Thesis of Doctoral (Ph.D.) Dissertation

EVALUATION OF PLANT NUTRIENT SUPPLY IN PRECISION AGRICULTURE WITH SPECIAL REFERENCE TO NITROGEN

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1. INTRODUCTION AND OBJECTIVES

Nowadays climate change causes more and more problems for crop production. Supply of essential nutrients for plants is one of the most cardinal factors in agrotechnical interventions. In addition, it carries one of the most significant risks, from both ecologically and economically point of views. In the first half of the 20th century, application of synthetic nutrients was at very low level. Then, this value multiplied during "Green Revolution" and then decreased significantly again in the 90's. According to the latest available data, more than 110 million tonnes of nitrogen were applied to soils worldwide as fertilizer. This could be harmful for both environment and economy. There is a necessity to reduce the use of N-containing fertilizers and to increase their application efficiency. However, reducing the amount of fertilizers and chemicals used alone will not solve the problem, so adapting other practices is essential in practical farming.

Success of our farming is fundamentally determined by adaptation ability of the crop genotype. In parallel with the spread of new breeding methods genetics of plants gone through on a significant development. This is particularly important for large-scale crops such as maize, which were grown on more than 197 million hectares worldwide in 2019 and produced 1.14 billion tonnes. Due to constant expansion of its uses and its role in the food chain, these numbers are expected to increase in the future.

Advances in technology in recent decades have made it possible to record accurate, site-specific plant conditions. Basic premise of precision farming is that we carry out the agrotechnical interventions differentiated according to the conditions of the field and the expected yield level. Our main goal is to achieve optimal yields and to optimize the application of input materials. The opportunities provided by technology have made precision technologies better and more applicable in practice, but continuous research and the discovery of newer and newer parameters are essential for its further development and improvement. Plant physiological researches had special importance in this, because it belongs to the technological basis of precision farming.

The field of our research was primarily outlined around corn and its nutrient utilization properties, our goals were:

- Investigation of physiological responses of maize genotypes to different amounts of N.
- We also aimed to identify parameters that can be used to characterize the N utilization by the plant, their plasticity under different conditions, and the limits

of adaptation. We aimed primarily to identify parameters that can be recorded *in vivo* and *in situ*, which can be used as a direct tool for precision crop production.

- Categorization of genotypes based on the results in terms of N use efficiency.
- Investigation of N use efficiency and other plant physiological parameters of maize genotypes with different N use efficiency with field conditions.
- Further goal was to determine whether the reduced nitrogen supply affects the intake of zinc, which is important for maize.

The unfavourable environment has a negative impact on the efficiency of nutrient utilization. The objectives of our research provide basic research results for applied research as well as for plant breeding, and also take into account the demands of climate change on crop production.

2. MATERIALS AND METHODS

2.1. Description of experimental sites

The location of our controlled experiments was at the climate room of the Department of Applied Plant Biology, Institute of Plant Science, Faculty of Agricultural and Food Sciences and Environmental Management, University of Debrecen. In the plant growth chamber, the light/dark period was 16 hours/8 hours. At day period, the light intensity was adjusted to 300 µmol $m^{-2}s^{-1}$. The periodicity of the temperature was 25/20 ° C (day / night) while the relative humidity (RH) was 65-75%. Plants were grown with hydroponic system. Two completely different experiments were performed under controlled conditions. In the experiment set up to test nitrogen use efficiency, plants were treated with two nitrogen (N) levels (optimal and reduced to ¼) and 22 maize (*Zea mays* L.) genotypes (Armagnac, DK 440, DKC 4590, DKC4490, Fornad, Loupiac, MV Danietta, MV Illango, MV Margitta, MVOlek, Neffel, NK Columbia, NK Thermo, Occitan, P0023, P0216, P9074, P9415, P9537, P9903, Renfor, Sushi). In the experiment set up to study the nitrogen-zinc interaction, Armagnac and P9903 were selected from genotypes. In this experiment, the plants were treated with different doses of nitrogen and zinc (Zn). The nitrogen treatments were the same as the N levels presented above. For zinc, 5 levels were used (0 µmol L^{-1} (0 Zn); 50 µmol L^{-1} (1/2 Zn), 100 µmol L^{-1} (Opt. Zn); 200 µmol L^{-1} (2x Zn); 500 µmol L^{-1} (5x Zn)). Sampling and measurement of various parameters were performed at the 5-leaf (V5) stage at the end of experiment.

Location of our experiment to characterize nitrogen utilization efficiency was at Experimental Station of Látókép, University of Debrecen, Institutes for Agricultural Research and Educational Farms, (47°30'N, 21°36'E; altitude: 111 mBf). The soil of the experiment site was calcareous chernozem. Three nitrogen levels were used in the experiment. In case of control plots 0 kg ha^{-1}, in the second fertilizer level 80 kg ha^{-1}, and in case of third level 160 kg ha^{-1} N active ingredient was applied. Three maize genotypes were examined during the experiment (Armagnac, Fornad, Loupiac). Research was carried out during 3 growing seasons (2018, 2019, 2020).

The nitrogen-zinc interaction experiment was set at the Exhibition Garden and Arboretum of Institutes for Agricultural Research and Educational Farms, University of Debrecen (47°33'N, 21°36'E; altitude 114 mBf). The soil of the experiment site was calcareous chernozem. Armagnac and P9903 genotypes were used for the experiment. Two levels of N were used: 40 and 160 kg ha^{-1}. Further treatments of experiment were the application method of Zn (without Zn, leaf fertilizer, soil fertilizer). Research was carried out during 2 growing seasons (2019, 2020). Samples were collected at 7 leaf (V7), tasselling (VT), silking (R1), blister (R2) and physiological maturity (R6) stages from the two mentioned experiments. Growth stages were identified according to HANWAY (1963).

2.2. Description of experimental methods

During our experiments, we examined the increase of plant dry matter in young plants with thermogravimetric method. Specific leaf area (SLA) was determined according to the method of GARNIER et al. (2001). The SPAD (Soil Plant Analysis Development) value was used to characterize the relative chlorophyll content. Values were measured with a SPAD 502 (Minolta, Japan). Qualitative and quantitative analysis of photosynthetic pigments was determined by the method of MORAN and PORATH (1980) and by the formulae of WELLBURN (1994). The *in vivo* chlorophyll fluorescence induction method was used to characterize the effects of potential nutrient stress on plants. A PAM 2100 (Walz, Germany) portable fluorometer was used in our experiments. In the laboratory experiment set up to test N use efficiency, the carbon and N contents of samples were determined with Variomax Cube CNS analyser (Elementar, Germany). Zn and N contents of samples from N-Zn interaction experiment were examined at the Agricultural Instrument Centre of DE-MÉK. The Gaussen-Bagnouls xerothermal index (BGI) was used to characterize drought months (BAGNOULS and GAUSSEN, 1953).

2.3. Statistical analysis

SigmaPlot for Windows 12 (Systat software) and SPSS Statistics 20 (IBM) were used for statistical analysis. According to the experimental settings, we evaluated the effect of our treatments on the given parameters using two- and/or three-factor analysis of variance (ANOVA). At the end of laboratory N utilization experiment, cluster analysis was performed based on 20 parameters using the Ward linkage method.

3. RESULTS

Our aim was to identify genotypes that can be characterized by efficient N utilization. Comparing the averages of N, dry matter of the shoot decreased by 30.5% due to reduced N supply. However, the mass of root dry matter increased or did not change in several genotypes (Armagnac, DK440, Neffel, P9074, P9537, Occitan) which could be a sign of ability to adapt to nutrient deficiencies. The shoot to root ratio value was 17.8% higher at the optimal N level, but even then, there were differences among genotypes (Figure 1). Optimal N supply induced shoot growth, while N deprivation resulted in increasing root growth, therefore, the difference increased between optimal and reduced N levels in case of shoot to root ratio. However, when significant difference was not found among the values of different N levels, a tolerance for N deficiency can be expected for example Armagnac, NK Columbia and P0216 genotypes.

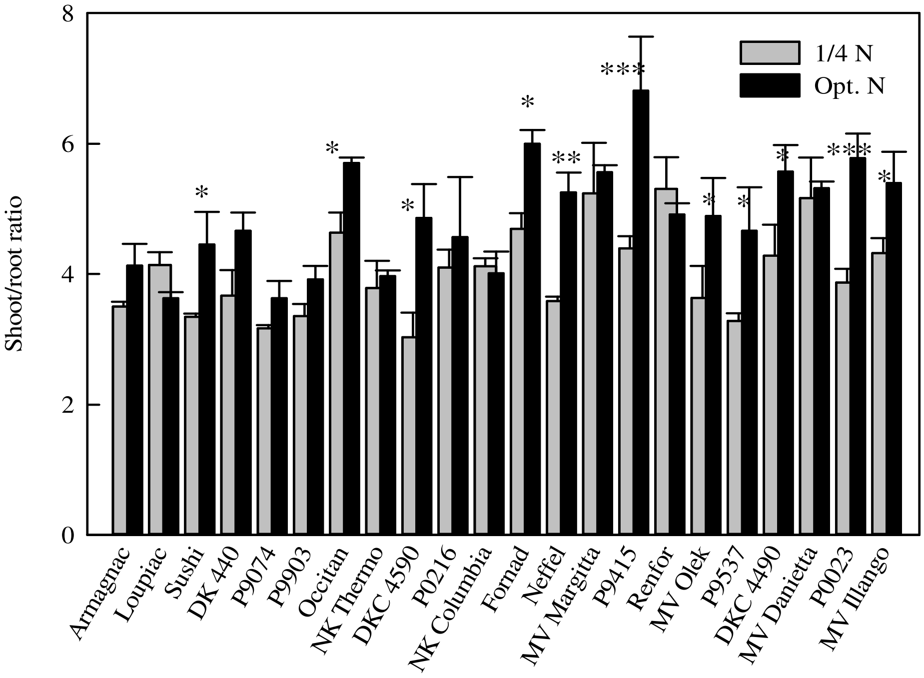

Figure 1. Changes of ratio between shoot dry weight and root dry weight (shoot/root ratio) due to N (optimal N: opt. N, reduced N: ¼ N), in genotypes (Armagnac, Loupiac, Sushi, DK440, P9074, P9903, Occitan, NK Thermo, DKC4590, P0216, NK Columbia, Fornad, Neffel, MV Margitta, P9415, Renfor, MV Olek, P9537, DKC 4490, MV Danietta, P0023, MV Illango) n=3, ±s.e., differences between N levels: P≤0.05*, P≤0.01**, P≤0.001***

The chlorophyll-a/chlorophyll-b ratio in the older leaf increased slightly with nitrogen removal, but this change was not statistically significant (Figure 2). Difference between genotypes was small as well. The highest value was found in NK Columbia. In MV Olek hybrid, this value was only 1.7 times the chlorophyll-b content.

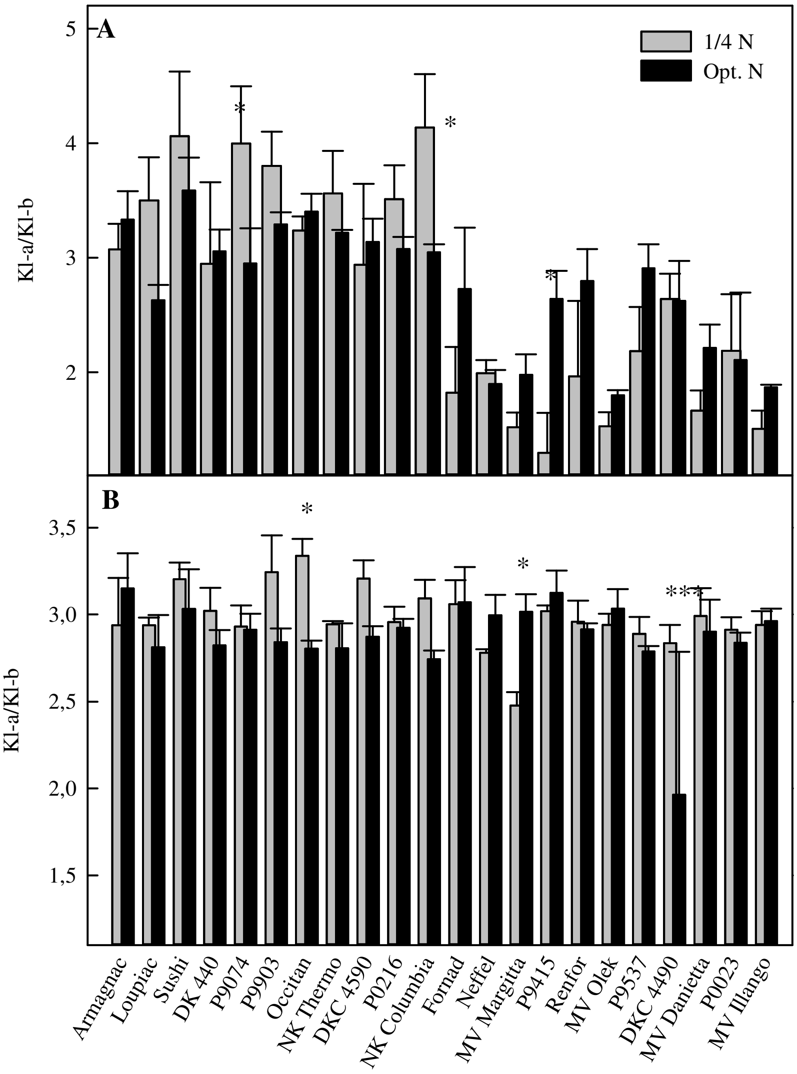

Figure 2. Changes of chlorophyll-a/chlorophyll-b ratio (Kla/Klb) in the older (A) and the younger (B) leaf due to N (optimal N: opt. N, reduced N: ¼ N), in genotypes (Armagnac, Loupiac, Sushi, DK440, P9074, P9903, Occitan, NK Thermo, DKC4590, P0216, NK Columbia, Fornad, Neffel, MV Margitta, P9415, Renfor, MV Olek, P9537, DKC 4490, MV Danietta, P0023, MV Illango) n=3, ±s.e., differences between N levels: $P \leq 0.05$*, $P \leq 0.01$**, $P \leq 0.001$***

Chlorophyll-a/chlorophyll-b ratio in the youngest fully-developed leaf was significantly higher at lower N level than in optimal conditions. Interaction between genotype and N treatment was also significant. Occitan and DKC4490 hybrids had significantly higher chlorophyll-a/chlorophyll-b ratios at reduced N level. In case of Occitan, the value was 2.81±0.04 at optimal N level, and 3.34±0.10 at reduced level. In contrast, in case of DKC 4490 1.96±0.82 was the value of chlorophyll-a/chlorophyll-b ratio at optimal N level, and 2.84±0.11 at reduced N level. Based on the results, it can be stated that there was a significantly higher N content in the shoot (Figure 3.) at the optimal N level (4.52% ±0.07) as compared to the reduced N level (2.79% ±0.11).

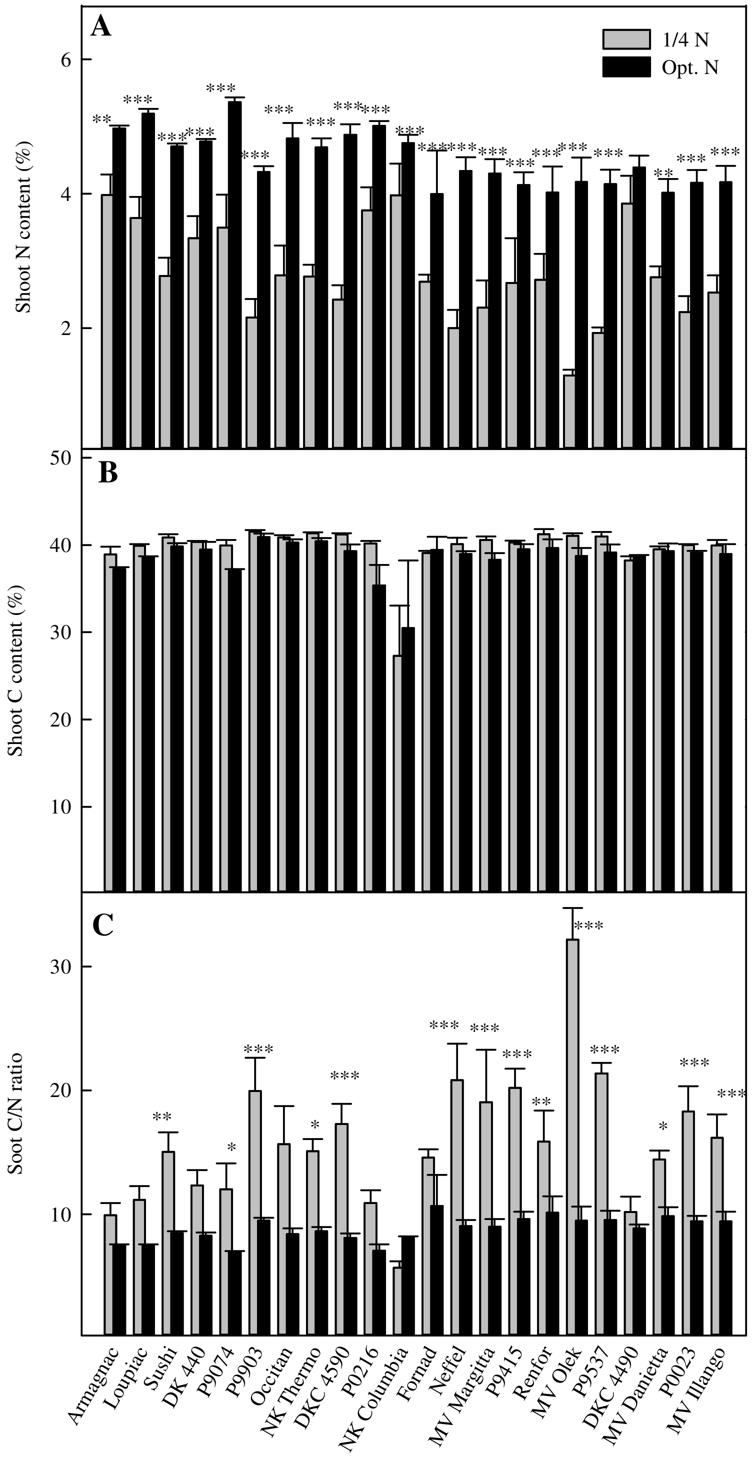

Figure 3. Changes of shoot N content (N (%)) (A) carbon content (C (%)) (B) C/N ratio (C) due to N (optimal N: opt. N, reduced N: ¼ N), in genotypes (Armagnac, Loupiac, Sushi, DK440, P9074, P9903, Occitan, NK Thermo, DKC4590, P0216, NK Columbia, Fornad, Neffel, MV Margitta, P9415, Renfor, MV Olek, P9537, DKC 4490, MV Danietta, P0023, MV Illango) n=3, ±s.e., differences between N levels: P≤0.05*, P≤0.01**, P≤0.001***

Based on the obtained results, the genotypes were grouped by Cluster analysis (Figure 4). As a result of the analysis, we distinguished 2 main groups, one of which is sensitive to N deficiency (Neffel, P9537, P9415, DK 440, P9074, DKC 4590, P9903, Occitan, NK Columbia, MVOlek, P0023), while the other group included genotypes characterized by good N use efficiency (Sushi, P0216, DKC4490, NK Thermo, MV Margitta, Loupiac, Fornad, Renfor, MV Danietta, Armagnac és MV Illango).

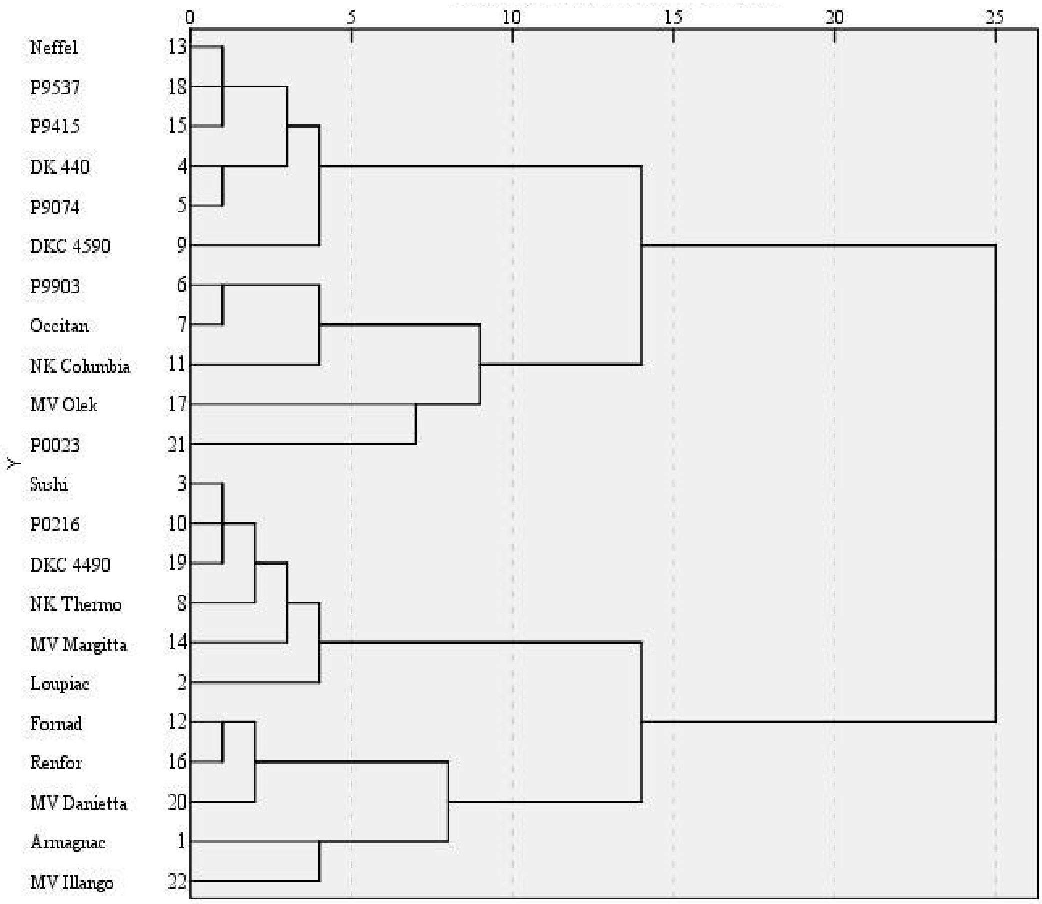

Figure 4. Results of cluster analysis based on shoot dry weight (g), root dry weight (g), shoot/root ratio, SLA (cm^2 g^{-1}), chlorophyll-a (mg ml^{-1}), chlorophyll -b (mg ml^{-1}), total chlorophyll (mg ml^{-1}), carotenoid (mg ml^{-1}), chlorophyll -a/ chlorophyll -b, and chlorophyll / carotenoid ratio, relative chlorophyll content, Fo, Fm, Fv, Fv/Fm, Fv/Fo, Fm/Fo parameters.

During the field experiments at the earliest measurement time (V7), different results were observed in the examined years. In 2018 at V7, the 160 kg ha^{-1} N significantly reduced the value of several parameters, thus higher dose of N had a negative effect on plants. For example, significantly higher SPAD values were observed at 0 (31.3%) and 80 kg ha^{-1} (22.4%) levels compared to 160 kg ha^{-1} N level. At VT phase, treatments had significant effect on several parameters. Effects of treatments on the examined parameters during the 3 years of the experiment are presented in Table 1.

Table 1. Changes of parameters measured at VT phase due to N (0 kg ha^{-1}, 80 kg ha^{-1}, 160 kg ha^{-1})) treatments and genotypes (Armagnac, Fornad, Loupiac) in 2018, 2019, 2020) n=4, ±s.e., differences between N: P≤0.05*, P≤0.01**, P≤0.001***

Parameter	2018			2019			2020		
	N	**Gen.**	**N x Gen.**	**N**	**Gen.**	**N x Gen.**	**N**	**Gen.**	**N x Gen.**
Fo	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fm	n.s.	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fv	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fv/Fm	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fv/Fo	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fm/Fo	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Ft	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	*	n.s.
ETR	n.s.	**	*	n.s.	**	n.s.	n.s.	n.s.	n.s.
Yield	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	n.s.
qP	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	***	n.s.
qN	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	n.s.
Fm'	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.	*	n.s.
SPAD	**	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Chl.-a	***	**	n.s.	***	**	n.s.	**	n.s.	n.s.
Chl.-b	***	**	n.s.	***	**	n.s.	**	n.s.	n.s.
Chlorophyll	***	**	n.s.	***	**	n.s.	**	n.s.	n.s.
Carotenoids	n.s.	*	n.s.	*	*	n.s.	***	n.s.	n.s.
Chl./Car.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Chl.-a/Chl.-b	***	*	n.s.	**	*	n.s.	**	n.s.	n.s.
SLA	***	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Measurements at the VT, R1, R2 stages showed a much clearer trend in the effect of N treatments than at V7 stage. Relative and absolute chlorophyll contents decreased significantly due to N deprivation. In contrast, differences were not found between 80 and 160 kg ha^{-1} levels. It can be stated that N demand of examined genotypes is lower than 160 kg ha^{-1}. Every year of this experiment, it was clearly shown that the chlorophyll-a/chlorophyll-b ratio increased with N deprivation. On average of the 3 experimental years, 16.7% increase at VT phase, while 17.1% increase at R1phase in chlorophyll-a/chlorophyll-b ratio were observed due to 0 kg ha^{-1} compared to 160 kg ha^{-1} N. The amount of grain yield per area had interesting values (Figure 5).

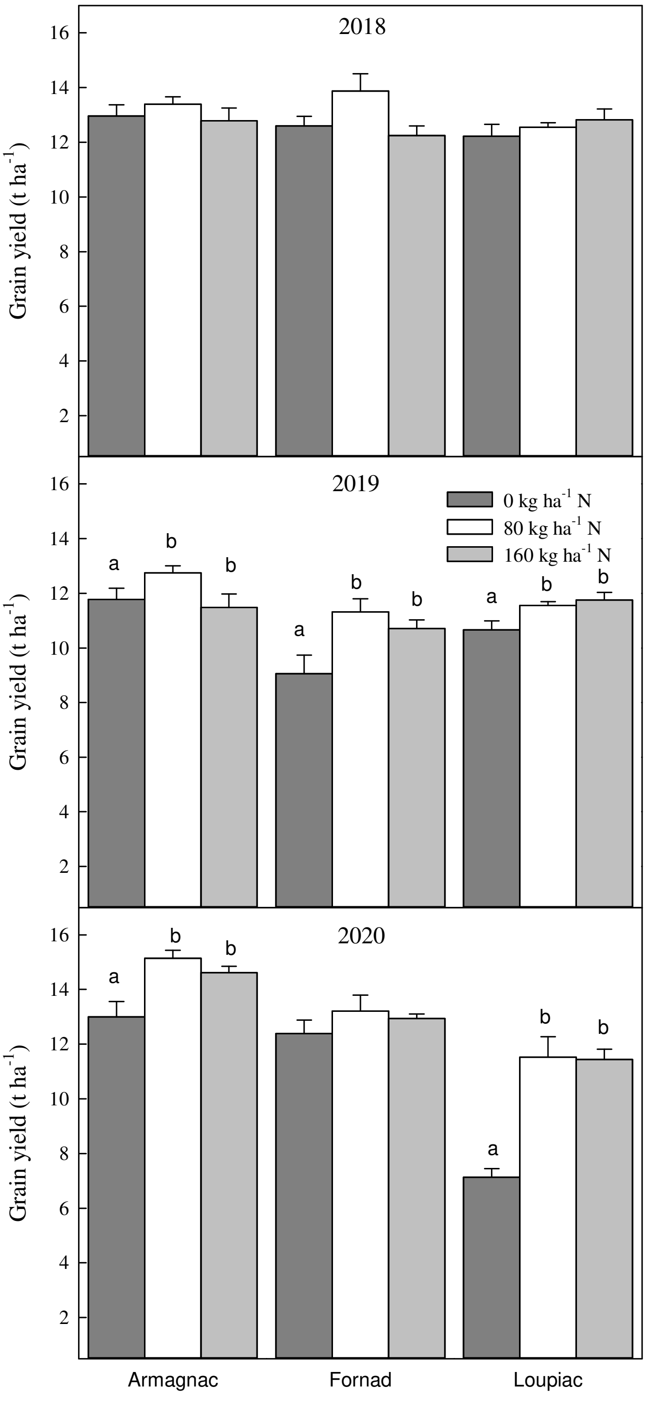

Figure 5. Changes of grain yield (t ha^{-1}) due to N (0 kg ha^{-1}, 80 kg ha^{-1}, 160 kg ha^{-1})) treatments in genotypes (Armagnac, Fornad, Loupiac) in 2018, 2019, 2020 n=4, ±s.e., differences among N levels: a,b ($P \leq 0.05$)

In the first experimental year, we could not detect a statistically significant difference between effects of genotypes or N treatments, however, the highest yield was measured at 80 kg ha^{-1} N level (13.3 t ha^{-1}±0.27). We observed that also in 2019 and 2020 as well. However, at this time the value of mentioned N level was significantly higher compared to the average of the plots without N application. It resulted in 17.9% increase in 2019 and 2020 on average. In these years on average, 160 kg ha^{-1} N level resulted in 13.7% increase compared to 0 kg ha^{-1} N level. However, statistically significant difference was not found between 80 and 160 kg ha^{-1} N levels. Moreover, at medium N dose the tendency of grain yield was higher than at the highest N level.

Different levels of Zn supply mainly affected the shoot and root dry matter and the Zn content in the organs during climate room experiments. Shoot dry weight was increased significantly (89.1%) due to higher N level but only at 5-fold Zn level in P9903 (Figure 6.).

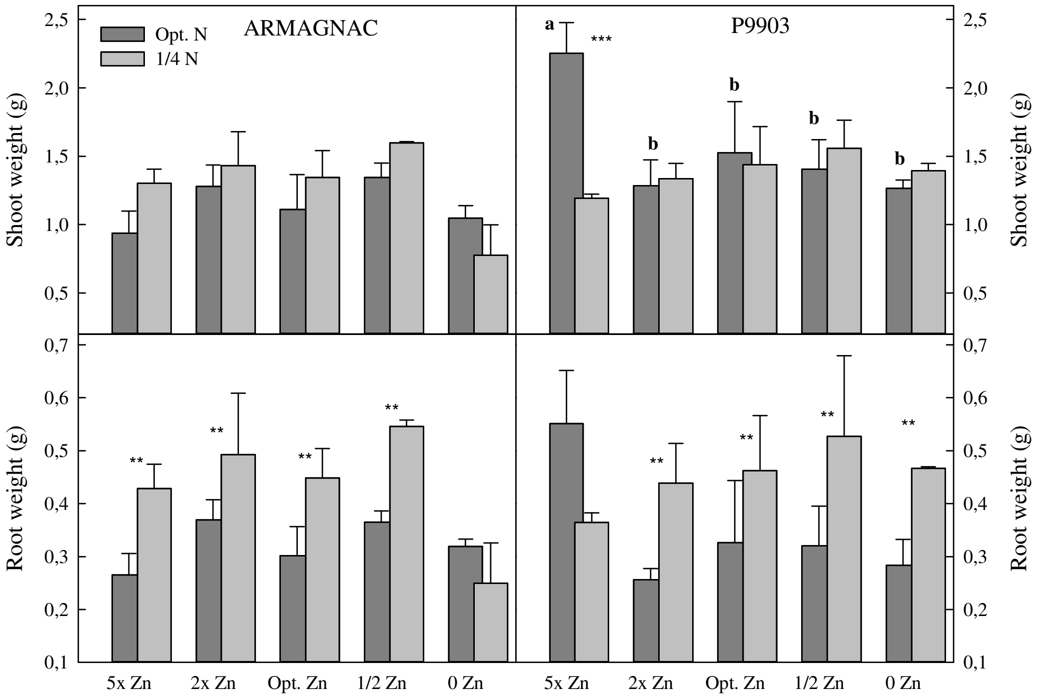

Figure 6. Changes of shoot and root dry weight (g) due to N (optimal N: opt. N, reduced N: ¼ N) and Zn (5xZn, 2xZn, Opt.Zn, 1/2 Zn, 0 Zn) treatments in genotypes (Armagnac, P9903) n=3, ±s.e., significant differences between N levels: $P \leq 0.05$*, $P \leq 0.01$**, $P \leq 0.001$***, significant differences among Zn levels: a,b, ($P \leq 0.05$)

At optimal N, the value measured at the 5-fold level of Zn was significantly higher compared to the other Zn levels ($P \leq 0.001$), which can be considered as intense nutrient reaction. The Zn content was significantly increased only at the 5-fold Zn treatment compared to other Zn levels ($P \leq 0.001$).

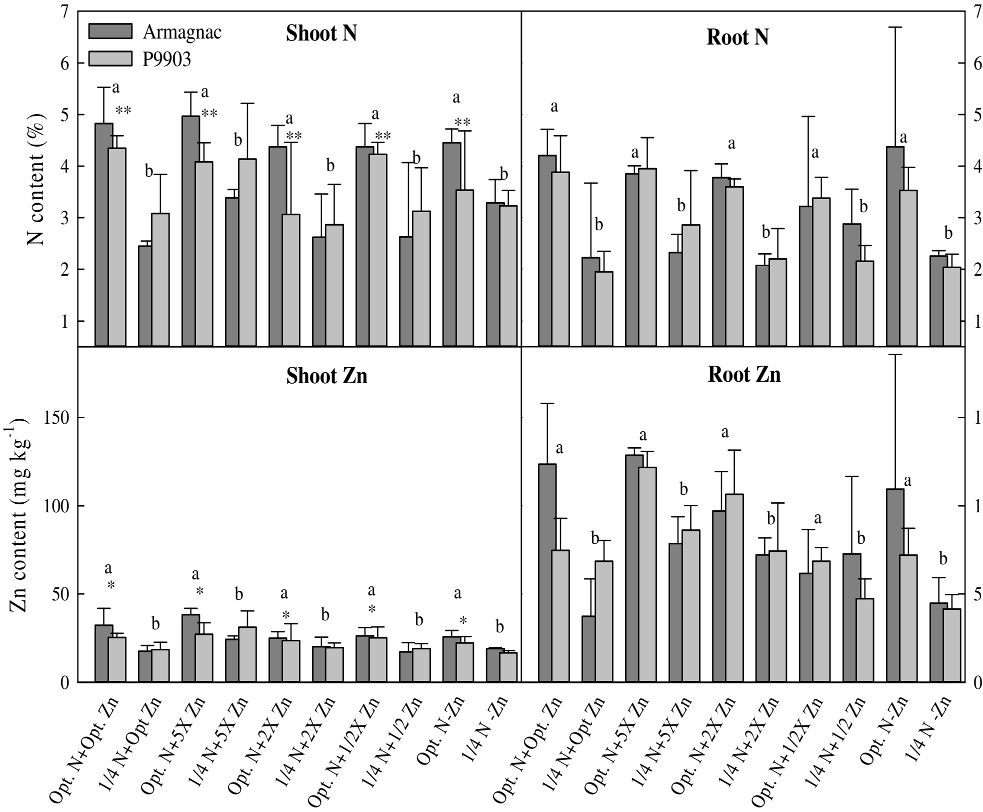

Figure 7. Changes of shoot and root N (m/m %) and Zn (mg kg^{-1}) content due to N (optimal N: opt. N, reduced N: ¼ N) and Zn (5xZn, 2xZn, Opt.Zn, 1/2 Zn, 0 Zn) treatments in genotypes (Armagnac, P9903) n=3, ±s.e., significant differences between N levels: $P \leq 0.05^*$, $P \leq 0.01^{**}$, $P \leq 0.001^{***}$, significant differences among Zn levels: a,b, ($P \leq 0.05$)

The N content of the root did not depend either on the genotype or on the Zn level. However, at the optimal N level (3.78% ± 0.16) a significantly higher N content than at the reduced N level was observed as expected. Five- and two-fold Zn levels significantly increased Zn content in the roots, but there was no difference between the two levels. Optimal N supply promoted Zn uptake. At the optimal N dose, 33.6% higher Zn content was measured in the shoot and the root on average (Figure 7.). SPAD value was significantly higher in P9903 (36.1±0.64) than in Armagnac (29.6±0.82). The effect of Zn was not significant. The effect of N deprivation could not be observed clearly in Armagnac, but P9903 was sensitive to it. Total chlorophyll content (14.8 mg ml^{-1} ±0.56) and carotenoids (3.03 mg ml^{-1} ±0.10) were higher in P9903 compared to Armagnac (Table 2.). Zn treatments did not result in phytotoxic symptoms. Higher levels of Zn were able to be taken up by plants, so it may be suitable for producing grain yield with higher biological value by translocating to the reproductive parts later.

Table 2. Changes of chlorophyll-a, chlorophyll-b, total chlorophylls, carotenoids contents and ratios of chlorophyll-a/chlorophyll-b and chlorophylls/carotenoids in the 3rd and the 5th leaves of maize plants due to N (optimal N, ¼ N) and Zn (5xZn, 2xZn, opt. Zn ½ Zn, 0 Zn) treatments in genotypes (Armagnac, P9903) n=3, ±s.e., significant differences: P≤0.05*, P≤0.01**, P≤0.001***

		Chlorophyll-a	**Chlorophyll-b**	**Chlorophyll**	**Carotenoids**	**Chl.-a/ Chl.-b**	**Chl/ Car**
3rd. leaf	**genotype**	***	***	***	***	n.s.	n.s.
	N	**	n.s.	*	n.s.	n.s.	***
	Zn	***	***	**	**	n.s.	n.s.
	genotype X N	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	genotype X Zn	n.s.	n.s.	n.s.	n.s.	*	n.s.
	N X Zn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	genotype X N X Zn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
5th leaf	**genotype**	***	***	***	***	***	n.s.
	N	n.s.	n.s.	n.s.	n.s.	n.s.	***
	Zn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	genotype X N	n.s.	n.s.	n.s.	*	n.s.	***
	genotype X Zn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	N X Zn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	genotype X N X Zn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Zn treatment was not yet performed in the early developmental stages of the field experiments, so we had the opportunity to study the N use efficiency of the selected two genotypes under field conditions in the early stages. In the first year of the experiment, none of the treatments had effect on the parameters, which can be attributed to the favourable weather conditions on the one hand, and to the favourable soil conditions on the other hand. From the second year of the experiment, the effect of reduced N supply and genotypes was detectable even in an early stage of development. Chlorophyll-a was decreased significantly due to N deprivation in P9903 (8.65%). At the second sampling date, in 2019 growing season, most of the parameters examined were not significantly affected by the applied treatments. An exception to this is the SPAD, where we found a significantly higher value in hybrid P9903 (58.8 ± 0.92) than in Armagnac (56.3 ± 0.85). At the same sampling time, similar trend was observed in 2020 growing season. Significantly higher SPAD value was observed in P9903 (61.2±0.85) compared to

Armagnac (58.7±1.04). Significant difference (11.2%) was found due to optimal N level compared to lower N level in P9903 when Zn was applied as a leaf fertilizer. In 2019 at R1 stage, significant differences were observed due to treatment only in chlorophyll fluorescence induction method parameters (Table 3.).

Table 3. Changes of Fo, Fm, Fv, Fv/Fm, Fm/Fo, Fv/Fo, Ft, ETR, Yield, qP, qN, Fm’ parameters due to N (160 kg ha^{-1}, 40 kg ha^{-1}) and Zn (without Zn, soil fertilizer, leaf fertilizer) treatments in genotypes (Armagnac, P9903) in 2019. n=3, ±s.e. significant differences: P≤0.05*, P≤0.01**, P≤0.001***

2019	Fo	Fm	Fv	Fv/Fm	Fm/Fo	Fv/Fo	Ft	ETR	Yield	qP	qN	Fm'
N	n.s.	n.s.	*	**	**	**	n.s.	n.s.	*	*	***	***
Zn	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	n.s.
Genotype	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	*
N X Genotype	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N X Zn	**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Zn X Genotype	n.s.	*	*	*	*	*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
N X Zn X Genotype	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Results in 2020 were different. Chlorophyll-a (4.42%), chlorophyll-b (10.5%) and total chlorophyll (6.38%) were decreased significantly due to N deprivation. Significantly higher carotenoids content was observed in P9903 hybrid with Zn applied as soil fertilizer (4.12±0.14 mg ml^{-1}) compared to Armagnac (3.45±0.22 mg ml^{-1}). Chlorophyll-a/chlorophyll-b ratio was significantly higher with N deprivation (2.31±0.07) compared to optimal N (2.12±0.04). SPAD value was significantly higher (6.77%) in case of P9903. None of the treatments caused any statistically significant differences in fluorescence parameters in 2020 in R1 phase. In 2019, significantly higher (+2.5 t ha^{-1}) grain yield was obtained at optimal N level as expected (Figure 8.). Significant differences were not found in 2020. The experiment proved the differences between genotypes. This supports the previous experiments’ conclusions. Effects of Zn treatments were not remarkable in the investigated parameters. Although we found interaction between Zn and N, and between Zn and genotypes in several cases, yet this did not show a clear tendency.

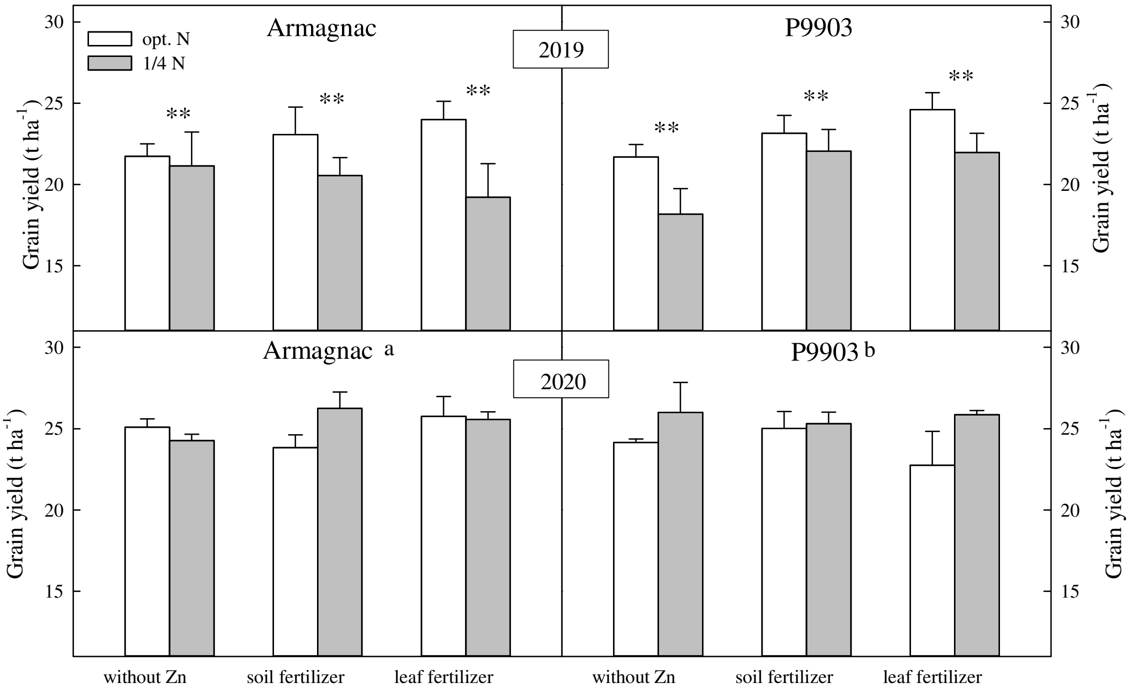

Figure 8. Changes of grain yield (t ha^{-1}) at R6 due to N ((opt. N: 160 kg ha^{-1}), 1/4 N (N adag: 40 kg ha^{-1})) and Zn (without Zn, soil fertilizer, leaf fertilizer) treatments in genotypes (Armagnac, P9903) in two experimental years (2019, 2020) n=3, ±s.e., differences between N levels: $P \leq 0.05$*, $P \leq 0.01$**, $P \leq 0.001$***, differences between genotypes: a,b ($P \leq 0.05$)

The summary of our results clearly supported the importance of genotypes as a factor in nutrient management in crop production systems. Maize genotypes with favourable N use efficiency may play an important role in intensive and extensive field crop production in the future. Our research provides fundamental research results for applied research as well as for plant breeders. Furthermore, our research takes into account the demands for crop production due to climate change and global food problems. Primarily, we wanted to identify *in vivo* and *in situ* parameters which could be direct and indirect tools for precision agriculture techniques.

4. NEW SCIENTIFIC RESULTS

1. Parameters examined in our laboratory experiment were able to characterize nitrogen use efficiency of examined genotypes. Among the parameters, the shoot dry mass decreased by an average of 30.5% due to N deprivation. An increase in the SLA value clearly indicated the symptoms of N deprivation, while the SPAD value decreased significantly ($P<0.001$), and the parameter changed differently on the different examined leaves. Total chlorophyll content also decreased significantly (42.8%) in plants treated with a low N dose. The parameter system used by us can be used to characterize the nitrogen utilization of a given genotype, which is supported by our field results.
2. Examined genotypes were classified to “sensitive to N deprivation” and “efficient N usage” groups. Neffel, P9537, P9415, DK 440, P9074, DKC 4590, P9903, Occitan, NK Columbia, MV Olek and P0023 genotypes belong to sensitive group. While Sushi, P0216, DKC4490, NK Thermo, MV Margitta, Loupiac, Fornad, Renfor, MV Danietta, Armagnac and MV Illango belong to the efficient group. Although maize hybrids used in the field N utilization experiment, respond differently to our treatments in several plant physiological parameters but we did not discover a clearly interpretable tendency in the responses of the genotypes for reduced N application. The three investigated hybrids (Armagnac, Fornad, Loupiac) belonged to less sensitive for N deficiency group, so it could be predicted that there would be less difference between them. In arable land conditions, I recommend setting up experiments lasting longer than the examined three-year period, under different agroecological conditions, thereby facilitating the drawing of accurate conclusions regarding the N utilization of the genotypes.
3. Zn had no significant effect on N uptake of the examined genotypes characterized with different N use efficiency, however, uptake of Zn was improved by optimal N level. The shoot contained 6.8 mg (+ 33.5%) more Zn per kilogram, while for the root this difference was close to 34 mg (+ 54.5%).
4. Zn levels in controlled experiment did not cause significant effect on plant physiological processes of examined genotypes at the early development stage, but plants were able to absorb higher rate of Zn. This was proven by the Zn content analysis of shoot and root. The 5-fold Zn level resulted in +27.7 mg more Zn in root and +6.8 mg more Zn in Soot compared to control Zn level. Thus, excess Zn can affect quantitative and qualitative parameters of grain yield at later stages of

development. Examined parameters were not affected by Zn applied as leaf or soil fertilizer under field conditions in soils with optimal Zn content. In the first year of N-Zn interaction field experiment, several parameters decreased due to N deprivation. P9903 was more sensitive to N deficiency than Armagnac at vegetative stages. Grain yield decreased due to N deprivation in both experimental years, however, statistically significant effect was found only in the first year, where 11% (2.5 t ha^{-1}) yield loss was caused by N deprivation.

5. In three years of field experiments in case of Armagnac Fornad and Loupiac genotypes, 0 kg ha^{-1} N had adverse impact on plant physiological parameters and on grain yield as well 11.9% and 8.13% yield losses were observed compared to 80 and 160 kg ha^{-1} N levels. However, significant difference was not found between 80 and 160 kg ha^{-1} N levels. Therefore, it can be concluded that the investigated genotypes can achieve 12 t ha^{-1} grain yield in the mentioned conditions with the application of 80 kg ha^{-1} N.
6. Chlorophyll-a/chlorophyll-b ratio was increased due to N deprivation in field experiments as well. Chlorophyll-b was more sensitive to N deprivation than chlorophyll-a.

5. PRACTICAL USABILITY OF RESULTS

1. One of the main conclusions of our experiments is that chlorophyll-b is more sensitive to N deprivation than chlorophyll-a. Most of the chlorophyll measuring devices detect an average decrease in green colour. According to our results, a device that detects changes in the colour range of chlorophyll-b would be more suitable to detect N deficiency. We could detect the possible N deficiency more efficiently earlier, thus allowing the necessary agrotechnical interventions to be carried out. Instrumental, non-destructive measurement of chlorophyll-b content can be a useful and a very important parameter for precision agriculture.
2. Plant physiological and plant morphological parameters in the controlled experiments were suitable to characterize N use efficiency of maize.
3. Plant physiological parameters were not affected by excessive Zn levels under field or laboratory conditions. However, our results suggest that up to 5-fold Zn was accumulated by experimental plants. The application of 3% ZnSO4 in a dose of 4 l ha^{-1} could have a positive effect on the grain quality and did not cause a significant economic burden if applied in combination with other agrotechnical interventions like weed control.
4. Abandonment of N fertilization resulted in a significant reduction in most examined parameters, so, omission of this operation is not recommended. However, grain yield did not differ significantly between 80 and 160 kg ha^{-1} N levels in the examined genotypes. Application of higher levels than 80-100 kg ha^{-1} N is not recommended in favourable soil conditions and in case of genotypes with optimal N use efficiency. The N application beyond the mentioned N level contains significant economic and ecological risks due to increasing frequency of extreme weather conditions and the increasing cost of input materials.

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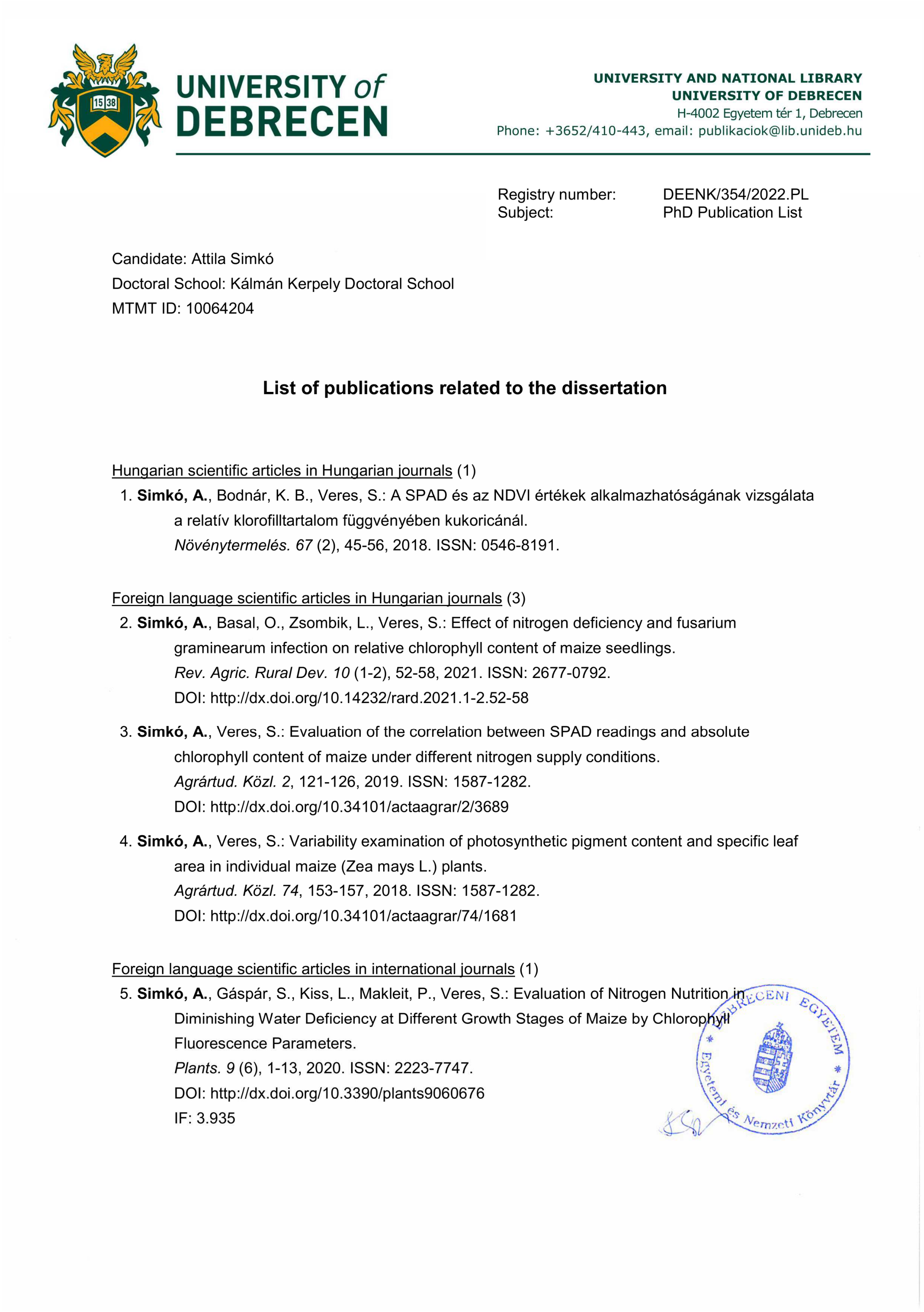
7. PUBLICATION LIST



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List of publications related to the dissertation

Hungarian scientific articles in Hungarian journals (1)

1. **Simkó, A.**, Bodnár, K. B., Veres, S.: A SPAD és az NDVI értékek alkalmazhatóságának vizsgálata a relatív klorofilltartalom függvényében kukoricánál.
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