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Effect of Different Nitrogen Supply on Maize Emergence Dynamics, Evaluation of Yield Parameters of Different Hybrids in Long-Term Field Experiments

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Abstract: This paper aims to examine the effect of various nitrogen (N) supply treatments on the date of emergence of maize hybrids classified in different age groups. The study site was at the University of Debrecen's Látókép Experiment Station in Hungary. The date of emergence of the tested maize hybrids was monitored under control (0 kg N ha^{-1}), 120 kg ha^{-1} N + PK, and 300 kg ha^{-1} N + PK nutrient levels in a long-term field experiment. In 2020, maize hybrids (H1 = FAO 490; H2: FAO 420–440; H3 = FAO 420; H4 = 490; H5 = 320–340; H6 = FAO 350–370) growing under natural precipitation supply conditions without irrigation were included in the study. During the days of emergence, different moisture, protein, oil, starch, and yield production levels were observed, according to the variance analysis. In diverse maize hybrids, increasing or decreasing fertilizer treatment resulted in diverse productivity metrics. Regression analysis revealed that the day of emergence had a greater impact on protein, moisture, starch, and oil content than N fertilizer; however, yield production was influenced by N fertilization, rather than day of emergence. Regarding productivity parameters, this study suggests that H1 has the best productivity until the fourth day of emergence.

Keywords: maize; fertilizer; regression analysis; grain yield

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1. Introduction

Today, the pressure on populations is increasing due to ever-increasing social and living standards and the demand for land for industrial investment activities [1]. The growing vigor in demand for maize also shows that this diversified plant plays a major role in the food processing industry [2]. In terms of maize cultivation technology, an evolving trend has emerged over the last decade. It is necessary to use hybrids that are resistant to external factors in extreme weather. An expanding hybrid palette is available for farmers. Site-specific crop production needs to be implemented in a way that is optimally adapted to production objectives and ecological conditions; the production process is affected by site-specific nitrogen application, and significant positive yields have been obtained with the help of soil moisture monitoring systems under irrigated conditions [3]. In this study have been carried out to understand the effects of stress on the germination and emergence of plants [4]. However, it should be mentioned that there is a close relationship between the response of plants to stress and the germination vigor of seeds [5,6]. According to Macaloney et al. [7], the maize germination process is divided into three stages: first stage: the swelling of the grains, abundant water uptake; second stage: the elongation of existing cells; third stage: the division of meristem cells. Murungu et al. [8], according to their experiment, stated that soils with finer soil grains have better seed-to-soil grain contact, i.e., faster water uptake and germination. As a result of physical soil tests, the water holding, water absorption, and water conductivity capacities of soils can be determined. The physical diversity of each soil can also be inferred from its ability to supply and store nutrients. The mechanical (grain) composition of the soil is the ratio of individual particles of different sizes to each other; this ratio largely determines water and nutrient management and other physical and chemical properties of the soil [9]. Some of the precipitation seeps into the soil, so the soil moisture is partly adhered to the soil particles and partly present in the gaps between the particles. Seed and plants can utilize this water in the gaps between soil grains [10]. Rapid germination affects the emergence of a homogeneous stock, which can be achieved under better conditions. Fast and even germination is the most important factor for successful crop production, while germination problems appear due to slow, uneven, unreliable germination, and seeds with low germination vigor [11]. Low post-sowing temperatures (8–12 °C) have a negative effect on the physiological activity of germinating maize seeds, also affecting the various organisms in the soil which promote germination [12,13]. A positive correlation between seed size and total oil content can be detected along with germination vigor. Chemical analyses suggested that both lower stearic-and oleic acid in the seed and high levels of linoleic acid in the seed were also effective for spring germination. These traits are characteristic of genotypes with strong growth potential at the beginning of the germination period, even under frequent cool conditions [14]. During the growth of the maize plant, the critical stages are germination and emergence, which also affect the quality and quantity of yield [15]. Soil water content is a key factor in seed germination and seedling emergence in semi-arid areas [16]. Optimizing the sowing date has a positive effect on maize yield and adaptation to changing conditions. The results of the performed experiment revealed the effect of grain yield on the starch content and protein and oil production in one hectare [17]. The number of days from sowing to emergence (NDSSE) is one of the most important agronomic traits in the cultivation of maize (*Zea mays* L.), which is also reflected in the obtained yield [18]. At germination, the primary root is part of the whole root system, followed by the seminal roots, mesocotyl-borne roots, the crown and finally the brace roots to acquire water, NO_3^-, NH_4^+, P, and K [19]. Schneider and Gupta [20] conclude in their experiment that soil moisture sufficiently satisfied the water capacity of the field, and average soil temperature was the most strongly determining factor influencing germination time, when aggregate size was between 1.0 and 6.8 mm. Sowing is considered to be the first critical and most sensitive part of a plant's life cycle, and seeds are often exposed to adverse environmental effects that pose a threat to plant germination [21,22]. Sun et al. [23] emphasized that root growth—even at this early stage—responded to the low N content applied, indicating that this method could be used with a high-permeability phenotype maize germplasm under laboratory conditions. Based on statistical analysis of the data, the days to appearance were not significantly ($p > 0.05$) affected by N and P fertilizers. The maximum number of days (9 days) was recorded for short growing season hybrids, i.e., FAO 400–500 and local varieties. The minimum was recorded for FAO 400–500 hybrids. The number of days to emergence was recorded after treatment with N + PK at 100 kg ha^{-1}. The effect of N + PK (150:150) did not appear until a few days had passed. The combined effect of cultivars and NP levels had a non-significant ($p > 0.05$) effect on the days until emergence due to the fact that the seeds have their own nutrient reserves and do not need an external energy source [24]. N-fertilization was the most influential factor for the number of seeds per m^2. Kernel weight was lowest in the N0 (0 kg ha^{-1} N) control treatment and was significantly higher in the N60 (60 kg ha^{-1} N) and N120 (120 kg ha^{-1} N) treatments [25]. The appearance of seedlings was not homogeneous at 150:120 N:P doses. The efficiency of nitrogen utilization was moderate under the effect of the high nitrogen dose. At the low N dose, the incorporation of N into the plant was better detected. A safer emergence was achieved at a dose of 90:90 kg N:P ha^{-1} [26]. Hussain [27] reported that 100 kg N ha^{-1} resulted in complete, homogeneous germination of maize, thereby stimulating plant growth and also positively influencing yield. Siddique et al. [28] investigated the effect of increasing doses of N treatments (0, 56, 84, 112 kg ha^{-1}) on the quantity and quality of maize yields. It was concluded that increasing N doses had a positive effect on yield and crude protein content. Several researchers (in Látókép, Debrecen, east of Hungary and Kincaid, Haven, KS, USA) put forward that, in the case of harmonic NPK macronutrient fertilization, the nutrient intake per 100 kg ha^{-1} of maize grain yield is lower than in the case of unilateral fertilization (N, P, and K separately) [29–32]. The heterogeneity of emergence dynamics was compared with yield and quality. The main aim of the experiment was to clarify the effect of nitrogen and the maturity group of the hybrids for the emergency vigor and yield parameters in a long-term field experiment.

2. Materials and Methods

During the examination, a 0.5 cm tall seedling from the soil surface can be considered as a germinated plant. Germination was measured from the first seedling every day for 5 days at the same time in the morning, at 9 am, so that the results could be compared for the analyses. The experiment was established in 1986; this experiment was carried out in the 2020 growing season. The location of the experimental plots was randomized. Four replicates were used with two rows in each plot. Seed parameters (width, height, diameter, weight) were examined with a digital caliper and balanced under laboratory conditions. Minitab Statistical Software 19 was used for analysis.

Examinations were performed at the Látókép Experiment Site of the University of Debrecen, Hungary (47°33′ N, 21°26′ E, 111 m asl). The soil of the long-term fertilization experiment is calcareous chernozem with excellent properties [30]. With regard to the main soil properties, the soil has a pH of 5.8 (slightly acidic), which is optimal for the macro- and micronutrient uptake of the crops. In terms of its physical variety, the soil is clayey adobe, with an Arany's plasticity index of 38, and the total amount of water-soluble salts was 0.02%, i.e., low salt content. The organic matter content is around 2.1%. We measured the available soil moisture at different soil layers (0–100 cm). Four homogenized, randomized soil samples were taken for each treatment at a depth of 0–30 cm. Soil parameters were measured by an accredited soil lab in Hungary—Mertcontrol HL-LAB, Ltd. Examined the soil samples on the basis of the listed Hungarian standards: MSZ-08-0206-2:1978, MSZ-08-0205:1978, MSZ-08-0206-2:1978, MSZ-08-0206-2:1978, MSZ 08-0210:1977, MSZ 20135:1999, MSZ 20135:1999, MSZ 20135:1999, MSZ-08-0206-2:1978. (Table 1).

Table 1. The physical and chemical parameters of the soil under different nitrogen supply conditions. Notes: pH (KCL)—potassium chloride soluble pH; KA—Arany's plasticity index.

N-Stages	pH (KCl 1:2, 5)	K_A	Salt Content (m/m%)	$CaCO_3$ (m/m%)	Organic Matter (m/m%)	Nitrogen (mg/kg)	Sulfur (mg/kg)	Potassium Oxide (mg/kg)	Sodium (mg/kg)	Phosphorus Pentoxide (mg/kg)
0	6.15	38.56	<0.02	<0.1	2.16	1.17	0.78	185.28	13.59	52.90
2	5.70	40.28	<0.02	<0.1	2.23	2.30	6.88	277.44	9.55	146.65
5	5.57	36.81	<0.02	<0.1	2.02	2.11	2.81	277.02	9.22	129.12

The average of the total water volume of the soil was 18.51 m/m%. The highest amount (20.28 m/m%) of water was located at a depth of 10–60 cm. In addition, in the deeper layer of the soil (60–100 cm), the moisture content was lower, with the average moisture content being 17.1 m/m%.

Sowing was performed on 17 April 2020 with 74,000 seeds per hectare. The number of days between sowing and the beginning of germination was 12 days, meanwhile the average daily maximum temperature was 26.1 °C degrees. The experiment was carried out in 1248 plots, with 5 rows of borders on each side, eliminating the border effect. Plot size was 7.6 m^2 (1.52 × 5 m). The row spacing was 76.2 cm, while the stem spacing was 18 cm. Measurements were made in a polyfactorial long-term nutrient replenishment experiment for the hybrid-specific analysis of control (0 kg ha^{-1}), as well as nutrient doses of 120 kg ha^{-1} N + PK and 300 kg ha^{-1} N + PK [33]. Phosphorus and potassium were applied in each plot in the form of autumn basic fertilizer (P_2O_5: 184 kg ha^{-1}; K_2O: 216 kg ha^{-1}). No nutrients have been applied to the control plots since setting up the nutrient experiment in 1986. The performed measurements were set up under natural precipitation supply conditions without irrigation in the maize hybrid population (H1 = FAO 490; H2 = FAO 420–440; H3 = FAO 420; H4 = FAO 490; H5 = FAO 320–340; H6 = FAO 350–370) in 2020. The applied hybrids are used in a large area in Hungary; therefore, it is important to choose a hybrid according to the extreme climatic conditions.

In the winter months of 2020 (January–February), the total precipitation was 59.6 mm, which was lower than the multiple-year average (67.2 mm). The spring precipitation deficit resulted in delayed emergence. Regarding the summer months, precipitation in June (112.5 mm) was above the average of the last 30 years (79.5 mm). The amount of precipitation in July (182 mm) was almost three times as high as the multiple-year average (65.7 mm). The amount of precipitation in August (70 mm) was above the multiple-year average (60.7 mm). The temperature was above average in the autumn months (September: 17.8 °C; multiple-year average: 16.4 °C; October: 11.7; multiple-year average: 11.2 °C), to which the increasingly extensive roots of maize plants were relatively well able to adapt. From this point—almost in the full vegetation period of maize—temperature values significantly exceeded the values characteristic of the region. During the first half of June to harvest, there was abundant rainfall and constantly warm weather. In the weeks before harvest, the favorably high temperature and decreasing humidity helped the maize maturing process (Figure 1) [34].

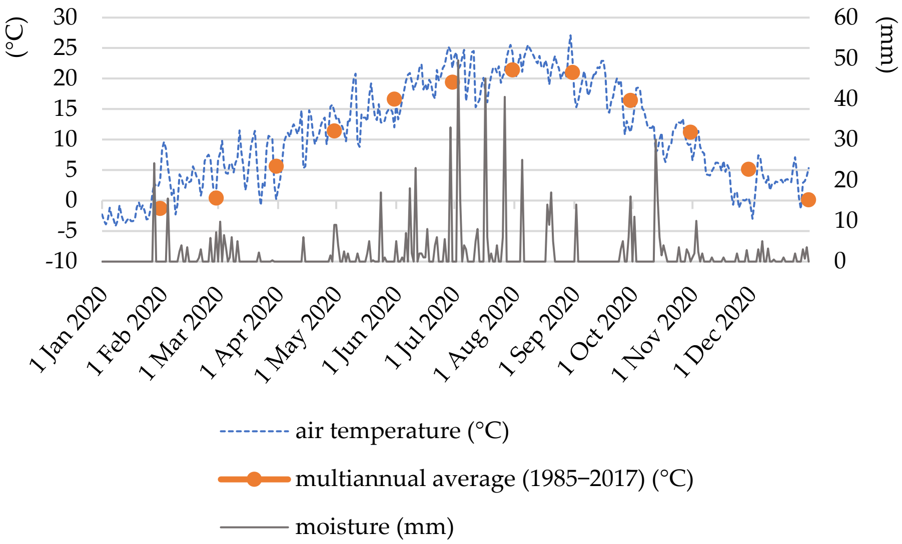

Figure 1. Precipitation and temperature values in 2020, Látókép Crop Production Experimental Site, University of Debrecen.

The effect of the meteorological parameters of the different growing seasons significantly affect the yield of maize production regardless of the level of fertilization. Irrigation can be used to increase yields above 100% in some extreme growing seasons. Based on the effect of fertilization measured in a long-term experiment, it increased production volume by 25% on a 20-year average [35].

As regards the examined hybrids, H1 is Optimum® AQUAmax® (Willmington, IL, USA) certified [36], H2 has excellent seed grain quality and good water release ability [37], H3 has a strong root system and optimal initial vigor of development [38], H4 is similar to H3 [39], characterised by excellent initial vigor of development, H5 has great adaptability for a dry-continental climate [40], and H6 has great drought resistance [40].

For sample processing, Haldrup precision equipment was used for crumbling and the main yield parameters of the cobs (1000 grain weight, total seed count of the cob, and weight of the ear) were measured separately. The system is automated. In addition, the grain counter shows reliable results with high precision, even at high speed. The quality parameters of the grains were determined using a Perten Inframatic 9520 cereal cab. The device is capable of examining the ash, protein, and moisture content of flour and meal samples, as well as other content values. The instrument analyses samples in the range of 570–1100 nm and can obtain 30 spectra per second at 5 nm intervals [41]. Harvest was performed on 24 October 2020.

ANOVA is a statistical test to determine the difference between the means of two or more independent statistical populations. In other words, the variance analysis technique is used to compare two or more groups to see if there are significant differences between them. Factor analysis is used to reduce many variables into fewer numbers of factors. This method extracts the highest common variance from all variables and puts them into a standard score. We can use this score to estimate all variables for further analysis. Linear regression is a linear model approach between the variable "response" and one or more "explanatory" variables. Regression is often used to discover the model of a linear relationship between variables. In this case, it is assumed that one or more descriptive variables whose value is independent of the other variables or under the researcher's control can be effective in predicting the response variable whose value does not depend on the explanatory variables under the control of the researcher. The purpose of regression analysis is to identify the linear model of this relationship. All analyses were carried out with Minitab and SPSS software.

3. Results

The Tukey grouping analysis was based on the parameters of different seed genotypes. The analysis showed that crop height had the highest value at H1. There was a similar value to H2 and H5 together, H3 and H6 also had the same height value, and H4 showed a minimum value. In addition, the analysis showed that H4 had the highest width value. H1 and H5 had similar values, while H2, H3, and H6 had similar width values. Based on the Tukey grouping, H5 had the highest diameter value. The lowest values were measured in H2 and H4. Similar values of this trait were measured in H1, H3, and H6. According to the results of the analysis, H1 had the highest weight value, while the lowest values were found in H3 and H6. However, this trait showed different values from H1 to H6 (Figure 2).

Variance analysis showed that the day of emergence was significant in terms of grain yield, protein, oil, starch, and moisture content. The effect of N fertilizer was significant on grain yield and protein. The interaction of days of emergence and hybrid effect was significant with respect to moisture, oil, protein, starch, and grain yield. The interaction of N fertilizer and days of emergence was significant in relation to grain yield. The interaction between N fertilizer, days of emergence, and hybrids is significant in relation to grain yield, too. In general, day of emergence is significant with respect to all productivity parameters included in this study. The effect of hybrids is not significant in terms of moisture, oil, protein, or starch content, nor grain yield. In addition, the nitrogen fertilizer effect was not significant in terms of moisture, oil, and starch content. The interaction of hybrids, nitrogen fertilizer levels, and day of emergence was not significant in terms of the productivity parameters (Table 2). Tukey grouping analysis showed that the third day of emergence had the higher productivity parameters. Parameters include moisture, protein, oil, starch, and grain yield. In addition, the second day of emergence had higher productivity. After that, the sixth, the fourth, and the fifth day had outstanding productivity. The third day of emergence had desirable stability on the productivity parameters (Table 3).

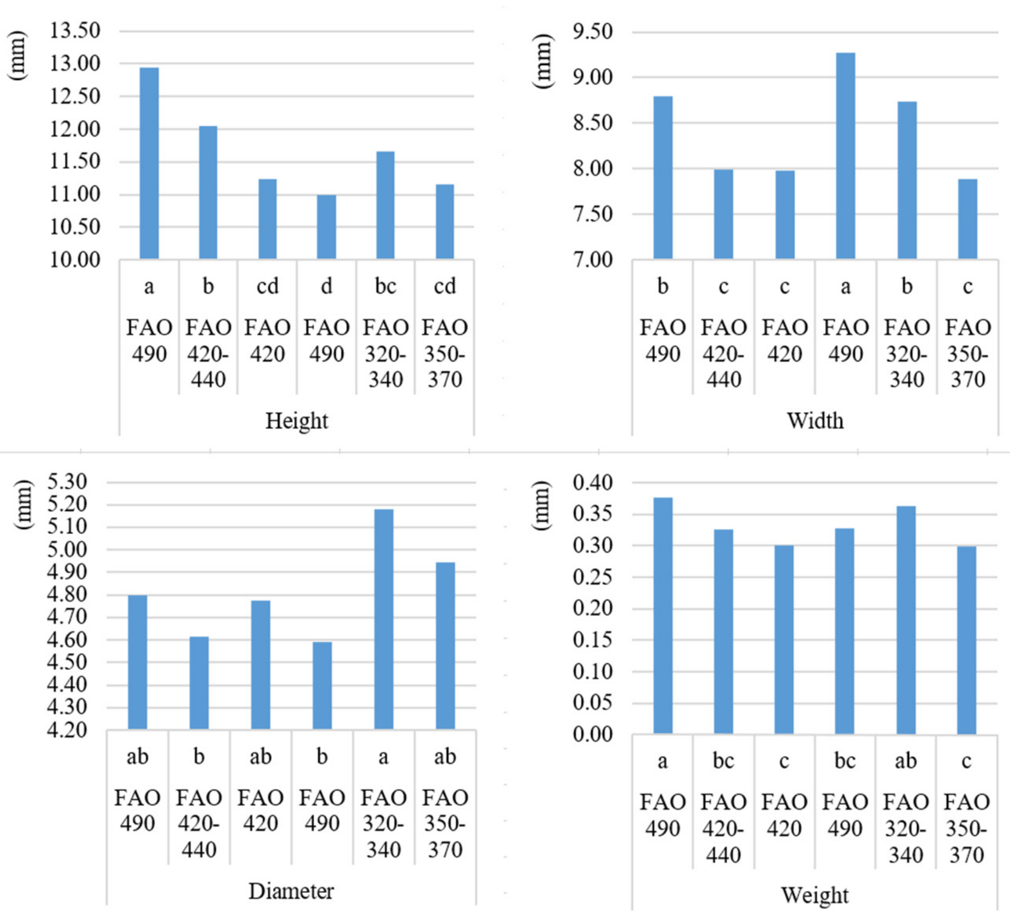

Figure 2. Results of comparing the seed parameter differences of hybrids. Notes: Treatments with the same letter are not significantly different.

Table 2. Variance analysis of productivity parameters.

Source	df	Moisture	Oil	Protein	Starch	Grain Yield
Hybrid	5	0.09	0.52	0.47	0.08	0.88
N	2	0.10	0.00	6.36 **	0.10	82.17 **
Day of emergence	5	49.68 **	49.06 **	50.01 **	51.05 **	38.74 **
Hybrid *	10	0.14	0.15	0.19	0.08	0.20
Hybrid *day of emergence	25	12.69 **	12.26 **	12.74 **	12.95 **	11.32 **
N*day of emergence	10	1.20	1.03	1.33	1.13	3.54 **
Hybrid *N *day of emergence	50	1.09	1.06	1.16	1.08	1.50 *

Significant levels: * $p < 0.05$; ** $p < 0.01$.

Table 3. Tukey grouping based on day of emergence.

Parameters	Day of Emergence	N	Mean	Grouping				
Moisture	3	72	4.59	A				
	6	72	3.71		B			
	2	72	3.07		B			
	1	72	2.21			C		
	5	72	2.05			C		
	4	72	1.36				D	
Oil	3	72	0.79	A				
	6	72	0.63		B			
	2	72	0.53		B			
	1	72	0.38			C		
	5	72	0.35			C	D	
	4	72	0.23				D	
Protein	3	72	1.52	A				
	6	72	1.25		B			
	2	72	1.01			C		
	1	72	0.73				D	
	5	72	0.68				D	
	4	72	0.44					E
Grain yield	3	72	2.68	A				
	6	72	2.19		B			
	2	72	1.77		B	C		
	1	72	1.36			C	D	
	5	72	1.20				D	E
	4	72	0.79					E
Starch	3	72	17.68	A				
	6	70	14.16		B			
	2	72	11.69		B			
	1	72	8.41			C		
	5	72	7.89			C		
	4	72	5.20				D	

Regression analysis indicated that the regression model was significant with respect to grain yield. Additionally, N fertilizer was significant with respect to grain yield and protein content. The regression model was not significant in terms of moisture, oil, protein, and starch content. Coefficient regression shows which factor had a more pronounced effect or which was essential for this study. In this case, the coefficient of the N fertilizer effect was 0.24, and the day of emergence effect was 0.01. Consequently, the N fertilizer had a more pronounced effect on grain yield. The effect of the day of emergence resulted in a higher index of oil, protein, and starch content. The effect of the N fertilizer achieved was maximal with respect to the index for grain yield and moisture. This study showed that the effect of hybrids on productivity parameters is not significant based on the performed regression analysis (Table 4).

Table 4. Regression analysis of productivity parameters.

Parameters	Source	Df	F	Equation
Grain yield	Regression	7	8.21 **	1.040 + 0.2478 N + 0.0151 day of emergence
	N	1	55.22 **	
	Day of emergence	1	0.14	
	Hybrid	5	0.39	
Oil	Regression	7	0.19	0.4751 + 0.00002 N + 0.0040 day of emergence
	N	1	0.00	
	Day of emergence	1	0.14	
	Hybrid	5	0.24	
Protein	Regression	7	1.01	0.7950 + 0.0398 N+ 0.0160 day of emergence
	N	1	5.44 **	
	Day of emergence	1	0.61	
	Hybrid	5	0.20	
Moisture	Regression	7	0.09	2.676 + 0.0155 N+ 0.0355 day of emergence
	N	1	0.10	
	Day of emergence	1	0.34	
	Hybrid	5	0.04	
Starch	Regression	7	0.08	10.29 + 0.028 N + 0.139 day of emergence
	N	1	0.02	
	Day of emergence	1	0.35	
	Hybrid	5	0.04	

Significant levels: ** $p < 0.01$.

The interaction of nitrogen fertilizer, hybrids, and days of emergence indicates that FAO 490 (H1) had a desirable productivity from the fourth day of emergence in terms of protein, moisture, oil, and starch content, as well as grain yield. However, increasing the days of emergence from four to six did not result in significant productivity. FAO 310–3440 had a maximum productivity from the third day of emergence to the sixth day of emergence on productivity parameters. The FAO 350–370 showed higher productivity parameters from the third to the fifth day of emergence. FAO 420–440 had the best productivity from the first to the third day of emergence with increasing fertilizer doses. The maximum productivity in parameters was observed on the sixth day of emergence. FAO 420 showed maximum productivity on the third day of emergence with increasing fertilizer levels. FAO 490 (H1) showed higher productivity from the first day of emergence to the sixth day of emergence with increasing fertilizer levels (Figures 3–7).

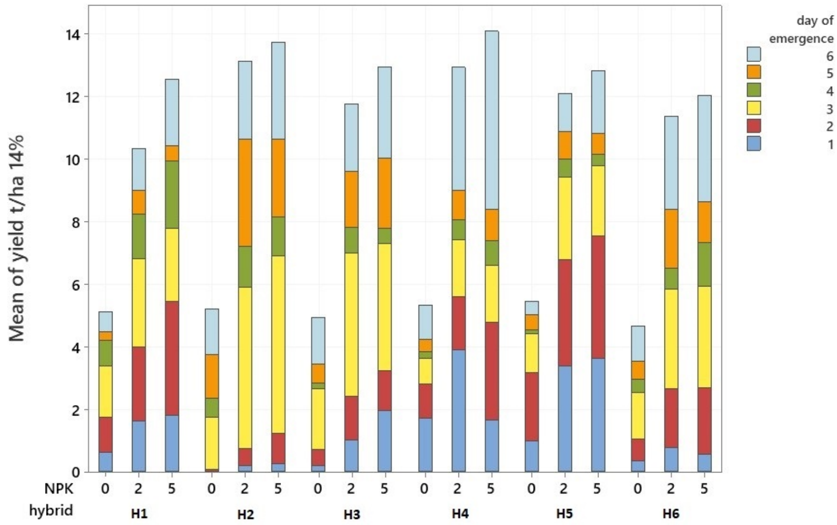

Figure 3. Interaction between hybrids, fertilizer, and day of emergence on yield.

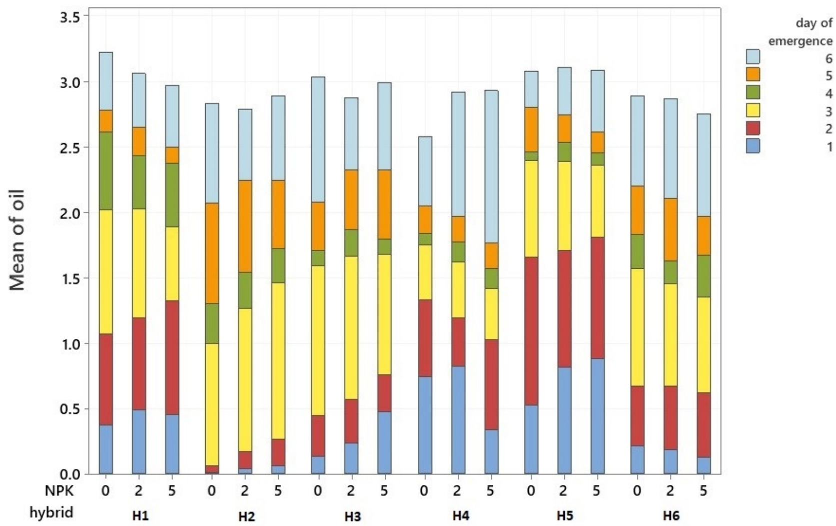

Figure 4. Interaction between hybrids, fertilizer, and day of emergence on oil content.

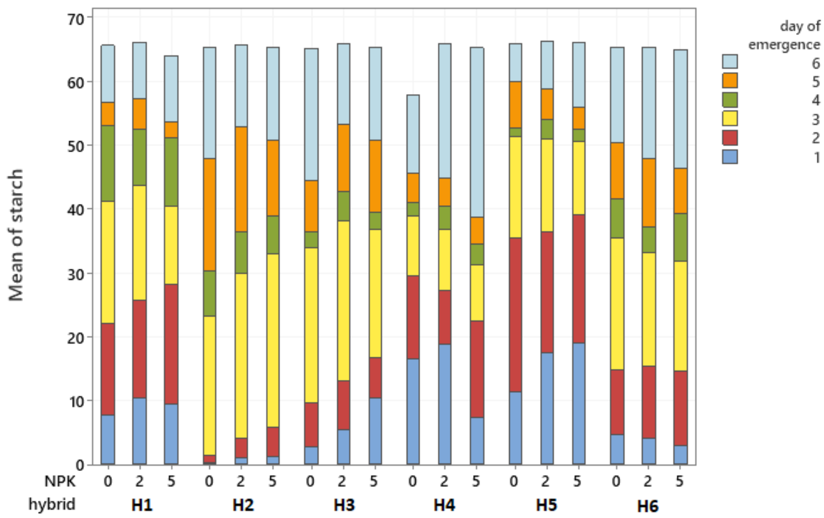

Figure 5. Interaction between hybrids, fertilizer, and day of emergence on starch content.

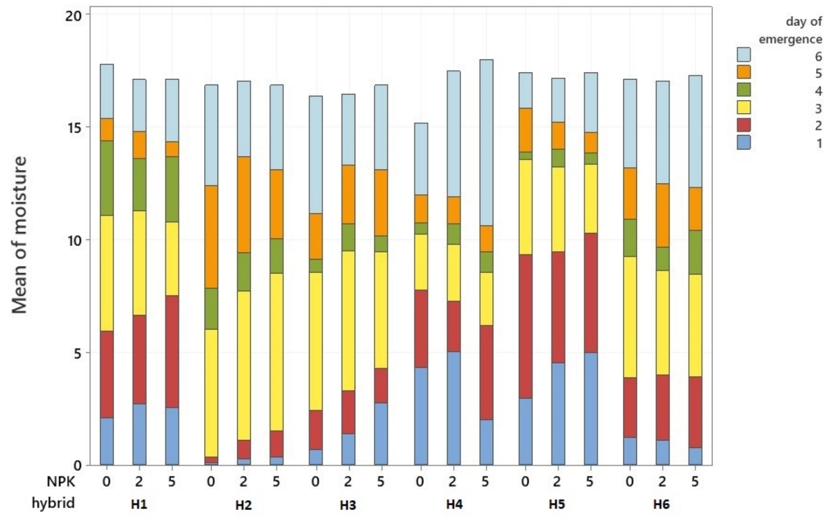

Figure 6. Interaction between hybrids, fertilizer, and day of emergence on moisture content.

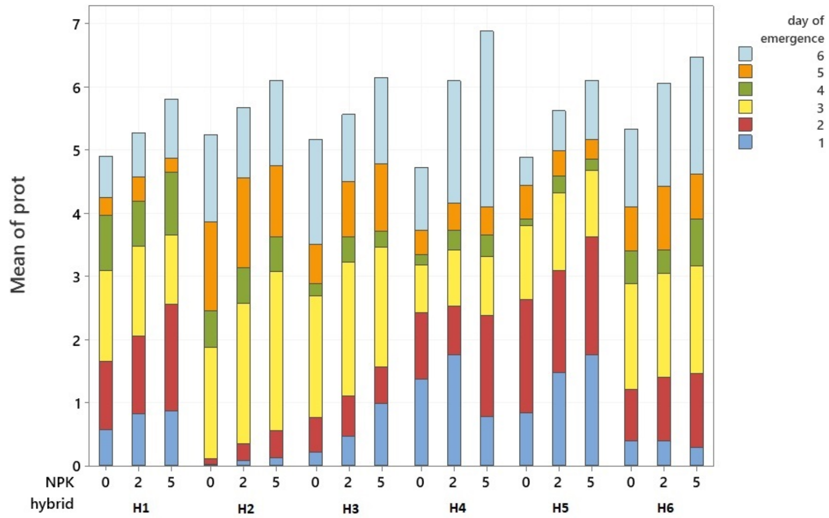

Figure 7. Interaction between hybrids, fertilizer, and day of emergence on protein content.

4. Discussion

Germination is the first developmental stage in the plant, one of the most essential and sensitive stages in the plant life cycle, and a critical process in seedling emergence [42]. Thus, this stage is one of the most vulnerable and critical stages in the plant's life. Germination is related to the genetic characteristics of the plant and is highly dependent on the factors that affect the seed life cycle. This influence starts from the mother plant and continues until the seed absorbs water [43]. Variance analysis showed that the day of emergence had various impacts on moisture, protein, oil, and starch content, as well as the yield of maize hybrids. Increasing or decreasing the fertilizer dose resulted in a variety of effects on production metrics for the different maize hybrids. Tukey grouping indicated that the third day of emergence had an essential effect on productivity parameters. Increasing N fertilizer doses influenced the day of emergence and the productivity of maize hybrids. Research in arid and semi-arid regions suggests that poor seed establishment is a common cause of poor crop yields [44]. Seed germination is a complex and dynamic stage of plant growth, which can improve yield production [45]. Abbasian et al. [46] examined the germination and seedling emergence of maize and stated that seedling emergence indices in the field did not significantly correlate with the standard germination test. The seedling growth analysis test involves measuring seedlings' length and dry weight in the standard germination test, which is used as a test for the vigor of seeds and seedlings [47]. Regression analysis showed that the day of emergence had a more pronounced effect than N fertilizer on protein, moisture, starch, and oil content. In addition, N fertilizer treatment had a more pronounced effect than day of emergence on grain yield. In this case, it can be concluded that the effect of N fertilizer and day of emergence together can be more pronounced on productivity parameters. Therefore, the applied N fertilizer could have a maximum effect on the day of emergence to harvesting time and yield. Nitrogen is a fundamental nutrient that enhances plant growth and greenness. Nitrogen reserves of the main root probably affect the rapid recovery of the plant due to defoliation [48]. This study showed that FAO 490 (H1) has maximum productivity from the first day to the fourth day of emergence on productivity parameters. FAO 350–370 showed excellent productivity after the fourth day of emergence with increasing N fertilizer doses. FAO 420–440 and FAO 420 had the best productivity from the first day to the sixth day of emergence. H8 showed maximum productivity from the third day to the sixth day of emergence with increasing fertilizer levels. In the case of this hybrid, the first day to the third day of emergence did not have a more pronounced effect on yield parameters. In general, the highest maize grain yields were achieved at early and optimal sowing times, resulting in significant yield reductions in late and very late sowing. Hybrids with higher FAO numbers had much higher grain yields than the early maturity group hybrids [25]. At the time of harvesting, sowing time had the greatest effect on grain moisture content. Based on the sowing date × hybrid interaction, it could be concluded that the grain moisture content increased in the higher FAO hybrids to a greater extent late or very late compared to lower FAO hybrids [49]. Sowing maize earlier resulted in larger crops and improved yield production, while crops sown later were smaller and had reduced yields. Yield reduction is often due to non-uniform density in the field; thus, researchers have investigated the effect of non-uniform density on yield by simulating different crop densities and using different crop dates [50]. In addition, some researchers have reported that the application of nitrogen fertilizer at 100 kg/ha compared to non-use of fertilizer causes an increase in average yield and day of emergence [51–53]. The decrease in grain yield and growth characteristics of the Sc. 301 hybrid maize was stated to be due to the reduced emergence and establishment of seedlings with weak vigor. Therefore, this study aimed to evaluate the effect of the size and shapes of different seed masses of the Sc. 704 hybrid maize, with an initial germination percentage of 1, 2, and 2% broad, medium, and round seed mass, on some characteristics of seedlings [54].

5. Conclusions

This study showed that the third day of emergence is the most important and had the greater impact on the measured parameters. Nitrogen fertilizer had a significant effect on the days of emergence by improving nutrient conditions for the germination of maize. Maize hybrids show a different trend in emergence dynamics depending on nitrogen fertilizer treatments, which farmers should consider. In this study, FAO 420–440 presented the highest percentage of emerged maize crops, resulting in higher stability.

Author Contributions: Á.I. and J.N. supervised the experiment and wrote the manuscript, C.B. and A.S. (Adrienn Széles) and A.S. (Atala Szabó) performed the experiments and collected samples in the field, S.M.N.M. made a statistical analysis and reviewed the manuscript to submit, L.R. made the figures and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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