Agronomy Research **19**(4), 1698–1710, 2021
https://doi.org/10.15159/AR.21.148

Hybridspecific nutrient interactions and their role in maize yield quality

C. Bojtor[1,*], Á. Illés[1], É. Horváth[1], J. Nagy[1] and L.C. Marton[1,2]

[1]University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management, Institute of Land Use, Engineering and Precision Farming Technology 138 Böszörményi St, H-4032 Debrecen, Hungary
[2]Eötvös Loránd Research Network, Centre for Agricultural Research, Agricultural Institute, 2 Brunszvik St, H-2462 Martonvásár, Hungary
[*]Correspondence: bojtor.csaba@agr.unideb.hu

Received: February 1st, 2021; Accepted: November 1st, 2021; Published: November 4th, 2021

Abstract. Different fertilization levels can be used according to the intensity of the plant production technology. The interactions among all the essential nutrients, the different ways of antagonisms and synergisms can weaken or strengthen the physiological processes of the plants, affecting significantly the efficiency of the production. Complex mineral profile of the vegetative (stalk, leaves) at V2, V4, V8, VT and R6 stages in 4 replications and generative (grain, cob -R6 stage only) plant parts of maize were determined in accredited laboratory with ICP-MS, ICP-OES microwave-assisted multielement analysis for metal components (P, K, mg, Ca, S, Zn, Fe, Cu, Mn, Ni, Mo) and Dumas method for nitrogen. Results showed that the effect of different nitrogen fertilization levels was significant on grain yield, protein, oil and moisture content. Significant differences were measured between the different maize genotypes in grain yield, protein and starch content. In the experiment all essential nutrient concentrations were measured, and the important nutrient ratios of macro- and micronutrients for the physiological development of maize were calculated. These nutrient stoichiometric ratios were evaluated according to their scale of influence in the yield formation. It can be concluded that different nitrogen fertilization levels affected significantly the essential nutrient ratios of the vegetative and generative plant parts of three maize hybrids in the growth period. Although different variables of nutrient stoichiometric ratios and yield parameters did not highly correlated, Pearson's correlations suggest that K:Mg and K:Zn ratio of stalk would be related with grain yield (R = 0.32; 0.34; 0.39 and 0.35; 0.37; 0.30, respectively) marking them as important parameters for novel nutrient stoichiometry research. Analysing the optimal nutrient ratios related to the yield quality and their interaction with the fertilization practices can give certain recommendations to the farmers to implement hybrid- and site-specific nutrient management strategies, reducing the environmental impact of the over-fertilization.

Keywords: fertilization efficiency, macronutrients, nitrogen use; nutrient ratios, stoichiometry.

INTRODUCTION

Maize (*Zea mays* L.) is currently one of the most important food crops in the world, maximizing its productivity and yield while maintaining the quality is one of the primary goals of corn producers (Xin et al., 2016).

The future of modern multinutrient management strategies is not clear. Cropping systems in the world are diverse and can be divided into at least two different levels of intensification, low and high input fertilization systems (Fischer & Connor, 2018). Optimal nutrient supply is a key circumstance to achieve high yields with good quality parameters. Soil - plant interactions and the complex use of nutrients during the growing season - beside other environmental factors – can determine the plant’s health conditions, having a high impact on the dry matter and grain yield accumulation of maize (Bojtor et al., 2020).

Optimum nitrogen concentration in crop plants can improve leaf development as well as photosynthesis, and delay the leaf senescence during the grain filling stages in maize (Liu et al., 2017). It also influences the utilization of phosphorus, potassium, and other minerals in plants. Optimal quantity of these macro- and micronutrients in the soil cannot be utilized efficiently in case of nitrogen deficiency. Therefore, nitrogen deficiency can result in reducing maize yields (Humtsoe et al., 2018). Lamptey et al. (2017) confirmed that the grain yield of maize is sensitive to changes in yield components and nitrogen content in the leaf. According to their results small stresses on these traits can result in significant impacts on grain yield.

High nutrient use efficiency is an economic and environmental goal of sustainable cropping systems (Gastal et al., 2015). There are a lot of factors mediating nutrient use efficiency, such as genotype (Wang et al., 2017), enough precipitation (Hoogmoed et al., 2018) and availability of other essential nutrients (Duncan et al., 2018). Different nutrient ratios are widely used to investigate their limitation for crops. N:P ratio has been used to quantify N and P deficiency in plants (Bélanger et al., 2017). Some studies also investigated the N:S ratio in maize (Salvagiotti et al., 2017; Carciochi et al., 2019). The aim of exploit the site-specific nutrient sources of the soil is one of the primary intentions of the modern agricultural practice. To achieve this goal the use of different maize genotypes that have better nutrient use efficiency is an important strategy with the correct management of the plant’s nutritional status. (Ortas, 2018). Analysis of different stoichiometric ratios of the plant’s organs of the essential macro- and micronutrients and testing the site-specific characteristics of the different production areas is an important research topic as a genotype-specific nutritional diagnostic tool.

Generally, obtaining relevant information about hybrid-specific nutrient interactions can help the farmers creating an environmentally favorable fertilization process in their farm practice.

The connection between different nutritional levels in the main phenological stages and yield quality parameters can provide the optimal nutritional bases to achieve high quality yield. The main objective of this study was to clarify - through a controlled long-term field experiment - the role of different nitrogen fertilization levels in the interrelationship among the essential macro-meso and micronutrients of maize hybrids during the vegetation period, focusing mainly on the nutrient status of the hybrids and on the way it can refer to the producible yield and its quality parameters at an early development stage.

MATERIALS AND METHODS

Experimental area

The experiment was performed at the Látókép Crop Production Experimental Site of the University of Debrecen (47° 33' N, 21° 26' E, 111 m above sea level) in 2019 with 3 different maize (*Zea mays* L. H1 = FAO360, H2 = FAO420, H3 = FAO490) hybrids and 3 different nitrogen fertilization levels, as a part of a long-term 6-level multifactorial fertilization experiment founded in 1983 (Nagy, 2019). In the experiment, phosphorus (P) and potassium (K) doses were constant at an optimal level for the plant by autumn application (184 kg ha^{-1} and 216 kg ha^{-1}, respectively), and nitrogen (N) varied between 0 and 300 kg ha^{-1} as follows (0, 60, 120, 180, 240 and 300 kg ha^{-1} N), with spatially randomized plots in four replications (Table 1). There was no nutrient replenishment in the soil on the control plots for 30 years.

Table 1. NPK doses of the multifactoral fertilization experiment of the Látókép Crop Production Experimental Site, Debrecen, Hungary. Bold fonts mark the treatments used in the present study

Fertilization level	N (kg ha^{-1})	P_2O_5 (kg ha^{-1})	K_2O (kg ha^{-1})	Total (kg ha^{-1})
N0	**0**	**0**	**0**	**0**
N1	60	184	216	460
N2	**120**	**184**	**216**	**520**
N3	180	184	216	580
N4	240	184	216	640
N5	**300**	**184**	**216**	**700**

Soil parameters of the experimental area

From the pedological point of view, the area is calcareous chernozem formed on loess ridges, with an average liquid limit (LL = 43–45), average humus content ($Hu_{\%}$ = 2.7–2.8), and with a humus layer thickness of 80 cm. Ammonium lactate soluble P_2O_5 and K_2O are 133 and 240 mg kg^{-1}, respectively, with a bulk density of 1.4–1.45 g cm^{-3} in the cultivated soil layer and 1.25–1.29 g cm^{-3} in the lower layers. In the 0–200 cm soil layer, which is relevant for the water supply of plants, the soil can hold 600–650 mm water, 50–60% of which is disponible for the plants. The average depth of groundwater is 3–5 m, and even in rainy periods it does not rise above 2 m. of depths (Nagy, 2019).

Weather conditions of the experimental area

The experimental area typically has a continental climate, with frequent occurrence of various weather extremes, such as changes in the amount and distribution of precipitation and fluctuations in temperature values within and outside the vegetation period. Only 311.8 mm total precipitation fell during the growing season between 01/04 and 31/10, and there were only 15 days with more than 5 mm, and 4 days with more than 15 mm precipitation (Fig. 1).

Experimental design

The experiment was split-plot design with four replicated plots for the optimal statistical evaluation. The main factor of the experiment was the fertilization (plots), the sub-factors were the hybrids (sub-plots). The size of the experimental plots was 7.2 m^2, with plant density of 73,000 plants ha^{-1} (2 rows x 5 m sized microplots, with approx. 50 plants in each one). Sowing date was 20.04.2019., with a co-application of tefluthrin-based soil disinfectant (15 kg ha^{-1}). In the present study total control plots (0 kg ha^{-1}) a medium, generally common nitrogen dose (120 kg ha^{-1}) and a high nitrogen dose (300 kg ha^{-1}) were selected for plant sampling to examine the effect of nitrogen fertilization on the nutrient ratios of maize. 5 different sampling dates were used in the experiment according to the main phenological stages of maize.

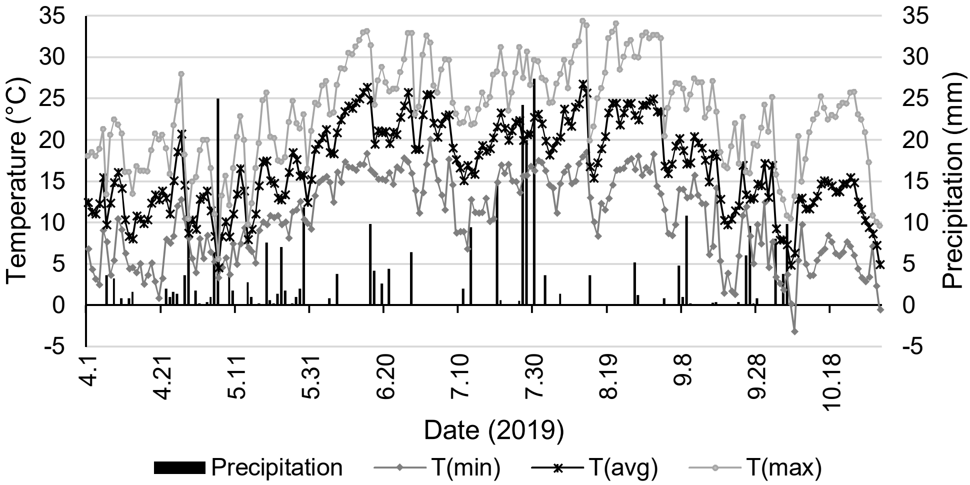

Figure 1. Precipitation and temperature data of the experimental area. 2019, Debrecen, Hungary. T(min) = Daily minimum temperature; T(avg) = Daily average temperature; T(max) = Daily maximum temperature.

Plant sampling

Plant samples were taken 5 times during the growing season, at V2, V4, V8, VT and R6 phenological stages in 4 replication. All plant samples were separated into leaf, stalk, and at the R6 stage into cob and grain too, then dried at 60 °C to a constant weight, weighed to obtain the dry matter of the plants (DM), and ground into fine powder.

Macro- and micronutrient analysis

Complex mineral profile of the vegetative (stalk, leaves) and generative (grain, cob) plant parts were determined in accredited laboratory with ICP-MS, ICP-OES (P, K, mg, Ca, S, Zn, Fe, Cu, Mn, Ni, Mo) using microwave-assisted multielement analysis (Tarantino et al., 2017) and Dumas method for N (Ebeling, 1968).

Sample preparation for the laboratory analysis was as follows: two parallel measurements were made from each sample. 0.4 g sample was weighed, and then 2 mL of high purity water and 4 mL of cc. nitric acid were added to the samples. The adequate digestion program was chosen according to the Milestone Ultrawave microwave system manual (Milestone Inc., USA). Sample extraction with microwave digestion was performed at 200 °C, with a holding time of 10 minutes. ICP-OES analysis was used for the determination P, K, mg, Ca, S, Fe, and Mn concentration. Sample of 5 mL was pipetted into a plastic test tube, and 5 mL of deionized water, 0.2 mL of the acid mixture, and 0.2 mL of 100 ppm Y-containing ISTD (internal standard) were added to the sample. The mixture was homogenized, and then it was put into the 5900 ICP-OES (Agilent Technologies Inc., USA). The Zn, Cu, Mo and Ni concentration of the samples were determined from the homogenized mixture with 7900 ICP-MS

(Agilent Technologies Inc., USA). Extracted sample of 5 mL, 1 mL of the acid mixture, and 4 mL of deionized water were added to the test tube for the analysis. A sample of each matrix type was prepared twice. Blank samples were prepared in each series of measurements by measuring water of the same quantity instead of a sample. Due to possible inhomogeneity, two parallel digests were made from each sample and the final result was calculated from the average of these.

N concentration was measured using the Dumas combustion method. Samples were subjected to oxidative digestion at a high temperature (900 °C) with a controlled oxygen supply The resulting flue gases passed through a copper oxide-platinum catalyst using a CO_2 carrier gas, thus ensuring complete oxidation. After the subsequent reduction processes and the purification of the carrier gas, the nitrogen content remaining in the CO_2 carrier gas was detected in a thermal conductivity detector (VELP NDA 702, Velp Scientific, Italy). The N_2 volume provided an electrical measurement signal, from which the N content of the various burned samples was measured and calculated based on a preprepared calibration curve.

Nutrient concentration ratios for all nutrient concentration combinations were calculated for each sample for the five different development stages, and their interrelationship with different yield parameters was evaluated according to its scale of influence and presented in this study.

Determination of yield parameters

Maize grain yield was determined through a plot-size harvester (Sampo SR2010, Sampo Rosenlew, Finland). The yield quality parameters (protein, starch, oil, and moisture content) were measured with rapid, non-destructive near-infrared transmittance (NIT) technology (Perten DA 7250, PerkinElmer Ltd, Waltham, USA).

Statistical analysis

Data normality was tested by Kolmogorov–Smirnov test. Analysis of variance *(ANOVA)* test was used to evaluate the effect of different nitrogen fertilization doses and different genotypes on the nutrient ratios of the different plant parts. General model of the analyses used fixed effects (Fertilization, Hybrid), interactions (HxF) and random effects (runs nested into 5 times) based on the four replications of each measured parameter. When the F value was significant, *Fisher's LSD* test was used to compare the means and find the least significant difference between them $LSD = \mathrm{t}_{\nu,\alpha}\sqrt{\mathrm{MS}_{\mathrm{S(A)}}\ \frac{2}{S}}$ (Williams & Abdi, 2010). *Pearson's* correlation analysis was used to examine the interactions between the nutrient ratios and the different yield parameters (Nahler, 2009). Significant differences are marked by letters (a, b, c, d) and bold fonts in the manuscript, respectively. Statistical analyses were conducted in R 3.2.4. (Team, 2016a), with RStudio graphical interface (Team, 2016b) and in Minitab Statistical Software.

RESULTS AND DISCUSSION

Simple and multiple effects of fertilization and genotypes on yield parameters

Results of the *ANOVA test* showed that the effect of different nitrogen fertilization levels was significant on grain yield, protein, oil and moisture content. Significant differences were measured between the different maize genotypes in grain yield, protein and starch content. The multiple effects of hybrids and fertilization levels were significant on the oil and moisture content (Table 2).

Increasing nitrogen level can primarily affect the organic matter accumulation and thus, the yield formation of the different crops. However, well-balanced, complex macro- meso and micronutrient replenishment is needed to achieve high yield in more consecutive years, returning the nutrients took away from the production areas with the high yields.

Data evaluation with *Fisher's LSD test* showed the exact differences between the yield parameters. The two higher fertilization levels increased significantly the grain yield of all genotypes, and the results of the H1 genotype was significantly higher at all the three N levels than the values of the other two hybrids.

At both increased N levels the H2 genotype had statistically proven higher protein and moisture content compared to the control. The highest protein content, 9.08% was measured in H3 at N5 level. Starch content decreased significantly in H1 genotype at both N levels compared to the control. Increasing N levels affected differently the protein and starch content of the grain, causing higher protein and lower starch content, respectively (Table 3).

Table 2 Effect of different N fertilization levels on the yield parameters of the hybrids

	Hybrid	Fertilization	HxF
Grain yield	10.90^{***}	26.78^{***}	0.51
Protein	7.70^{**}	21.91^{***}	2.07
Starch	2.97	8.41^{**}	1.11
Oil	9.93^{**}	1.81	3.26^{*}
Moisture	46.06^{***}	3.06	2.79^{*}

F values of the *ANOVA test* of the yield parameters. H = Hybrids; F = Fertilization. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 3. Differences of yield parameters between hybrids and fertilization levels with *Fisher's LSD* method

		Grain yield (t ha^{-1})	Protein (%)	Starch (%)	Oil (%)	Moisture (%)
N0	H1	8.39^{b}	8.13^{c}	63.1^{ab}	4.08^{c}	14.0^{c}
	H2	5.49^{c}	6.88^{d}	63.2^{a}	4.13^{bc}	14.9^{a}
	H3	4.67^{c}	8.13^{c}	62.1^{bcd}	4.05^{c}	14.8^{a}
N2	H1	12.37^{a*}	8.63^{abc}	61.8^{cd*}	4.25^{ab}	14.0^{c}
	H2	9.27^{b**}	8.30^{bc***}	62.2^{abc}	4.05^{c}	14.4^{b*}
	H3	8.83^{b**}	8.90^{ab}	61.0^{d}	4.08^{c}	14.8^{a}
N5	H1	11.90^{a*}	8.85^{ab}	61.6^{cd*}	4.30^{a*}	14.0^{c}
	H2	10.29^{ab***}	8.80^{ab***}	61.7^{cd*}	4.10^{c}	14.3^{bc**}
	H3	10.28^{ab***}	9.08^{a*}	61.8^{cd}	4.05^{c}	14.8^{a}

Means that do not share a letter are significantly different. Values marked with asterisks are statistically significant compared to control (N0). $LSD_{(yield)} = 1.76$; $LSD_{(protein)} = 0.38$; $LSD_{(starch)} = 0.67$; $LSD_{(oil)} = 0.15$; $LSD_{(moisture)} = 0.31$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Our results are directly in line with previous findings by Széles et al. (2018), that the higher nitrogen fertilization level can mainly increase grain yield, but the effect on grain protein content was not uniformly significant, it is affected by other agronomic and environmental factors. Plant nutrition has a great impact on yield formation, and the stability of different genotypes is a main parameters influencing the farmer's hybrid choice (Shojaei et al., 2021).

Simple and multiple effects of sampling time, fertilization and genotypes on the nutrient ratios of stalk, leaves, grain and cob of maize

In the experiment all essential nutrient concentrations were measured. Among them, the important nutrient ratios of macro- and micronutrients for the physiological development of maize were calculated and presented in the tables below. Results of the *ANOVA* of the stalk shoved that between the sampling times and the fertilization levels all presented nutrient ratios had significant differences, with exception of one value at $P < 0.001$ level. Between the different maize genotypes, significant differences were measured in the N:Mg, N:Ca, P:S and P:Fe ratios. The interaction of different development stages and genotypes affected significantly the P:S, P:Zn and P:Fe ratios.

While the analysis of fertilization level - sampling time interaction showed statistically verified differences between all presented nutrient ratios, except the N:Mg. Results of this analysis suggest that for the nutrient composition of stalk during the growing season the increasing nitrogen fertilization level is a higher influencing factor than choosing the adequate hybrid (Table 4). Ma et al. (2016) confirmed that grain yield, dry matter production, N and P uptake were affected by the N fertiliser application rate. Significant genotype effect was measured on grain yield, and shoot N and P contents, and also the N:P ratios increased with higher N fertiliser rates. The critical N and P values on the plant parts and their stoichiometric connection to other essential macro- and micronutrients is an important topic to further studies for a nutrient diagnosis system in maize.

Table 4. Effect of different N fertilization levels on the nutrient ratios of stalk

	Stalk						
	ST	H	F	STxH	STxF	HxF	STxHxF
N:Mg	53.60^{***}	3.50^{*}	22.58^{***}	0.77	1.21	0.83	0.44
N:Ca	48.26^{***}	3.33^{*}	15.05^{*}	1.09	2.13^{*}	0.77	0.35
N:Zn	14.44^{***}	0.97	14.09^{***}	1.15	4.73^{***}	0.31	0.73
P:K	47.55^{***}	2.21	17.67^{***}	1.07	2.21^{*}	0.53	0.53
P:Mg	48.90^{***}	0.04	10.19^{***}	1.89	2.23^{*}	0.25	0.24
P:S	19.66^{***}	4.25^{*}	18.35^{***}	2.35^{*}	4.70^{***}	0.98	0.42
P:Zn	27.44^{***}	0.50	19.56^{***}	2.11^{*}	8.12^{***}	1.06	1.37
P:Fe	65.67^{***}	4.10^{*}	12.63^{***}	2.47^{*}	5.99^{**}	1.57	1.34
K:Mg	25.02^{***}	0.68	28.73^{***}	1.26	3.07^{**}	0.84	0.73
K:Zn	30.19^{***}	1.15	27.27^{***}	0.72	4.93^{***}	0.39	0.59

F values of the *ANOVA test*. ST = Sampling times; H = Hybrids; F = Fertilization. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Analysis on the leaf nutrient ratios showed that the different sampling times and fertilization levels and their multiple effects significantly influenced all of them at $P < 0.001$ level, except the N:Mg and N:Ca ratios in their common effect. Between the three genotypes statistically proven differences could be measured in the N:Mg, N:Ca, P:K, P:S, P:Zn and P:Fe ratios.

Determining the adequate genotype for each production area is essential to achieve successful plant production. The nutrient ratios between genotypes, and the genotype - sampling times multiple effects resulted in significant differences in six of the ten important nutrient ratios (Table 5). Ortas (2018) experienced significant increases in grain yield, N, P and K concentrations of leaves as an effect of K fertiliser treatments, and it also increased the K and mg concentrations of seeds. Using different genotypes led to statistically proven higher values in N, K, Fe and Zn concentrations and ratios with increased K fertilization. Qiang et al. (2019) also found significant differences between genotypes in leaf N concentration which parameter contributed greatly to higher yields and increasing nitrogen and water use efficiency (NUE, WUE).

Table 5. Effect of different N fertilization levels on the nutrient ratios of leaves

	Leaves						
	ST	H	F	STxH	STxF	HxF	STxHxF
N:Mg	96.50***	4.08*	32.88***	0.67	1.26	1.32	0.57
N:Ca	317.33***	11.61***	16.59***	4.51***	1.65	0.65	0.80
N:Zn	7.25***	0.39	10.32***	1.19	6.23***	0.83	0.73
P:K	27.74***	5.64**	23.92***	6.04***	7.60***	0.69	0.68
P:Mg	108.82***	0.98	20.84***	1.75	3.96***	0.95	0.49
P:S	25.28***	3.80*	34.93***	3.41**	10.78***	0.04	0.42
P:Zn	40.22***	9.42***	17.14***	5.75***	8.61***	0.75	0.88
P:Fe	43.68***	5.61**	15.98***	5.14***	8.35***	3.15*	1.55
K:Mg	130.10***	2.24	46.22***	1.20	4.25***	0.31	0.42
K:Zn	50.18***	2.44	27.42***	2.41*	6.64***	0.20	0.34

F values of the *ANOVA test* of the nutrient ratios of leaves. ST = Sampling times; H = Hybrids; F = Fertilization. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

The analysis of variance of the grain and cob indicated significant differences between the three maize genotypes in the P:K, K:Mg, K:Zn and P:K, P:Fe, K:Mg and K:Zn ratios, respectively. The increasing N fertilization levels affected the nutrient ratios of grain and cob similarly, causing statistically confirmed differences in the P:S, P:Zn, P:Fe and K:Zn values. Examination of the multiple effects of the two factors showed that in the grain the P:S, in the cob the K:Mg and K:Zn ratios had significant changes, making these nutrient ratios important in the assessment of genotype-specific fertilization management systems (Table 6).

Table 6. Effect of different N fertilization levels on the nutrient ratios of grain and cob

	Grain			Cob		
	H	F	HxF	H	F	HxF
N:Mg	0.95	0.78	0.93	0.14	2.56	0.75
N:Ca	0.51	0.72	0.15	0.21	0.79	0.26
N:Zn	1.12	2.87	0.46	1.43	5.24*	1.03
P:K	9.99***	1.94	0.75	5.96**	0.08	0.94
P:Mg	0.56	2.53	0.12	0.65	1.14	0.35
P:S	0.98	5.24**	3.30**	3.05	6.15**	0.76
P:Zn	0.68	16.33***	1.12	1.07	16.42***	2.41
P:Fe	1.99	5.81**	0.24	6.89**	6.45**	0.86
K:Mg	6.25**	2.46	0.21	3.54*	1.59	3.47*
K:Zn	3.57*	8.26**	0.56	4.99*	10.76***	4.52**

F values of the *ANOVA test* of the nutrient ratios of grain and cob. H = Hybrids; F = Fertilization. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Results of correlation analysis of the vegetative parts of maize with yield parameters

Correlation analysis indicates the relationship between the different nutrient ratios and the yield parameters, how similarly their extent varies. Analysing the hybridspecific nutrient interactions and their connection to yield parameters can contribute to implement fertilization strategies helping the farmers to increase the yield and its quality parameters. The mean nutrient ratios of the five sampling times and three fertilization levels were correlated to the grain yield, protein and starch content of maize. There are high differences between the exact nutrient concentrations during the growing season in the five sampling times, so we intended to identify the significant correlations to find the connections between the yield parameters and values measured during the vegetative growth period.

According to the results of correlation analysis between grain yield and nutrient ratios of stalk, the H1 genotype positively correlated with N:Zn, and K:Zn at $P < 0.01$, and negatively with P:S. The H2 genotype also had positive correlation at $P < 0.01$ with N:Mg, K:Mg and K:Zn, and negative correlation with P:K ratio. H3 grain yield had close positive correlation only with K:Mg ratio. Correlation between K:Mg ratio and grain yield was significant in all three hybrids, marking it as an important parameter for future research. Protein content had important correlation values only with the nutrient ratios of the H2 genotype, significantly positive at $P < 0.01$ with N:Mg, K:Mg and K:Zn, and negative with P:K and P:S, respectively. Starch content of the grain had only negative notable correlation values, N:Zn in H1, and K:Mg, K:Zn in H2 genotype (Table 7).

Table 7. *Pearson's R* values between the means of nutrient ratios of maize stalk with different fertilization levels and the yield parameters

	Grain yield			Protein			Starch		
	H1	H2	H3	H1	H2	H3	H1	H2	H3
N:Mg	0.30*	0.28**	0.22	0.14	0.37**	0.14	-0.27*	-0.32*	-0.11
N:Ca	0.29*	0.18	0.21	0.10	0.27*	0.11	-0.28*	-0.21	-0.06
N:Zn	0.41**	0.23	0.25	0.14	0.27*	0.11	-0.33**	-0.19	0.03
P:K	-0.24	-0.33**	-0.24	-0.29*	-0.35**	-0.13	0.27*	0.28*	0.09
P:Mg	0.16	0.22	0.11	0.12	0.30*	0.19	-0.18	-0.27*	-0.10
P:S	-0.31**	-0.29*	-0.23	-0.21	-0.36**	-0.11	0.28*	0.28*	0.15
P:Zn	0.26*	0.29*	0.21	0.16	0.30*	0.18	-0.25	-0.19	0.05
P:Fe	-0.17	-0.28*	-0.11	-0.15	-0.30*	0.02	0.12	0.22	-0.04
K:Mg	0.32*	0.34**	0.39**	0.23	0.44**	0.28*	-0.31*	-0.41**	-0.20
K:Zn	0.35**	0.37**	0.30*	0.18	0.41**	0.19	-0.32*	-0.31**	-0.03

$^{*}P < 0.05$; $^{**}P < 0.01$.

Gökkuş et al. (2016) also revealed that the high protein and high oil genotypes had stalk and leaves of higher nutritional parameters. It is important to study the exact nutrient characteristics to understand the factors influencing yield quality.

Correlation analysis between the mean nutrient ratios of leaves and yield parameters showed that in grain yield H2 negatively correlated with P:K, P:S and P:Fe, H3 with P:S ratios at $P < 0.01$, and positively with N:Mg, N:Zn, P:Zn and K:Zn ratios at $P < 0.05$ level. Protein and starch content had significant correlation with H2 genotype in case of protein P:K, P:S and P:Fe negatively, and P:S with starch, positively, all of them at $P < 0.01$ level. (Table 8). Shao et al. (2020) reported significant positive correlation between grain yield and N, P and K accumulation and remobilization efficiency during the growing season with pre- and post silking measurements.

Table 8. *Pearson's R* values between the means of nutrient ratios of maize leaves with different fertilization levels and the yield parameters

	Grain yield			Protein			Starch		
	H1	H2	H3	H1	H2	H3	H1	H2	H3
N:Mg	0.21	0.21	0.28*	0.21	0.24	0.27*	-0.27*	-0.19	-0.16
N:Ca	0.07	0.06	0.18	0.13	0.08	0.13	-0.14	-0.05	-0.06
N:Zn	0.16	0.23	0.31*	0.02	0.23	0.20	-0.10	-0.17	-0.18
P:K	-0.20	-0.39**	-0.31*	-0.16	-0.36**	-0.22	0.25	0.32*	0.07
P:Mg	0.14	0.15	0.18	0.21	0.17	0.23	-0.20	-0.13	-0.19
P:S	-0.26*	-0.43**	-0.35**	-0.09	-0.46**	-0.27*	0.22	0.41**	0.05
P:Zn	0.03	0.09	0.27*	0.01	0.07	0.22	-0.06	0.01	-0.26*
P:Fe	-0.10	-0.32**	-0.23	0.17	-0.34**	-0.21	0.00	0.24	0.05
K:Mg	0.19	0.27*	0.25	0.23	0.30	0.26*	-0.29*	-0.24	-0.14
K:Zn	0.15	0.28*	0.31*	0.10	0.29*	0.23	-0.19	-0.20	-0.19

$^{*}P < 0.05$; $^{**}P < 0.01$.

Nitrogen use efficiency, the relationship and co-limitation of nitrogen with other macro- and micronutrients is an important research area in terms of sustainable agriculture, agriecosystem modelling and scailing them into global level (Elser et al., 2010; Khoshgoftarmanesh et al., 2011; Weih et al., 2018). Different studies of the nutrient stoichiometry revealed that N:P, N:S and P:S relationships were allometric and not modified by P deficiency. The lower N:P value observed under N limitation was mainly caused by a higher P content, but the higher N:P value observed under P limitation was mainly caused by a higher N content. The P:S stoichiometric relationship may be used as a 'post-mortem' tool for identifying S responsive sites using grain nutrient analysis (Yan et al., 2016; Salvagiotti et al., 2017; Carciochi et al., 2018). N:S stoichiometry can be used for characterizing plant S nutritional status when N is not a limiting factor for plant growth, eg. in areas or crop production technologies with high nitrogen fertilization supplies (Divito et al., 2016; Arata et al., 2017, Carciochi et al., 2020). Nutrient stoichiometry can be affected mainly by soil microbial activity (Auwal et al., 2021), soil type and its nutrient status (Bruns & Ebelhar, 2006), crop rotation systems (Ma et al., 2003; Xia et al., 2013; Ma & Zheng, 2018) and the plant's water use efficiency (Yan et al., 2016). As a conclusion, the optimal crop nutrition systems have great importance on the morphological parameters, quality and quantity parameters of the plants, which need a well-balanced fertilization practice (Illés et al., 2020; Mousavi et al., 2020; Bojtor et al., 2021) Nutrient stoichiometry can provide a reliable scientific tool to monitor the plant's health status, and make adequate decisions about the optimal fertilization management strategies.

CONCLUSIONS

The findings of this study focused on how the increased nitrogen fertilization levels affected significantly the essential nutrient ratios of the vegetative and generative plant parts of maize. The optimal nutrient supply and ratios of these nutrients of the vegetative plant parts is essential for the optimal dry matter accumulation, which allows to maximize the leaf area and also the photosynthetic capacity, providing the possibility of achieving high yields with remarkable quality parameters. Results of this study could be validated in further novel research and a new perspective of an optimal fertilization dosage could be achieved. Analysing the optimal nutrient ratios related to the yield quality and their interaction with the fertilization practices can give certain recommendations to the farmers to implement hybrid- and site-specific nutrient management systems, reducing the environmental impact of the over-fertilization.

ACKNOWLEDGEMENTS. The research was supported by the ÚNKP-21-3-II New National Excellence Program of the Ministry for Innovation and Technology from the source of the National Research, Development and Innovation Fund. Project no. TKP2020-IKA-04 has been implemented with the support provided from the National Research, Development and Innovation Fund of Hungary, financed under the 2020-4.1.1-TKP2020 funding scheme.

REFERENCES

Arata, A.F., Lerner, S.E., Tranquilli, G.E., Arrigoni, A.C. & Rondanini, D.P. 2017. Nitrogen× sulfur interaction on fertiliser-use efficiency in bread wheat genotypes from the Argentine Pampas. *Crop and Pasture Science* **68**(3), 202–212.

Auwal, M., Singh, B.P., Chen, Z., Kumar, A., Pan, S., Luo, Y. & Xu, J. 2021. Nutrients addition regulates temperature sensitivity of maize straw mineralization. *Journal of Soils and Sediments*, 1–13.

Bélanger, G., Ziadi, N., Lajeunesse, J., Jouany, C., Virkajarvi, P., Sinaj, S. & Nyiraneza, J. 2017. Shoot growth and phosphorus–nitrogen relationship of grassland swards in response to mineral phosphorus fertilization. *Field Crops Research* **204**, 31–41.

Bojtor, C., Illés Á., Nagy, J., Marton, L.C. 2020. Effect of nitrogen fertilisation on dry matter incorporation and micronutrient uptake of different maize hybrids. *Növénytermelés* **69**(3), 1–22.

Bojtor, C., Illés, Á., Nasir Mousavi, S.M., Széles, A., Tóth, B., Nagy, J. & Marton, C.L. 2021. Evaluation of the Nutrient Composition of Maize in Different NPK Fertilizer Levels Based on Multivariate Method Analysis. *International Journal of Agronomy*, 2021. https://doi.org/10.1155/2021/5537549

Bruns, H.A. & Ebelhar, M.W. 2006. Nutrient uptake of maize affected by nitrogen and potassium fertility in a humid subtropical environment. *Communications in soil science and plant analysis* **37**(1–2), 275–293.

Carciochi, W.D., Calvo, N.I.R., Wyngaard, N., Divito, G.A., Eyherabide, M. & Echeverría, H.E. 2019. Prognosis and diagnosis of sulfur status in maize by plant analysis. *European Journal of Agronomy* **108**, 1–10.

Carciochi, W.D., Salvagiotti, F., Pagani, A., Calvo, N.I.R., Eyherabide, M., Rozas, H.R.S. & Ciampitti, I.A. 2020. Nitrogen and sulfur interaction on nutrient use efficiencies and diagnostic tools in maize. *European Journal of Agronomy* **116**, 126045.

Carciochi, W.D., Wyngaard, N., Divito, G.A., Cabrera, M.L., Calvo, N.I.R. & Echeverría, H.E. 2018. A comparison of indexes to estimate corn S uptake and S mineralization in the field. *Biology and fertility of soils* **5**4(3), 349–362.

Divito, G.A., Echeverría, H.E., Andrade, F.H. & Sadras, V.O. 2016. N and S concentration and stoichiometry in soybean during vegetative growth: Dynamics of indices for diagnosing the S status. *Field Crops Research* **198**, 140–147.

Duncan, E.G., O'Sullivan, C.A., Roper, M.M., Biggs, J.S. & Peoples, M.B. 2018. Influence of co-application of nitrogen with phosphorus, potassium and sulphur on the apparent efficiency of nitrogen fertiliser use, grain yield and protein content of wheat. *Field crops research* **226**, 56–65.

Ebeling, M.E. 1968. The Dumas method for nitrogen in feeds. *Journal of the Association of Official Analytical Chemists* **51**(4), 766–770.

Elser, J.J., Fagan, W.F., Kerkhoff, A.J., Swenson, N.G. & Enquist, B.J. 2010. Biological stoichiometry of plant production: metabolism, scaling and ecological response to global change. *New Phytologist* **186**(3), 593–608.

Fischer, R.A. & Connor, D.J. 2018. Issues for cropping and agricultural science in the next 20 years. *Field Crops Research* **222**, 121–142.

Gastal, F., Lemaire, G., Durand, J.L. & Louarn, G. 2015. Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. In *Crop physiology Applications for Genetic Improvement and Agronomy*, Academic Press, San Diego, pp. 161–206.

Gökkuş, A., Kahriman, F., Alatürk, F. & Ali, B. 2016. Variation of nutritional values in leaves and stalks of different maize genotypes having high protein and high oil during vegetation. *Agriculture and Agricultural Science Procedia* **10**, 18–25.

Hoogmoed, M., Neuhaus, A., Noack, S. & Sadras, V.O. 2018. Benchmarking wheat yield against crop nitrogen status. *Field Crops Research* **222**, 153–163.

Humtsoe, B.M., Dawson, J. & Rajana, P. 2018. Effect of nitrogen, boron and zinc as basal and foliar application on growth and yield of maize (Zea *mays* L.). *Journal of Pharmacognosy and Phytochemistry* **7**(6), 01–04.

Illés, Á., Mousavi, S.N., Bojtor, C. & Nagy, J. 2020. The plant nutrition impact on the quality and quantity parameters of maize hybrids grain yield based on different statistical methods. *Cereal Research Communications* **48**(4), 565–573.

Khoshgoftarmanesh, A.H., Schulin, R., Chaney, R.L., Daneshbakhsh, B. & Afyuni, M. 2011. Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture. In *Sustainable Agriculture* Volume **2**. Springer, Dordrecht, pp. 219–249.

Lamptey, S., Yeboah, S., Li, L. & Zhang, R. 2017. Dry matter accumulation and nitrogen concentration in forage and grain maize in dryland areas under different soil amendments. *Agronomy Research* **15**(4), 1646–1658.

Liu, T., Huang, R., Cai, T., Han, Q. & Dong, S. 2017. Optimum leaf removal increases nitrogen accumulation in kernels of maize grown at high density. *Sci. Rep.* **7**, 39601.

Ma, B.L., Ying, J., Dwyer, L.M., Gregorich, E.G. & Morrison, M.J. 2003. Crop rotation and soil N amendment effects on maize production in eastern Canada. *Canadian Journal of Soil Science* **83**(5), 483–495.

Ma, B.L., Zheng, Z.M., Morrison, M.J. & Gregorich, E.G. 2016. Nitrogen and phosphorus nutrition and stoichiometry in the response of maize to various N rates under different rotation systems. *Nutrient cycling in agroecosystems* **104**(1), 93–105.

Ma, B.L. & Zheng, Z. 2018. Nutrient uptake of iron, zinc, magnesium, and copper in transgenic maize (Zea mays) as affected by rotation systems and N application rates. *Nutrient Cycling in Agroecosystems* **112**(1), 27–43.

Mousavi, S.M.N., Illés, Á., Bojtor, C. & Nagy, J. 2020. The impact of different nutritional treatments on maize hybrids morphological traits based on stability statistical methods. *Emirates Journal of Food and Agriculture*, 666–672.

Nagy, J. 2019. Complex long-term experiments on soil use, water and nutrient management at the University of Debrecen since 1983. *Növénytermelés* **68**(3), 5–28.

Nahler, G. 2009. Pearson correlation coefficient. In Dictionary of Pharmaceutical Medicine. Springer, Vienna, pp. 132–132.

Ortas, I. 2018. Influence of potassium and magnesium fertilizer application on the yield and nutrient accumulation of maize genotypes under field conditions. *Journal of Plant Nutrition* **41**(3), 330–339.

Qiang, S., Zhang, Y., Fan, J., Zhang, F., Xiang, Y., Yan, S. & Wu, Y. 2019. Maize yield, rainwater and nitrogen use efficiency as affected by maize genotypes and nitrogen rates on the Loess Plateau of China. *Agricultural Water Management* **213**, 996–1003.

Salvagiotti, F., Prystupa, P., Ferraris, G., Couretot, L., Magnano, L., Dignani, D. & Gutiérrez-Boemoem, F.H.G. 2017. N: P: S stoichiometry in grains and physiological attributes associated with grain yield in maize as affected by phosphorus and sulfur nutrition. *Field Crops Research* **20**3, 128–138.

Shao, H., Shi, D., Shi, W., Ban, X., Chen, Y., Ren, W., Chen, F. & Mi, G. 2020. Nutrient accumulation and remobilization in relation to yield formation at high planting density in maize hybrids with different senescent characters. *Archives of Agronomy and Soil Science* 1–17.

Shojaei, S.H., Mostafavi, K., Omrani, A., Omrani, S., Nasir Mousavi, S. M., Illés, Á., Bojtor, C. & Nagy, J. 2021. Yield Stability Analysis of Maize (*Zea mays* L.) Hybrids Using Parametric and AMMI Methods. Scientifica, 2021. https://doi.org/10.1155/2021/5576691

Széles, A., Horváth, É., Vad, A. & Harsányi, E. 2018. The impact of environmental factors on the protein content and yield of maize grain at different nutrient supply levels. *Emirates Journal of Food and Agriculture*, 764–777.

Tarantino, T.B., Barbosa, I.S., Lima, D.D.C., Pereira, M.D.G., Teixeira, L.S. & Korn, M.G.A. 2017. Microwave-assisted digestion using diluted nitric acid for multi-element determination in rice by ICP OES and ICP-MS. *Food analytical methods* **10**(4), 1007–1015.

Team, R. 2016a. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. R Foundation for Statistical Computing. URL: http://www.R-project.org/

Team, R. 2016b. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA, USA. URL: http://www.rstudio.com/

Wang, Z., Sadras, V.O., Hoogmoed, M., Yang, X., Huang, F., Han, X. & Zhang, S. 2017. Shifts in nitrogen and phosphorus uptake and allocation in response to selection for yield in Chinese winter wheat. *Crop and Pasture Science* **68**(9), 807–816.

Weih, M., Hamnér, K. & Pourazari, F. 2018. Analyzing plant nutrient uptake and utilization efficiencies: comparison between crops and approaches. *Plant and Soil* **430**(1), 7–21.

Williams, L.J. & Abdi, H. 2010. Fisher's least significant difference (*LSD*) test. *Encyclopedia of research design* **218**, 840–853.

Xia, H., Zhao, J., Sun, J., Xue, Y., Eagling, T., Bao, X., ... & Li, L. 2013. Maize grain concentrations and above-ground shoot acquisition of micronutrients as affected by intercropping with turnip, faba bean, chickpea, and soybean. *Science China Life Sciences* **56**(9), 823–834.

Xin, L., Liu, J., Liu, S., Chen, Y., Nan, Z., Yuan, M., & Chen, J. 2016. Effects of Combined Application of Straw and Organic Fertilizer on Grain Yield and Quality under Wheat Maize Rotation System. *Acta Agriculturae Boreali-Sinica*, 06.

Yan, W., Zhong, Y., Zheng, S. & Shangguan, Z. 2016. Linking plant leaf nutrients/stoichiometry to water use efficiency on the Loess Plateau in China. *Ecological Engineering* **87**, 124–131.