yield genotypes. The reliability of prediction of the grain protein content at harvest was weak in both years in the non-irrigated treatments, while it was close (2007) and average (2008) in both years in the non-irrigated treatments.

Conclusion

The study shows that the larger or smaller shortage of nutrients (nitrogen) and the disorder of photosynthetic activity are general phenomena. There can be various reasons for these problems, e.g. there can be halts in the nutrient uptake of plants. This problem can follow from undeveloped roots or it can evolve due to unfavourable weather. As a result of these factors, nutrient uptake is not able to properly satisfy the intensive green mass growth; therefore, the nutrients taken up by plants are unable to get incorporated into the plants quickly enough. Nutrient shortage and unfavourable environmental stress effects could lead to disorders in the photosynthetic activity. As a result, weaker shoot growth and smaller green mass will be developed which limits yield and deteriorates quality. Therefore, timely intervention will be the most determinant factor affecting yield in the further development of maize.

Material 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, altitude 111 m), in the growing seasons of 2007 and 2008. The experimental station is located in a moderately warm and dry production area at the north-eastern part of the Great Hungarian Plain. The irrigation × fertilisation field experiment had a strip plot experimental design with four replications. Plot size was 15 m^2.

Soil

The soil of the experimental site was lowland pseudomyceliar chernozem (Mollisol-Calciustoll or Vermustoll/Pachic or Typic, silt loam, USDA '90 taxonomy). The field capacity of the examined soil layers can be characterised by 0.27–0.34 $cm^3\ cm^{-3}$ moisture content, and 0.10–0.12 $cm^3\ cm^{-3}$ for the wilting point. The average pH_{KCl} value of soil is 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 is 39; the total amount of water-soluble salts (anions and cations) is 0.04%. The calcium-carbonate content in the upper 80 cm of the soil is around 0%, whereas it is 12% from a depth of 100 cm. Organic matter content in the upper 20 cm layer of the soil is 2.3% and it does not exceed 1% at a depth of 120 cm. The potassium supply of the soil is favourable, whereas its P supply is moderate.

Experimental details

Six fertiliser treatments (0, 30, 60, 90, 120, 150 kg N ha^{-1}) were applied in the field experiment in both years. The entire amount of fertiliser (ammonium nitrate) was applied in the spring, 3 weeks before sowing. Two different genotype maize hybrids (Debreceni 377 and Mv 277) belonging to the same ripening group (FAO 310) were sown on 24th April 2007 and 22nd April 2008, at 70 000 seeds per hectare, following winter ploughing and spring seedbed preparation. The preceding crop was maize. Irrigation was performed using Valmont linear irrigation equipment. The amount of irrigation water applied in the growing season of 2007 was 110 mm, with 4 applications (25 mm on 27 April, 30 mm on 16 May and on 10 June, respectively, and 25 mm on 26 June). 25 mm irrigation water was used in one application on 11 May 2008. Maize was harvested on 8 October in both years. The harvested grain yield was determined at 14% moisture content.

Weather

The heat unit for the whole growing season was calculated on the basis of the following equation:

$$\text{Heat Unit} = \sum_{i=1}^{n} \frac{\left(T_{max} - T_{min}\right)}{2} - T_{basis}$$

where T_{max} = maximum daily temperature [^oC], T_{min} = minimum daily temperature [^oC], $T_{bázis}$ = 10 ^oC (Davidson and Campbell, 1983; Gallagher, 1979; Nield and Seeley, 1977).

Several methods (Penman, 1948; Thornthwaite, 1948; Mckenny and Rosenberg, 1993; Szász, 1977) make determination of potential evapotranspiration (PET) possible. The method of Szász (1977) was used, as it provides a highly accurate estimation.

$$PET=\beta[0{,}0095(T-21)^2(1-R)^{2/3}f(v)]$$

where PET= potential evapotranspiration [mm day^{-1}], 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. The temperature and rainfall conditions of the vegetation periods of 2007 and 2008 were totally opposite to each other (*Figure 1)*. In 2007, the value of the effective heat sum was 158 ˚C lower and the amount of rainfall was 200 mm lower than in 2008. The difference between rainfall and PET values was -367 mm in 2007, while it was -96 mm in 2008. The weather was colder and drier in the growing season of 2007 than in 2008. The nitrogen nutrition index (NNI) is used to characterise plant levels of nitrogen supply. Its determination calls for destructive measurement methods, therefore, quickly and easily measurable indexes are needed in practice (Justes et al., 1997). The Soil Plant Analysis Development (SPAD) chlorophyll meter was developed in Japan, to evaluate the level of nitrogen supply in rice (Chubachi et al., 1986; Huang et al., 2008) and this instrument was also deemed to be reliable in maize (Yadava, 1986; Piekielek and Fox, 1992; Schepers et al., 1992; Feil et al., 1997). Plant chlorophyll (CMR value) is a useful measurement factor, as it is closely correlated with the nitrogen content of plants (Auernhammer, 2001; Berzsenyi and Lap, 2005). We measured the relative chlorophyll concentration of the maize leaf using the SPAD-502 (Minolta, Japan) portable chlorophyll meter at the R1 growth stage of maize Ritchie et al., 1997). We carried out the CMR measurements on the basis of the guidelines by Costa et al. (2001), taking readings from the leaf below the ear on each crop, using 20 crops per fertiliser treatment. We took yield samples of the two maize hybrids in both years from all treatments and measured the grain protein content using Foss Infratec^{TM} 1241 meter based on near-infrared spectroscopy (NIRS) and near-infrared transmittance (NIT).

Statistical analysis

We used a *general linear model* (GLM) to evaluate the correlation between the dependent variable (Chl content, yield, protein content) and the production factors (fertiliser, irrigation, genotype). This method is the alloy of the conventional variance analysis and the linear regression analysis and provides the opportunity to indicate the effect of the production factors on the Chl content, yield and the grain protein content, as well as to test the reliability of the linear model. We used *the Duncan's test* to compare the mean values of yield and protein content. In this test, homogeneous groups are created due to which the Chl content, yield data and protein content not differ from each other at the 5% level of significance. This method can also be used in multiple comparisons of the mean values of treatments, as the probability of alpha error is not cumulated. We used a *linear function* to evaluate the correlation between the Chl content and the grain protein content, the Chl content and yield, the protein content and yield. The following linear function was used:

$y = b_0 + b_1Chl$

where, y= yield (t ha^{-1}), b_0= invariant, b_1= coefficient of the linear term

We fitted the functions using regression analysis, by minimising the sum of squared deviation. The accuracy of the fitting of the functions was determined by the R value and the error mean square. We used SPSS for Windows to carry out the evaluation.

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