Theses for doctoral dissertation (PhD)

EXAMINATION AND EVALUATION OF THE MAIN VALUE INDICATORS OF MAIZE HYBRIDS IN LONG-TERM FIELD EXPERIMENTS

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1. INTRODUCTION

Today, increasing the productivity of crop production is essential to feed a growing population, but the scope for increasing the area under cultivation is limited. We need to produce the food, feed and industrial raw materials needed to meet the needs of a growing population on a constantly reducing available land.

The number of fodder maize hybrids available for farmers in Hungary and the EU exceeds 400. However, it is difficult for farmers to find their way around the large number of different hybrids. Each hybrid differs in their abiotic and biotic resistance and the length of the growing season and the related physiological parameters are less well known.

The environment for agricultural production is in a dynamic change. Increases in the cost of inputs and decreases in available land have led to an increase in the value of produced crops. The primary challenge for farmers is to apply a well thought-out management system, to meet the needs of hybrids and to make optimal use of inputs. An ever-expanding range of hybrids is available to farmers (*Nagy,* 2021). Precision farming and technological advances enable and require more efficient ways of meeting the needs of arable crops. Reaching this objective requires knowledge of the genetic parameters of the plants/varieties/hybrids and their responses to different environmental stresses (*Nagy et al.* 2020). Site-specific crop management should be implemented in a way that is optimally adapted to production objectives and ecological conditions. Based on their studies, *Széles et al*. (2019) suggest that the efficiency of fertiliser use should be determined for each hybrid used in cultivation.

Farmers face a difficult decision when choosing a variety/hybrid because of the large number of varieties and the lack of information to help them determine their stress tolerance values for a given growing area, soil type and climatic conditions. In many cases, professional considerations are overshadowed and influenced by marketing activities, leading to the selection of genotypes that are not suited to the specific area, soil or climatic conditions, and thus to a significant reduction in the success and quality of production, as well as yield safety.

The genetic potential and stress tolerance value varies between hybrids. In different crop years, and in particular in unfavourable crop years, the variation resulting from tolerance is larger than in an average crop year.

The frequency of extremes in the weather is increasing. Reducing the amount and distribution of rainfall during the growing season is a major challenge for farmers. The winter refill of soils is decreasing, which increases the role of growing season rainfall, but its rate is often insufficient and does not come at the phenologically significant time. The weather parameters measured during the growing season tend towards extremes, and the choice of a suitable hybrid for the growing area and climatic conditions to compensate for this phenomenon is a major factor in successful crop production.

Nowadays, maize cultivation is specialised, with the genotype often determined by industry or the consumer market. Criteria such as the nutritional value of the crop for industrial or other human uses, or the choice of genotype for economic reasons linked to grain moisture at harvest, thus increasing cost-proportional profitability, are becoming more important. The production of starch and isoglucose will be promoted as a low-cost alternative to beverages and foods containing refined sugar.

Objectives of the research

My aim is to determine the most sensitive phenological periods for crop production by means of enzyme activities and stress factors measured at the sampling times in field experiments with the hybrids under study, and to examine the plant physiological and yield changes in different hybrids in response to different amounts and rates of macroelement fertilisation. Based on the obtained results, my aim is also to provide farmers with recommendations on fertiliser rates and dosages and to draw attention to the technologically important phenological stages. I hypothesised that abiotic and biotic stress factors affecting the crop at important times and periods will largely determine crop success, including yield, quality and yield safety.

2. MATERIAL AND METHODS

2.1 Location and basic parameters of the long-term experiment

Two forage maize (*Zea mays* L.) hybrids of different maturity groups were used as experimental plants. The two hybrids have different FAO number and maturity group: hybrid 1 - FAO 360 (characterised by high yield, excellent water release, stress tolerance, yield safety, wide yield optimum, favourable fertiliser response); hybrid 2 - FAO 490 (characterised by high yield, good water release, stress tolerance, yield safety, excellent vigour and fertiliser response).

The experiments were carried out at the University of Debrecen, Institutes for Agricultural Research and Educational Farm, Debrecen Edcuational Farm and Landscape Research Institute (DTTI), Látókép Crop Production Experiment Site (47° 83, 030" N, 21° 82, 060" E, 111 m a.s.l.). The experimental area is an excellent site for field crop production, with appropriate agrotechnical, biological and soil conditions.

The field experiment that served as the basis for my research was established in 1983 by Prof. Dr. János Nagy, and has been continued for 39 years with the same parameters, nutrient replenishment system, site, tillage and agrotechnology. The total area of the experiment is more than 1.3 ha, with 1248 plots. The agro-technical operations of the experiment consist of a series of operations adapted to a conventional farming model based on tillage, in this case ploughing. Since its inception, the experiment has been conducted in monoculture.

Five replications were used for leaf sampling, with the fifth replication randomly selected from the 4-replication plot design. I used Microsoft Excel for performing random selection.

For maize, I have studied five major phenological stages – 4-leaf (V_4), 6-leaf (V_6), 8-leaf (V_8), 14-leaf (V_{14}) and silking (R_1). The heat amounts differed in the two examined years. At the V_4 phenological stage, the examined hybrids required less heat sum in the 2020 growing year and reached the 4-leaf phenological stage earlier. These dynamics were similar throughout the growing season. In the growing year 2020, the plant population developed faster and less heat sum was sufficient to reach the respective phenological stages. The actual maturity date of the black layer formation in the growing year 2020 is 03.09.2020, while it was reached later in 2022, on 11.09.

The first three samples were taken from the 4th fully developed leaf, the 4th sample from the 10th fully developed leaf, and the last sample from the leaf opposite the ear.

2.2 Agrotechnical parameters

	Nitrogen doses with high phosphorus and potassium levels				Nitrogen doses with proportional phosphorus and potassium levels		
(kg/ha)	**N**	**P_2O_5**	**K_2O**	**(kg/ha)**	**N**	**P_2O_5**	**K_2O**
N 0	0	0	0	**NPK 0**	0	0	0
N 2	120	184	216	**NPK 2**	60	46	54
N 5	300	184	216	**NPK 5**	150	115	135

Table 1. Nutrient supplementation levels used in the experiment in active ingredient amounts per macro-element

In the first part of the experiment (first experiment), the effect of nitrogen was tested at constant high levels of 0-300 kg/ha (N dose experiment) ((control N0, P_2O_5 0 and K_2O 0: N2 120 kg/ha, N, P_2O_5 (184 kg ha^{-1}) and K_2O (216 kg ha^{-1}): N5 300 kg/ha N - provocative fertiliser levels) P_2O_5 (184 kg/ha) and K_2O (216 kg/ha)). Fertiliser levels marked N5 are not practically applicable, they are specifically applied as experimental provocative levels of nitrogen, which do not comply with modern principles of reduced input and fertiliser use.

The second part (second experiment) measured the optimum nitrogen:phosphorus:potassium (NPK) ratio for maize (control N:0, P:0, K:0; NPK 2 - 60 kg/ha N, 46 kg/ha P_2O_5 , 54 kg/ha K_2O; NPK 5 150 kg/ha N, 115 kg/ha P_2O_5 , 135 kg/ha K_2O), as well as the negative control without fertiliser supplementation. Phosphorus and potassium fertilisers were applied before winter ploughing and nitrogen fertiliser in spring before sowing.

In the first part of the experiment, we provided constant high levels of phosphorus and potassium to the plants independently of nutrient levels, with no inhibitory factors and no nutrient antagonism for uptake. In the second half of the experiment, where we increased phosphorus and potassium levels in proportion to nitrogen, a fertiliser dose close to practical management could be tested (Table 1).

2.3 Lipid peroxidation measurement and sample handling protocol

The lipid peroxidation value was calculated from the amount of malondialdehyde (MDA) with an extinction coefficient of 155 mM^{-1} cm^{-1} , determined as the fresh weight of MDA in μmol*g^{-1} FW (Fresh Weight) as described by *Heat - Packer* (1968).

To measure lipid peroxidation (LP), I used 100 mg veinless leaf samples. The samples were rubbed in a grinder with 0.25% concentrated thiobarbituric acid (TBA) and 10% concentrated trichloroacetic acid (TCA) with the addition of liquid nitrogen, and centrifuged at 10 800 rpm for 25 min at 4 °C using an Esco Versati MCR-88-8 refrigerated benchtop centrifuge. The supernatant was pipetted and a 200 µl plant sample was obtained. I mixed 800 µl with 0.5% TBA and 20% TCA and placed in a Julabo Pura 10 laboratory water bath at 95 °C for 30 min in a hermetically sealed Eppendorf freezer tube. The samples were placed on ice and then centrifuged at 10 800 rpm for 10 min at 4 °C in preparation for measurement and the supernatant was pipetted. Absorbance was measured at 532 nm using a Thermo Scientific Evolution 350 UV-Vis spectrophotometer. The blank sample was measured at 600 nm.

Calculation formula: MDA concentration (mM) = (ABS 532 - ABS 600) / 155

2.4 Enzyme tests

2.4.1 Ascorbate peroxidase (APX) measurement and sample handling protocol

The activity of ascorbate peroxidase (APX) was determined by the rate of decrease in absorbance. APX was characterised by the decrease in absorbance measured at 290 nm over 60 s according to the method of *Nakano - Asada* (1981) with modifications by *Janda et al.* (1999). During sample processing, 500 mg of plant sample was rubbed together in a mortar with isolating buffer. For the rubbing, I used liquid nitrogen and 0.02 g of 0.1-0.4 mm fraction size quartz sand of laboratory purity to achieve adequate homogeneity following the guidelines of *Illés et al.* (2021). As a next step, I centrifuged the sample at 2 °C for 20 min and stored on an ice bed until measurement. During the measurement, 2 ml of Tris-HCl solution and 100 µl of ascorbate solution were added to 50 µl of plant sample. The 60 s absorbance decrease of the previous mixture was determined using a Thermo Scientific Evolution 350 UV-Vis spectrophotometer, and the 60 s decrease was measured by adding 100 µl $H_2 O_2$ to the same mixture. As a next step, I calculated the difference between the two values.

Ascorbate peroxidase (APX) is calculated as: enzyme activity (units/L) = (ΔABS × total test volume) / (Δt x ε x l x enzyme sample volume).

The extinction coefficient = ε of the substrates in M^{-1} cm^{-1} units and l is the diameter of the cell in cm. Enzyme activity (units) was defined as the amount of enzyme that oxidized 1 µmol of substrate per minute.

2.4.2 Superoxide dismutase (SOD) measurement and sample handling protocol

My study was based on the protocols of *Giannopolitis et al.* 1977 and *Beyer - Fridovic* 1989. Superoxide dismutase (SOD) activity was measured using 400 mg veinless leaf sample. Samples were homogenised with a buffer containing K_2HPO_4, polyvinylpyrrolidone (PVP), phenylmethylsulphonyl fluoride (PMSF) and ethylenediaminetetraacetic acid (EDTA) in a mortar using liquid nitrogen. The pH of the buffer was adjusted to 7.8 with phosphoric acid using a VWR 1100 L pH meter. The resulting mixture was centrifuged at 10 000 rpm for 14 min at 4 °C and the supernatant was pipetted. The absorbance was measured with a spectrophotometer at 560 nm. For the measurement, 2,675 ml of buffer solution, 250 µl of methionine, 25 µl of riboflavin, 25 µl of nitro-blue tetrazolium chloride (NBT) were added to a reaction tube, and finally 25 µl of plant sample. I then added the NBT to the blank sample, which had a combined volume of 2.7 ml. To the reaction mixture, which also contained the sample, I added NBT every minute to the SOD tube. Then, the samples were measured with a Thermo Scientific Evolution 350 UV-Vis spectrophotometer in the specified order every minute after 20 minutes. For spectrophotometer measurements, I used a quartz cell. The samples were measured in 3 replicates.

2.4.3 Yield composition analysis with a NIR apparatus

In addition to the yield measurement, starch, oil, protein, moisture content and v/v mass were determined using a FOSS Infratec calibrated grain analyser. Samples were measured using 1 kg of homogenised crop samples taken immediately after harvesting. The instrument weighed the sample one by one, divided into 10 sub-units, and averaged the results. Near-infrared (NIR) spectroscopy offers a non-destructive method for the determination of starch, oil, protein, moisture content and volume/volume weight (*Janni et al. 2008*).

3. RESULTS

3.1 Dynamics analysis for different phenological stages

Enzymes and lipid peroxidation rates tested at different phenological stages varied across nutrient levels, hybrids and averaged over cropping years. The activity of APX increases steadily as the phenological stages progress. V_4 increases from 9.35 units at phenological stage to 12.99 units at R_1 phenological stage. The activity of lipid peroxidation and SOD gradually decreased during the phenological stages.

In the 2019 cropping year, APX activity increased from V_4 phenological stage to V_6 phenological stage by 0.5 units, and then decreased by 3.1 units by the V_8 phenological stage. When analysing hybrids separately, I also observed different dynamics between cropping years in the average nutrient levels. APX activity had similar values at V_4 and V_6 phenological stage, and then decreased by 2.9 units at the V_8 phenological stage. After the phenological stage V_8, APX activity increased gradually until the phenological stage R_1. In the R_1 phenological stage, the APX activity was 13.38. The rate of lipid peroxidation and SOD activity of the FAO 360 hybrid resulted in a constant value averaged over the nutrient levels in the 2019 cropping year. At the end of the growing season, LP rate increased slightly and SOD activity decreased. A similar result was obtained in the studies of *Rácz* et al. 2021, where SOD activity decreased in both the nitrogen-containing top dressing and the control treatment as the phenological stage progressed between phenophases V12 and R1. In the 2019 growing season, the activity of APX in hybrid FAO 490 increased by 0.5 units from phenological stage V_4 to phenological stage V_6 and decreased by 3 units by the phenological stage V_8. Towards the end of the growing season, the APX activity increased to 12.91 units in R_1. The rate of lipid peroxidation and the dynamics of SOD activity differed from the phenological stage V_{14} to the phenological stage R_1 towards the end of the growing season. The rate of lipid peroxidation increased and the SOD activity decreased by the phenological stage R_1.

In the 2020 cropping year, APX activity gradually decreased, which can be attributed to the agrometeorological environment in the different growing seasons. The activity of APX decreased by more than 20% in the R_1 phenological stage compared to the V_4 phenological stage. In the case of the FAO 360 hybrid, the activity of APX decreased continuously throughout the growing season averaged over the nutrient levels in the 2020 cropping year.

Initially, it had a value of 15.4 units in the phenological stage V_4 and then gradually decreased. The dynamics of lipid peroxidation is not balanced in the 2020 cropping year for hybrid FAO 360, averaged over the different nutrient levels - it decreased and increased between sampling dates. At the end of the growing season, this value decreased similarly to APX and lipid peroxidation.

The activity of the FAO 490 hybrid APX showed opposite dynamics compared to the 2019 cropping year. APX activity started from a high level and then gradually decreased in average nutrient levels as the growing season progressed. Overall, SOD activity and lipid peroxidation rates showed a decreasing trend as the growing season progressed. From the beginning of the growing season, LP increased by 2 units from the V_4 to the V_6 phenological stage, and then decreased in a similar way to SOD activity by the V_8 phenological stage. LP rate and SOD activity also increased by the phenological stage V_{14} and decreased at the end of the growing season by the phenological stage R_1.

3.2 Analysis of physiological changes by nutrient level

During the performed analyses, the physiological parameters varied with hybrid, sampling date, crop year and nutrient levels. Research by *Krantev et al.* 2008 suggests that physiological parameters (MDA levels, APX activity) may vary in response to abiotic stress.

In the two long-term experiments used for my study, the different nutrient treatments needed to be evaluated separately. My study can be divided into two parts. In the first part I evaluate the effect of nitrogen levels at constant high phosphorus and potassium levels, in the second part I investigate the effect of a proportional NPK dose. The results are analysed in terms of sampling dates, nutrient treatments and hybrids.

In the 2019 cropping year, APX activity of the FAO 360 hybrid in the V_4 phenological stage increased at the N2 level with increasing nitrogen dose compared to the control. The N5 nitrogen dose slightly decreased APX activity compared to the control. The change in APX activity of the hybrid FAO 490 in response to nutrient treatments at the four-leaf phenological stage was similar to that of hybrid FAO 360. LP and SOD were similar for the two hybrids and did not change with increasing nutrient levels. In many cases, analysis of the means of the results masked the true differences and they were not detectable.

There was no difference in APX activity and lipid peroxidation between NPK 2 and NPK 5 nutrient levels in response to proportional NPK supply. SOD activity showed a slight increase with increasing proportional NPK levels of nutrients in the FAO 360 hybrid at the four-leaf phenological stage. The activity of APX increased slightly by 26% compared to the control with increasing nutrient levels in response to proportional NPK supply in the FAO 490 hybrid at the four-leaf phenological stage. Similar results were obtained by *Kaya et al.* 2020 in their studies where the amount of ROS, antioxidant compounds, increased under nutrient stress. In the six-leaf phenological stage, the activity of APX in the FAO 360 hybrid remained unchanged, but it decreased by 32% at the N2 nutrient level compared to the control. The rate of lipid peroxidation and SOD activity decreased proportionally with increasing nutrient level compared to the control. At the six-leaf phenological stage, APX activity measured in hybrid FAO 490 was different compared to hybrid FAO 360. In hybrid FAO 490, APX activity was higher at the control and high N5 nutrient levels than at the N2 nutrient level. The difference was 13 % higher compared to control and 11 % higher compared to N5. The lipid peroxidation rate and SOD activity were unchanged in the FAO 490 hybrid at the V_6 phenological stage. At the V_6 phenological stage, APX activity was higher than that of hybrid FAO 490 at NPK 2 levels similar to those of hybrid FAO 360. Compared to the control, APX activity was 68% higher at the NPK 2 nutrient level and 44 % higher at the NPK 5 nutrient level.

In the V_8 phenological stage, no significant change in APX activity was observed in the FAO 360 hybrid at constant high P and K levels compensated by increasing nitrogen levels. Lipid peroxidation rates increased slightly at N5 nutrient levels compared to the control. APX activity and lipid peroxidation rates measured in the hybrid FAO 490 increased (between nutrient levels N0 and N5) with increasing nutrient levels. SOD activity decreased slightly with increasing N levels.

In the V_8 phenological stage, there was no significant difference in APX activity and lipid peroxidation rates in response to proportional NPK supplementation in both examined hybrids. SOD activity decreased slightly with increasing nutrient levels. In the fourteen-leaf phenological stage, the APX activity of the FAO 490 hybrid measured at the N5 nutrient level decreased significantly by 21 % compared to the control and by 48 % compared to the N2 nutrient level. At the V_{14} phenological stage, the activity of APX increased gradually with increasing nutrient levels as a result of equal NPK ratio. The rate of lipid peroxidation was highest at the NPK 2 nutrient level and lowest in the control plot with 2,874 units. In the 2019 cropping year, the activity of APX increased gradually between sampling dates for all tested parameters, averaged across hybrids and nutrient levels. In the V_{14} phenological stage, much higher values were observed for hybrid FAO 490 compared to the APX activity measured for hybrid FAO 360. In comparison with the control population, more than 40% higher APX was measured than in hybrid FAO 490 with a value of 8.063. Consequently, at the V_{14} phenological stage, there was a significant physiological difference between the two hybrids as a function of nutrient levels. In the R_1 phenological period, the APX activity value of the FAO 360 hybrid showed a 50% increase compared to the control stand at N2 nutrient level. In addition, the lipid peroxidation rate and SOD activity did not change significantly. In the R_1 phenological stage, the APX activity of the FAO 490 hybrid was 76% higher compared to N2 and 60% higher compared to N5 in the control stand, similar to the FAO 360 hybrid. The rate of lipid peroxidation decreased slightly by 0.973 units at the N2 nutrient level compared to the control. Similar results were obtained in the studies of *Zhang et al.* 2007, where LP levels decreased with increasing nitrogen levels under water stress. They showed that the plant successfully compensated for the stress effect by reducing LP levels.

In the 2020 cropping year, the APX activity of the samples collected in the V_4 phenological stage was several times higher than the results obtained in the 2019 cropping year. The phenological stage V_4 had and higher temperature during the sampling period compared to the multi-year average, therefore; the the sum of temperatures associated with each phenological stage during the growing season was also higher. In the V_4 phenological stage, the activity of APX increased slightly by 1.2 units at the NPK 2 nutrient level compared to the control. The APX activity measured in the control population was the same as that measured at the NPK 5 nutrient level. Lipid peroxidation rates were low for all examined nutrient levels. SOD activity was nearly identical for the control and the two nutrient levels examined for the FAO 490 hybrid. In the V_6 phenological stage, APX activity increased gradually with increasing nutrient levels. The N2 nutrient level increased by 21 % compared to the control and the N5 nutrient level increased by 22% compared to the control. The rate of lipid peroxidation also increased with increasing nutrient levels compared to the control. The SOD activity varied between 4.055 and 4.501 in the V_6 phenological stage of the FAO 360 hybrid in the 2020 growing season, regardless of nutrient level. In the V_6 phenological stage, the activity of APX decreased by 2.3 units at the NPK 2 nutrient level compared to the control with equal rate of NPK nutrient supplementation. The APX activity at the NPK 5 nutrient level was 72% higher than the control stock in the FAO 360 hybrid. At the V_6 phenological stage, the activity of APX did not increase at NPK 5 compared to NPK 2. This is in contrast to the results obtained in case of the short maturity hybrid. The rate of lipid peroxidation was 2 units higher at the NPK 5 nutrient level compared to the control. In the V_8 phenological stage, the activity of APX in the FAO 360 hybrid was 3.4 units higher at N2 and 4.8 units higher at N5 compared to the control. The lipid peroxidation rate slightly increased by 0.9 units at the N5 nutrient level compared to the control. In hybrid FAO 490, the activity of APX was the same at the control and the N5 nutrient levels, but 3 units lower at the N2 nutrient level compared to the control and the N5 nutrient level. At the V_8 phenological stage, APX activity was different compared to the 2019 growing season. In the 2020 growing season, APX activity was higher. Compared to the control, APX activity increased gradually with increasing nutrient levels. The activity of APX was 1.8 units higher at NPK 2 and 5.3 units higher at NPK 5 nutrient levels compared to the control population. The rate of lipid peroxidation did not change with increasing nutrient levels, but there was a slight increase in SOD activity. A similar trend was measured for the FAO 490 hybrid. At the V_{14} phenological stage, APX activity increased with increasing nitrogen levels in hybrid FAO 360. Compared to the control, the amount of increase was 2.3 units at the N2 nutrient level and 6.4 units at the N5 nutrient level. The rate of lipid peroxidation also increased with increasing nutrient levels, by 0.5 units at the N2 nutrient level and by 2.6 units at the N5 nutrient level compared to the control. SOD activity decreased by 0.6 units. At the V_{14} phenological stage, the activity of APX increased with increasing nutrient levels in the 2020 growing season for the FAO 490 hybrid. The amount of increase was 1 unit at the N2 nutrient level compared to the control, and 5.2 units at the N5 nutrient level. Lipid peroxidation rate increased by 2 units compared to the control at the N5 nutrient level. SOD activity was the same at the N0 and N2 nutrient levels and decreased by 1.2 units at the N5 nutrient level compared to the control. At the V_{14} phenological stage, proportional NPK supply resulted in a significant increase in APX activity with increasing nutrient levels. At the NPK 2 nutrient level, APX activity decreased by 2.1 units and at the NPK 5 nutrient level by 7.7 units compared to the control. In the 2020 growing season, at the R_1 phenological stage, APX activity decreased slightly with increasing nitrogen levels at constant high phosphorus and potassium levels. The rate of lipid peroxidation increased with increasing nutrient levels. N2 nutrient levels increased by 0.6 units and N5 nutrient levels increased by 2.2 units compared to the control. The lipid peroxidation rate increased significantly at the end of the growing season with increasing NPK levels in equal proportions in the FAO 490 hybrid. At the NPK 2 nutrient level, lipid peroxidation increased by 1.3 units and at the NPK 5 nutrient level by 2.6 units compared to the control.

3.3 Analyses of variance

Phenological stages had a significant effect on the extent of lipid peroxidation. The combined analysis of the different hybrids and phenological stages had no effect on the lipid peroxidation rate, but adding fertiliser levels to the examined factors, they have a significant effect on the lipid peroxidation rate. Phenological stage and hybrid did not have an effect on APX activity either as a separate factor or together. Fertiliser levels alone and together with phenological stage had a significant effect on APX activity, but no real difference was found when hybrid and phenological stage were added. Lipid peroxidation rates were different at all time points relative to each other. There was little agreement between the V_6 and V_8 phenological stages. The rate of lipid peroxidation at R_1 was also different relative to all phenological stages.
SOD activity was identical in the V_8 and V_{14} phenological stages. The dates of V_4 , V_6 and R_1 were significantly different. When analysed by nutrient level, the two highest nutrient levels N5 and NPK 5 clearly affected APX activity. N0, N2, NPK 0 and NPK 2 did not result in significant changes when averaged across the examined growing years and hybrids. Also, when lipid peroxidation rates were examined, N5 and NPK 5 resulted in a different group for each nutrient level, and control plots were significantly different for each examined fertiliser level. When SOD activity was analysed in plots with constant high phosphorus and potassium levels, N2 nutrient levels did not result in significant changes averaged over hybrids and cropping years compared to the control. When analysed by phenological stage and hybrid, APX activity was the same in the FAO 360 hybrid in the V_4 and V_{14} phenological stages averaged over the examined cropping years and fertiliser levels. There was also similarity in the activity of APX in the V_6 and V_8 phenological stages for the FAO 360 hybrid, averaged over the different cropping years and fertiliser levels. For the FAO 360 hybrid, the R_1 period differed in APX activity from all phenological stages. The rate of lipid peroxidation was the same for the two examined hybrids in the V_4 phenological stage.

3.4 Correlation analysis

In the 2019 cropping year, the FAO 490 hybrid showed a decrease in oil and protein content with an increase in starch content and with high phosphorus and potassium levels on average. As the starch content of the crop increased, its oil and protein content decreased. Protein content increased with increasing yield and starch content decreased with increasing yield with a moderately strong significant relationship. In the 2020 cropping year, APX activity increased with increasing yield protein content in the FAO 360 hybrid. There was a significant increase in APX activity and grain moisture content with increasing yield averaged over the different nutrient levels. In the 2019 cropping year, FAO 360 hybrid with proportional phosphorus and potassium levels increased oil content with increasing yield protein content averaged over the various nitrogen dosess with increasing yield protein content at a moderately strong level of significance. There was a strong significant positive correlation with the increase in protein content as yield increased. As starch content increased, protein content decreased averaged over the different nutrient levels.

In the 2020 growing season, there was a positive correlation between yield and APX activity, with APX activity increasing as a result of increasing yield at a moderately strong level. As yield increased, protein content and grain moisture increased. As grain moisture increased, oil content decreased with proportional phosphorus and potassium levels averaged over the different nitrogen doses. No correlation was found between the other examined parameters as a function of the following factors.

4. CONCLUSIONS AND RECOMMENDATIONS

Today, agricultural production is in a drastically changing environment. Increasing food consumption, together with rising input costs and decreasing land availability, has led to an increase in the value of produced crops. The primary challenge for farmers is to apply well-considered, rational management systems, to better meet the needs of maize hybrids and to optimise input use.

The long-term experiment that was used for my investigation was set up in 1983 by Prof. Dr. János Nagy. For 39 years, the research has continued with unchanged parameters, the same nutrient supply system, soil cultivation and agrotechnology. In my study, I used forage maize hybrids with different FAO numbers in the 2019 and 2020 growing years. I measured SOD, APX activity and lipid peroxidation at 5 sampling dates in total and analysed the yield parameters taking these into account. In the experiment, 4 different nutrient treatments and a complete control stand for these treatments were used.

In the 2019 cropping year, the APX activity of the FAO 360 hybrid in the V_4 phenological stage increased with increasing nitrogen dose at the N2 level compared to the control, and N5 slightly decreased APX activity compared to the control. In response to proportional NPK supply, increasing nutrient levels increased APX activity by 26% compared to the control in the FAO 490 hybrid at the four-leaf phenological stage.

At the V_6 phenological stage, APX activity measured in hybrid FAO 490 differed from hybrid FAO 360. For hybrid FAO 490, APX activity was higher at the control and high N5 nutrient levels than at N2 nutrient levels, with a 13% difference compared to control and 11% difference compared to N5. The lipid peroxidation rate and SOD activity were unchanged in the FAO 490 hybrid at the V_6 phenological stage. Lipid peroxidation rate and SOD activity decreased proportionally with increasing nutrient levels compared to the control. In the phenological stage V_{14}, much higher levels of APX activity were measured in the FAO 490 hybrid compared to the APX activity measured in the FAO 360 hybrid. Compared to control stands, the APX activity was more than 40% higher in hybrid FAO 490 with a value of 8.063. As a function of this, at the V_{14} phenological stage, there was a significant physiological difference between the two hybrids, depending on the applied nutrient levels. In the R_1 phenological stage, the APX activity value of the FAO 360 hybrid was 50% higher than that of the control stand compared to the N2 nutrient level and 30% higher compared to the N5 nutrient level. The lipid peroxidation rate and SOD activity did not change significantly. Similarly to the FAO 360 hybrid, in the R_1 phenological stage, the APX activity of the FAO 490 hybrid was 76% higher and 60% higher in the control stand compared to N2 and N5, respectively. The rate of lipid peroxidation was slightly reduced by 0.973 units at N2 nutrient level compared to the control stand.

In 2020, in the phenological stage V_6, APX activity increased gradually with increasing nutrient levels. N2 nutrient levels increased by 21% compared to control and N5 nutrient levels increased by 22% compared to the control. The rate of lipid peroxidation also increased with increasing nutrient levels compared to the control. SOD activity varied between 4.055 and 4.501 in the V_6 phenological stage of the FAO 360 hybrid in the 2020 growing season, irrespective of nutrient level. In the V_6 phenological stage, the activity of APX decreased by 2.3 units at the NPK 2 nutrient level compared to the control with equal rate of NPK nutrient supplementation. The APX activity at the NPK 5 nutrient level was 72% higher than the control stand in the FAO 360 hybrid. In the V_6 phenological stage, the activity of APX did not increase at the NPK 5 compared to NPK 2. This is in contrast to the results obtained in the short maturity hybrid. At the V_{14} phenological stage, APX activity increased with increasing nitrogen levels in the FAO 360 hybrid. Compared to the control, the amount of increase was 2.3 units at the N2 nutrient level and 6.4 units at the N5 nutrient level. The rate of lipid peroxidation increased by 2 units compared to the control at the N5 nutrient level. SOD activity was the same at the N0 and N2 nutrient levels and decreased by 1.2 units at the N5 nutrient level compared to the control.

APX activity increases steadily as the phenological stages progress, increasing from 9.35 units at the V_4 phenological stage to 12.99 units by the R_1 phenological stage. The activity of lipid peroxidation and SOD gradually decreased during the phenological stages. In the 2019 cropping year, there were different dynamics than when cropping years were averaged. The activity of APX increased from the V_4 to the V_6 phenological stage by 0.5 units, then decreased by 3.1 units by the V_8 phenological stage. At the R_1 phenological stage, the measured APX activity was 13.38. The lipid peroxidation rate and SOD activity of FAO 360 hybrid resulted in a constant value averaged over the different nutrient levels in the 2019 cropping year. In the 2020 cropping year, APX activity decreased gradually, which could be attributed to the agro-meteorological environment present in the different growing season. The activity of APX decreased by more than 20% in the R_1 phenological stage compared to the V_4 phenological stage. In the case of the FAO 360 hybrid, the activity of APX decreased steadily throughout the growing season averaged over the different nutrient levels. From the beginning of the growing season, LP increased by 2 units from the V_4 to the V_6 phenological stage and then decreased in a similar way with SOD activity by the V_8 phenological stage. LP and SOD activity increased by the V_{14} phenological stage, followed by a decrease at the end of the growing season by the R_1 phenological stage.

Phenological stages significantly influenced the degree of lipid peroxidation. The combined analysis of different hybrids and phenological stages had no effect on lipid peroxidation rates, but when fertiliser levels are added to the analysed factors, a significant effect is shown on lipid peroxidation rates. Lipid peroxidation rates were different at all time points relative to each other. There was little similarity only at the phenological stages V_6 and V_8. The rate of lipid peroxidation at R_1 was also different relative to all phenological stages. When analysed by nutrient level, the two highest nutrient levels, N5 and NPK 5, clearly affected APX activity. Also, when lipid peroxidation rates were examined, N5 and NPK 5 resulted in a different group from all nutrient levels, and control plots were significantly different from all examined fertiliser levels.

In the 2019 cropping year, FAO hybrid 490 decreased oil and protein content as starch content increased, with high phosphorus and potassium levels averaged over the various nutrient levels. As the starch content of the crop increased, the oil and protein content decreased. As the yield increased, the protein content increased and the starch content decreased, with a moderately strong significant relationship. For the FAO 360 hybrid, with proportional phosphorus and potassium levels, averaged over the various nitrogen doses the yield oil content increased with increasing yield protein content at a medium strong significant level. As yield increased, there was a strong significant positive correlation with the increase in protein content. Protein content and grain moisture increased with increasing yield. In addition, as grain moisture increased, oil content decreased, with proportional phosphorus and potassium levels averaged over the different nitrogen doses. No correlation was found between the other examined parameters as a function of the analysed factors.

The two hybrids showed similar APX, LP and SOD dynamics in many cases averaged over the different nutrient levels. In the 2019 growing season, both hybrids FAO 360 and FAO 490 showed a decreasing trend in all physiological parameters as the growing season progressed. However, in 2020, all examined physiological parameters increased, averaged over the examined hybrids and nutrient levels between each phenological stage.

5. NEW SCIENTIFIC RESULTS

1. Based on the results of the crop year effect analysis, I concluded that APX activity decreased by 2.01 units between the phenological phases V_4 and R_1 in the 2020 cropping year, averaged over the examined hynrods and nutrient levels. In contrast, it increased by 4.56 units between the phenological phases V_4 and R_1 in the 2019 cropping year.

2. During my analyses, I found that lipid peroxidation and SOD activity decreased during the phenological phases. The APX activity increased slightly (0.5) from phenological phase V_4 to V_6 and decreased significantly by 3.1 units by phenological phase V_8.

3. Within the growing season, in important phenological phases such as the V_{14} phenological phase, the analysis of marker processes such as APX and LP can give the most accurate picture of the physiological processes affecting maize plants and thus their health.

4. Evaluating the data of the long-term experiments, I found that the lowest APX activity was shown by the average values of the control plot, with the highest value at the NPK 5 nutrient level of 9.14 units.

5. Correlation analysis based on nitrogen fertiliser levels showed that grain yield was in a significant correlation with APX activity and lipid peroxidation rate, but not with SOD activity.

6. Based on my examinations in the N-dose long-term experiment, I found that the APX activity of the FAO 490 hybrid was higher at control and the provocative high 300 kg/ha nitrogen nutrient levels (N5) than at N2 nutrient levels. The difference was 13 % higher compared to control and 11 % higher compared to N5. The obtained results demonstrate the beneficial effect of the 120 kg/ha nitrogen dose.

6. PRACTICAL USE OF THE RESULTS

1. The application of fertiliser at a proportional and appropriate rate (120 kg N/ha) is essential to achieve good yield, quality and safety. Excessive (300 kg/ha N) and deficient (0 kg/ha NPK) NPK application is harmful to the physiological processes of the plant, thereby affecting yield.

2. Physiological processes affecting plants during the growing season can have different effects on the development of certain yield parameters, such as those related to its quantity and quality. For this reason, a rapid and reliable analysis of certain marker physiological processes (APX, LP, SOD) within the growing season will help to eliminate yield losses from certain stress conditions.

3. A good indicator for maximising grain yield is the analysis of APX and LP activity in the V14 phenological phase. This examination provides a faster way to obtain feedback on the current physiological state of the crop stand than leaf analysis or soil analysis.

4. In my examinations, I have found that, in a long-term experiment, as a result of proportionate nutrient levels (150 kg/ha N, 115 kg/ha P_2O_5, 135 kg/ha K_2O), increasing nutrient levels increased the APX activity of the FAO 490 hybrid by 26% compared to the control at the early, 4-leaf phenological stage.

5. Correlation analysis revealed that, averaged over cropping years (2019-2020), hybrids (FAO 360, FAO 490) and nutrient levels, APX activity increased with increasing grain moisture (R=0.548) and decreased with increasing oil content (R=0.557).

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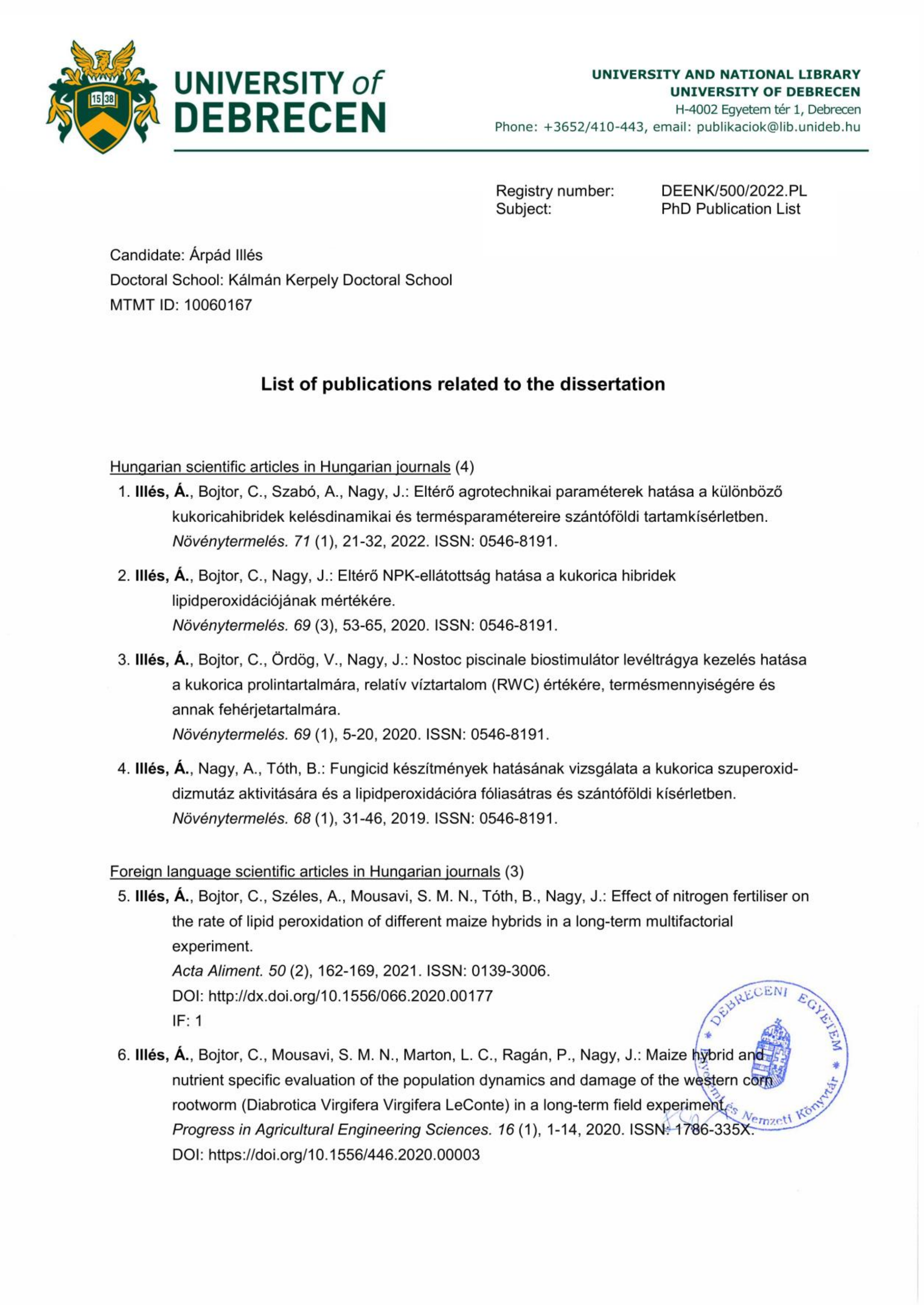
PUBLICATIONS ON THE SUBJECT OF THE THESIS



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List of publications related to the dissertation

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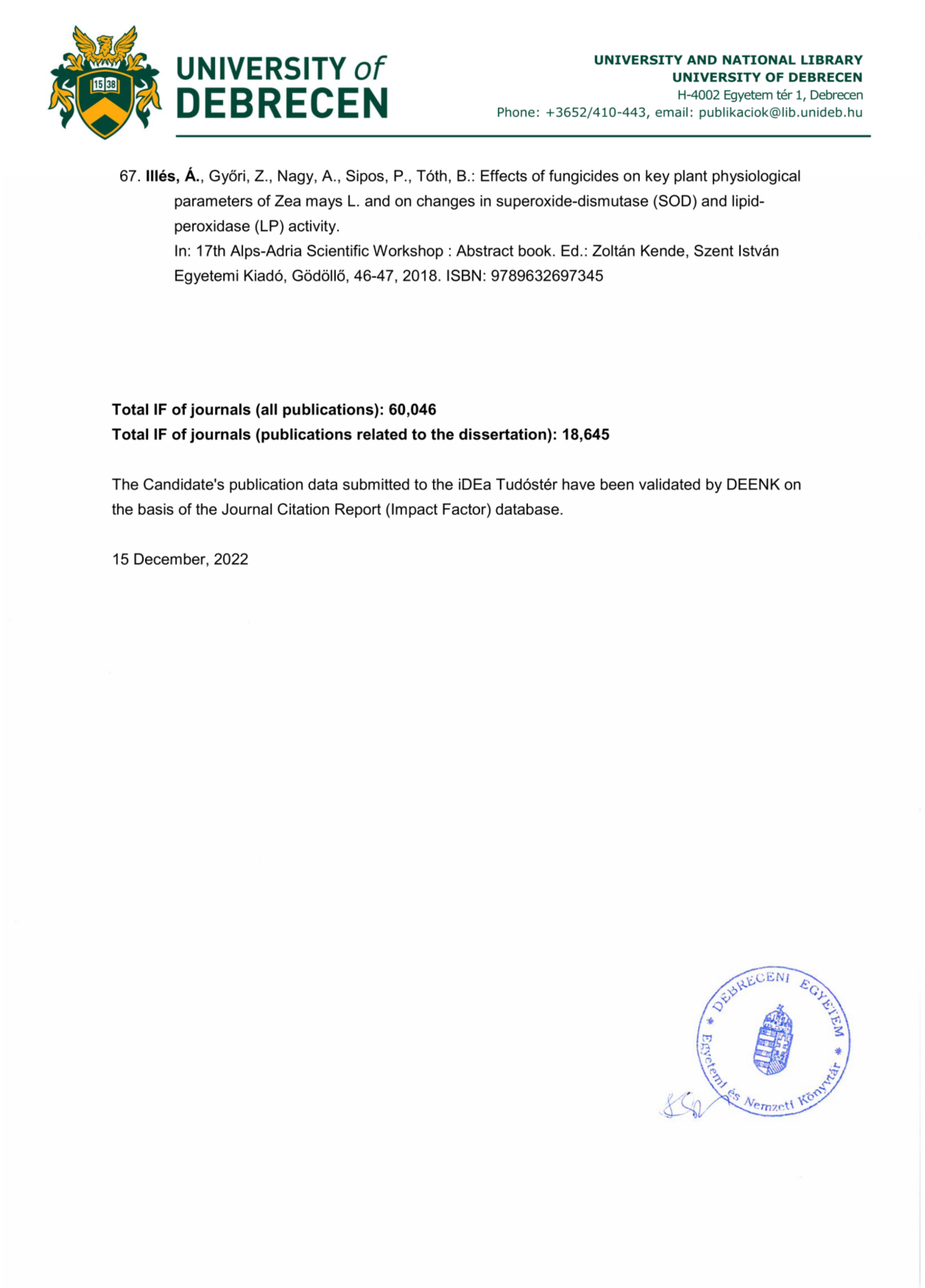

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