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Is It Possible to Replace Part of the Mineral Nitrogen Dose in Maize for Grain by Using Growth Activators and Plant Growth-Promoting Rhizobacteria?

Arkadiusz Artyszak * and Dariusz Gozdowski

Institute of Agriculture, Warsaw University of Life Sciences—SGGW, Nowoursynowska 159,
02-776 Warsaw, Poland; dariusz_gozdowski@sggw.edu.pl
* Correspondence: arkadiusz_artyszak@sggw.edu.pl; Tel.: +48-22-5932702

Received: 22 September 2020; Accepted: 23 October 2020; Published: 26 October 2020

Abstract: The European Green Deal presented by the European Commission aims to reduce nutrient losses by at least 50% while preventing the deterioration of soil fertility and reducing the use of fertilizers by at least 20% by 2030. Farmers in the EU must prepare for this. Studies carried out in several locations in Poland in 2017–2019 tested the possibility of replacing 30–40% of the dose of mineral nitrogen by Penergetic (K + P) growth activators alone and in combination with Azoter containing plant growth promoting rhizobacteria in the cultivation of maize for grain. It was confirmed that the two combinations allowed a higher yield of maize grain by 2.9% and 8.8%, respectively, compared to the full nitrogen dose. Positive changes in the content of some assimilable macro- and microelements and soil organic carbon (SOC), and an increase in soil pH, were also observed.

Keywords: growth activator; nitrogen; maize for grain; plant growth-promoting rhizobacteria

1. Introduction

In 2018, maize was cultivated on an area of 193.7 million ha worldwide. In the EU (28), it was grown on 8.25 million ha [1]. One of the main factors determining the yield of maize grain is nitrogen fertilization. In Poland, the maximum allowed amount of nitrogen available for maize (for both grain and silage) is 240 kg N ha^{-1}. Maize takes up 26 kg of nitrogen to produce of 1 tonne of grain with the corresponding biomass [2].

For environmental reasons, the EU aims to limit nitrogen doses, which entails the risk of reducing yields. Therefore, other, more environmentally friendly ways to increase the yield of maize grain are expected. One may be the use of plant growth promoting rhizobacteria (PGPR) [3–7]. The application of PGPR-containing biofertilizers reduces the need for expensive nitrogen fertilizers and facilitates phosphorus uptake by plants [8]. Many experiments have proven the beneficial use of PGPR on the growth and development of maize [9–14]. The two main aspects that most influence the success of inoculation are the effectiveness of the bacterial isolate and the proper application technology [15].

Another method may be the use of Penergetic growth activators, the beneficial effect of which has been confirmed in other crops [16–26]. There are no results of studies on their use in maize cultivation. It has been proven that the use of Penergetic growth activators alone or in combination with PGPR allows mitigation of the limited sugar beet yields caused by the 30% reduction of mineral nitrogen dose [25].

The aim of the research was to investigate the possibility of limiting fertilization with nitrogen in maize grown for grain through the use of growth activators and plant growth promoting rhizobacteria and to determine their impact on soil characteristics.

2. Materials and Methods

In 2017–2019, eight field experiments with maize for grain were conducted: four in 2017 (Pągów, Pityny, Rogów, and Strzyżowiec), 2 in 2018 (Rogów and Terebiń), and two in 2019 (Bukowina and Rogów) (Figure 1). The experiments were conducted on following soils: Pągów–Albic Podzols (sandy loam: clay–10%, sand–55%, silt–35%); Bukowina (loam: clay–20%, sand–35%, silt–45%), Pityny (sandy loam: clay–12%, sand–64%, silt–24%), Rogów (silty clay loam: clay–29%, sand–20%, silt–51%), Strzyżowiec (loam: clay–26%, sand–30%, silt–44%) and Terebiń (clay loam: clay–27%, sand–26%, silt–47%)–Endocalcaric Cambisol [27].

Figure 1. Locations of field experiments.

Soil samples were collected at t soil depths (0–30 and 30–60 cm) twice: immediately after harvesting the forecrop and after harvesting maize. At District Chemical–Agricultural Stations in Warszawa-Wesoła, Opole, and Gdańsk, the following soil parameters were evaluated: pH_{KCl}, potentiometrically in 1 M KCl [28], soil organic carbon (SOC) content [29], nitrate nitrogen ($N\text{-}NO_3$) and ammonium nitrogen (NH_4) [30], available phosphorus [31], available potassium [32], available magnesium [33], available B [34], available Cu [35], available Fe [36], available Mn [37], and available Zn [38].

The pH_{KCl} of the soil before the experiments ranged from 5.3 to 7.0 in the 0–30 cm layer and 5.2 to 7.6 in the 30–60 cm layer (Supplementary Table S1). The SOC content was in the range of 0.54–1.40 and <0.17–1.18% at 0–30 cm and 30–60 cm, respectively; the range of $N\text{-}NO_3$ was 2.1–46.8 and 1.8–22.1 mg kg^{-1}; $N\text{-}NH_4$ was 1.24–9.92 and <1.00–8.19 mg kg^{-1}; mineral nitrogen (Nmin) was 27–188 and 12–107 kg ha^{-1}; P was 47–109 and 13–82 mg kg^{-1}; K was 85–291 and 42–191 mg kg^{-1}; and Mg was 53–103 and 47–108 mg kg^{-1}. The ranges of available micronutrient content (mg kg^{-1}) were: B, 0.75–2.31 and 0.69–1.23; Cu, 2.0–6.6 and 1.7–6.9; Fe, 500–1424 and 400–1222; Mn, 92–199 and 34–122; and Zn, 4.0–16.3 and <1.8–17.7.

Optimal rainfall for maize grown for grain is 200 mm in July–August [39]. Such conditions were found in Pityny in 2017, and in Rogów and Terebin in 2018 (Supplementary Table S3).

The most common forecrops for maize were winter wheat (Pągów in 2017, Rogów in 2017 and 2018, Bukowina in 2019), and less often sugar beet (Strzyżowiec in 2017, Terebiń in 2018), maize for grain (Rogów in 2019), and potato (Pityny in 2017).

The characteristics of the technology used in the experiments are presented in Table 1. In the experiments, depending on the location, the following were used:

- Ammonium sulfate 32 (16% N in ammonium form and 16% N in nitrate form)
- Korn-Kali: potassium chloride with added magnesium salt (33.2% K, 3.6% Mg, 3% Na and 5% S)
- Mocznik (46% N in amide form)
- Polidap: ammonium phosphate (18% N in ammonium form, 20.1% P as mono- and diammonium phosphate, 2.8% S as sulfate)
- Polifoska 6 fertilizer (6% N in ammonium form, 8.7% P as mono- and diammonium phosphate, 24.9% K as potassium chloride, 2.8% S as sulfate)
- Potassium chloride (49.8% K as potassium chloride)
- Saletrzak Standard 27: ammonium nitrate with added dolomite flour containing calcium and magnesium (13.5% N in ammonium form and 13.5% N in nitrate form, 1.4% Ca, 2.4% Mg)
- RSM 28: urea-ammonium nitrate solution (7% N in nitrate form, 7% N in ammonium form, 14% N in amide form)

Table 1. Characteristics of maize production technology in the experiment (2017–2019).

Location	Forecrop	Side Yield of Forecrop, t ha^{-1}	Cultivar of Maize	Mineral Fertilization, kg ha^{-1}	Sowing Date	Harvest Date	Length of Vegetation Period (Days)
				2017			
Pagów	Winter wheat	6.00 (straw)	LG 30.215 (FAO 230)	N–126 (variant No 0) and 88 (variants No 1 and No 2); P–34; K–173; Mg - 14.4, Na- 12, S–20	26.04	19.10	176
Pityny	Potato	(haulm)	Ambrosini (FAO 220)	N–128 (variant No 0) and 90 (variants No 1 and No 2); P–17; K–50; S–6	12.05	25.10	166
Rogów	Winter wheat	6.00 (straw)	SY Talisman (FAO 220–230)	N–150 (variant No 0) and 90 (variants No 1 and No 2); P–72 K–72	20.04	06.11	200
Strzyżowiec	Sugar beet	40 (leaves)	SY Talisman (FAO 220–230)	N–56 (variant No 0) and 39 (variants No 1 and No 2); P–30 K–30	24.04	16.10	175
				2018			
Rogów	Winter wheat	6 (straw)	SY Talisman (FAO 220–230)	N–150 (variant No 0) and 90 (variants No 1 and No 2); P–72; K–72	18.04	28.09	163
Terebiń	Sugar beet	40 (leaves)	SY Talisman (FAO 220–230)	N–56 (variant No 0) and 39 (variants No 1 and No 2); P- 30; K–30	20.04	09.10	172
				2019			
Bukowina	Winter wheat	4.00 (straw)	Farmagic (FAO 240)	N–184 (variant No 0) and 129 (variants No 1 and No 2); P - 86; K–90	25.04	09.10	167
Rogów	Maize for grain	30 (straw)	SY Talisman (FAO 220–230)	N–150 (variant No 0) and 90 (variants No 1 and No 2); P–72; K–72	16.04	15.10	182

0: control; 1: Penergetic (K + P); 2: Penergetic (K + P) + Azoter.

Fertilizers were applied before and during sowing, and some nitrogen fertilizers were also applied as top dressing. Plants were also foliar fertilized with magnesium sulfate, zinc, and manganese in recommended doses. Foliar nutrition with microelements was carried out throughout the experiment and it was prophylactic, and its aim was to prevent possible deficiencies of these nutrients in plants.

Corn was sown with a precision seeder in the amount of 85,000 seeds per hectare, 3–4 cm deep in rows with 75 cm spacing. Protection against weeds, diseases and pests was carried out in accordance with the recommendations of the Institute of Plant Protection—National Research Institute in Poznań.

In the experiments, three treatments were applied:

Treatment 0, control: Full nitrogen fertilization dose depending on location, from 56 to 184 kg ha^{-1} N.

Treatment 1: Dose of mineral nitrogen reduced by 30% (40% in Rogów) compared to full dose before sowing and during vegetation, from 34 to 110 kg ha^{-1} N; Penergetic-K (400 g ha^{-1}) on the harvest residuals of the forecrop before it was mixed with the soil; Penergetic-K (400 g ha^{-1}) with the first herbicide spray; Penergetic-P (300 g ha^{-1}) with a second herbicide spray; and Penergetic-P (300 g ha^{-1}) 3 weeks later.

Treatment 2: Dose of mineral nitrogen reduced by 30% (40% in Rogów) compared to full dose before sowing and during vegetation, from 34 to 110 kg ha^{-1} N; Penergetic-K (400 g ha^{-1}) + Azoter (10 dm^3 ha^{-1}) on the harvest residuals of the forecrop before it was mixed with the soil; Penergetic-K (400 g ha^{-1}) + Azoter (10 dm^3 ha^{-1}) in spring with the first herbicide spraying; Penergetic-P (300 g ha^{-1}) with the second herbicide spray; and Penergetic-P (300 g dm^3 ha^{-1}) 3 weeks later.

Penergetic-K and Penergetic-P are growth activators, and their composition is withheld by the manufacturer. Penergetic International AG produces Penergetic-P and -K from bentonite clays subjected to the application of electric and magnetic fields. These products are used to improve the performance of organisms in the soil that decompose organic matter (Penergetic-K) or increase the photosynthetic efficiency of plants (Penergetic-P).

Azoter is liquid bio-fertilizer produced by AZOTER Trading s.r.o. (Slovakia). It contains the high density of vital microbes, which restore microbial activity in the soil and accelerate the decomposition of harvest residues, straw and organic matter in soil. Azoter is a bacterial preparation containing plant growth-promoting rhizobacteria (*Azotobacter chroococcum*, *Azospirillum brasilense*, and *Bacillus megaterium* at 4×10^9 colony-forming units (CFU) cm^{-3}, with pH of 5.8–8.5). Azoter is a gray-brown thick liquid with a characteristic molasses-bacterial smell, typical for this product.

The number of replications was 4 and the total number of plots was 12, each consisting of four rows. Each single plot had a of length 10 m and width of 3 m (30 m^2), of which 15 m^2 (2 middle rows) was for harvesting. At harvest, the plants were counted, their height was measured, and the number of cobs per plant was counted. The number of grains was counted in 10 randomly selected cobs. Then all cobs in the plot were hand husked, and the grain yield was weighed after cleaning it from impurities. The plants were cut and the straw yield was weighed. The grain moisture was determined with an electric hygrometer (Dramiński GMM mini). Then, 2 × 500 grains were collected to determine the weight of 1000 grains [40]. The yield of grain with the current moisture content was converted into the yield at a standard moisture content of 14% (the same was applied to the grain yield per plant/cob and the weight of 1000 grains).

The following measurements performed in the experiments: grain yield (t ha^{-1}), grain moisture (%), straw biomass (t ha^{-1}), plant height (cm), grain yield per plant (g plant $^{-1}$), weight of 1000 grains (g), number of grains per cob, items in cob^{-1}.

The data were analyzed using analysis of variance and multiple comparison of means using Tukey's honestly significant difference (HSD) procedure. The significance level for all analyses was set at 0.05. The analyses were performed using Statistica 13 software (TIBCO Software Inc., Palo Alto, CA, USA). Descriptive statistics, including minimum, maximum, standard deviation (SD), and coefficient of variation (CV), were calculated.

3. Results

In some locations, some of the assessed soil parameters were improved (Supplementary Table S2). There was an increase in the content of available magnesium, iron, phosphorus, potassium, and zinc, SOC, and soil pH compared to the control.

The density of maize plants in each location was approximately 85,000 per hectare. Plants that developed more than one cob were not found. The main differences between the studied combinations were observed for the grain yield and 1000 grain weight. Variant 1 caused a significant increase in the grain yield (by 2.9%), plant height (by 2.8%), grain yield per plant (by 2.7%), and 1000 grain weight (by 2.9%), and a significant reduction of grain moisture during harvest (by 6.1%) and straw yield (by 9.0%) compared to the control (Table 2, Figure 2).

Variant 2 contributed to a significant increase in grain yield (by 8.8%), plant height (by 4.4%), grain yield per plant (by 8.0%), 1000 grain weight (by 2.7%), and number of grains in the cob (by 6.5%), and a significant reduction in grain moisture during harvest (by 2.3%) and straw yield (by 11.7%) compared to the control variant. Variant 2 was characterized by significantly higher grain yield (by 5.7%), grain moisture (by 4.0%), plant height (by 1.6%), grain yield per plant (by 5.1%), and number of grains in the cob (by 5.4%) in relation to the variant with Penergetic. For almost all traits, a significant ($P < 0.005$) interaction between treatment and environment (year and location) was observed, which means that the effect of the treatments varied depending on the environmental conditions.

Among the examined traits, the highest variability was found in straw yield (CV = 28.7%) and number of grains in the cob (CV = 28.5%), and the lowest variability was observed for 1000 grain weight (CV = 4.4%) (Table 3).

Table 2. Influence of Penergetic activators (Penergetic International AG, Romanshorn, Switzerland) and Azoter bacterial preparation (Azoter Trading, Bratislava, Slovakia) on yield and traits of maize plants (2017–2019) and effects of treatment and environment (location × year) and their interaction.

	Treatment			***p*-Value Based on ANOVA**		
Trait	**0**	**1**	**2**	**Treatment (T)**	**Environment (E: Year x Location)**	**Inter-Action: TxE**
Grain yield (14% H_2O), t ha^{-1}	12.53 a*	12.89 b	13.63 c	<0.001	<0.001	0.205
Grain moisture, %	28.65 c	26.91 a	27.99 b	<0.001	<0.001	<0.001
Yield of straw, t ha^{-1}	31.91 b	29.05 a	28.18 a	<0.001	<0.001	<0.001
Height of plants, cm	260.20 a	267.50 b	271.75 c	<0.001	<0.001	<0.001
Grain yield per plant (14% H_2O), g	170.05 a	174.66 b	183.57 c	<0.001	<0.001	0.088
Weight of 1000 grains (14% H_2O), g	431.88 a	444.25 b	443.50 b	<0.001	<0.001	<0.001
Number of grains per cob, pcs.	391.1 a	395.2 a	416.7 b	<0.001	<0.001	0.017

* Same letters within rows indicate lack of significant difference between means at α = 0.05.

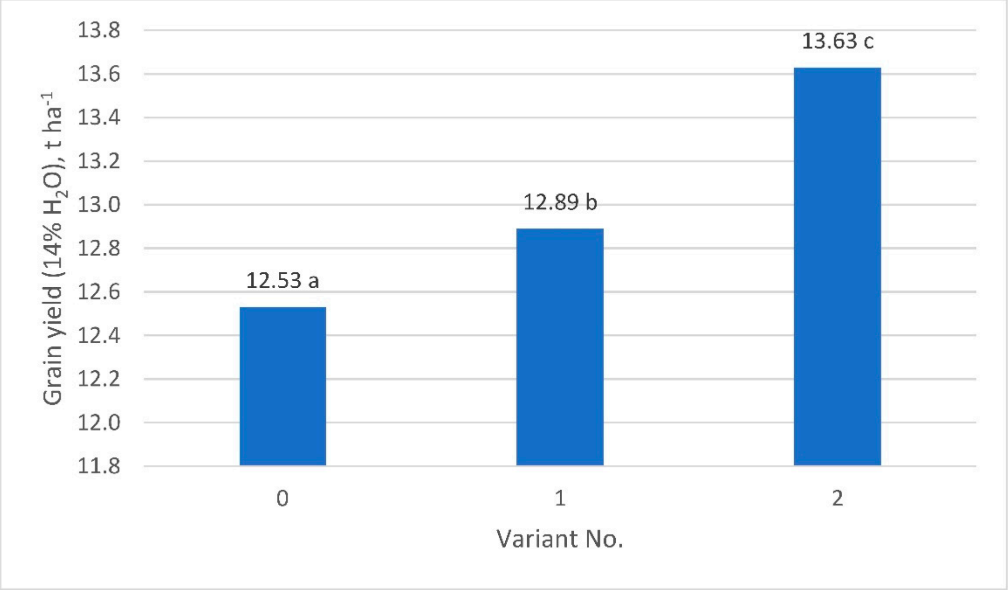

Figure 2. Influence of Penergetic activators (Penergetic International AG, Romanshorn, Switzerland) and Azoter bacterial preparation (Azoter Trading, Bratislava, Slovakia) on yield and traits of maize plants (2017–2019). Same letters next to means indicate lack of significant difference between means at α = 0.05.

Table 3. Descriptive statistics for all experiments with maize (2017–2019).

Trait	Mean	Minimum	Maximum	Standard Deviation (SD)	Coefficient of Variation (CV), %
Grain yield (14% H_2O), t ha^{-1}	13.02	5.90	19.76	3.45	26.49
Grain moisture, %	27.85	20.80	45.20	5.55	19.91
Yield of straw, t ha^{-1}	29.72	17.00	51.50	8.53	28.72
Height of plants, cm	266.48	190.00	319.00	24.58	9.22
Grain yield per plant (14% H_2O), g	176.09	76.82	250.14	49.13	27.90
Weight of 1000 grains (14% H_2O), g	439.88	400.00	476.00	19.29	4.38
Number of grain s per cob, pcs.	401.0	174.6	618.4	114.1	28.45

4. Discussion

The growth activators used in the research accelerated the decomposition of organic matter in the soil and transformed some nutrients in the soil that were unavailable to plants into available forms, and improved their effective uptake by the plants. The bacterial strains *Azotobacter chroococcum* and *Azospirillum brasilense*, which are in the Azoter preparation, participate in the non-symbiotic fixation of atmospheric nitrogen, making it available to plants, and *Bacillus megaterium* participates in the release of phosphorus. Thanks to this effect, maize plants were able to take up a similar amount of nitrogen from the soil and have increased grain yield with nitrogen fertilization reduced by 30–40% compared to the control variant with a full nitrogen dose of mineral fertilizers applied. Similar results were observed for the other nutrients.

The improvement in the content of available macronutrients in the soil under the influence of the applied macronutrients observed in our research was consistent with the results of a similar experiment performed on the cultivation of sugar beet [25]. However, there was no increase in the content of nitrate nitrogen ($N\text{-}NO_3$), ammonium nitrogen ($N\text{-}NH_4$), or mineral nitrogen (Nmin). This may prove that nitrogen was taken up more effectively by maize plants when mineral nitrogen fertilization was lowered by 30–40%. Other authors observed an increase in the content of available phosphorus in the soil as a result of faster mineralization and the release of phosphorus due to the use of PGPR [41]. The use of solubilizing isolates increased the availability of Zn and K elements for plants [42] and the uptake of Zn by maize [43]. The increase in SOC that we obtained is similar to the results of [14], in which *Paraburkholderia nodosa* NB1 improved the total C and organic matter content of the soil.

The use of Penergetic-K and Penergetic-P growth activators with a 30–40% reduction in mineral nitrogen fertilization not only allowed the grain yield to be maintained at the level of the control variant with a full nitrogen dose, but even increased it significantly. The increase in yield was even greater when the Azoter preparation containing PGPR was additionally applied. The more favorable effect of the use of growth activators in combination with a preparation containing PGPR in relation to the growth activators alone was due to the availability of atmospheric nitrogen bound by *Azotobacter chroococcum* and *Azospirillum brasilense* and phosphorus as a result of solubilization by *Bacillus megaterium* for plants. The increased yield of maize grain observed in this case resulted from the increase of two components: the number of grains per cob and the weight of 1000 grains in variant 2. Some authors [14] argue that at least half of the dose of nitrogen and phosphorus could be reduced by using a combination of fertilization with beneficial bacteria. In previous studies, the highest increase in maize grain yield (19.7%) was obtained with a mixture of *Pseudomonas* PS2 and *Bacillus* Q7 with *Azotobacter chroococcum* [12]. In this case, however, no reduction in nitrogen fertilization was tested.

Another similar study evaluated the effects of five plant growth-promoting rhizobacteria (*Bacillus panthothenicus*, *Pseudomonas cichorii*, *Pseudomonas putida*, *Pseudomonas syringae*, and *Serratia marcescens*) on the growth and yield of maize. A half-dose of recommended NPK (13, 17, 17, kg ha^{-1}) was applied. The results showed that the *Serratia marcescens* + 50% NPK treatment yielded the best results for height, fresh underground biomass, dry aboveground biomass, dry underground biomass, and grain yield [44].

Co-inoculation with phosphate solubilizing microorganisms and plant growth promoting rhizobacteria increased the uptake of micronutrients by maize [45]. These mechanisms are still elucidated. Probably the greater amount of phosphorus available to plants stimulates the growth of the root system, which takes up more nutrients, and in this case Zn. Promising growth effects of *Pseudomonas* sp. DSMZ 13,134 on field-grown maize were obtained by Nkebiwe et al. [46]. *Bacillus subtilis* could be used as an inoculant for maize to protect against water stress [47]. Applying Azoter in combination with the growth activators Penergetic-K and Penergetic-P had a more beneficial effect on the biological yield of sugar beet and technological sugar yield than Penergetic (K + P) growth activators. [25].

5. Conclusions

The results obtained in the field experiments performed in this work prove that it is possible to reduce the dose of mineral nitrogen by 30–40% not only to maintain, but even to increase the yield of maize grain using Penergetic-K and Penergetic-P growth activators alone or in combination with Azoter preparation containing PGPR. Further research should focus on an attempt to explain the mechanism of growth activators in the cultivation of agricultural plants.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/10/11/1647/s1, Table S1: Soil conditions before establishing experiment with maize for grain (2016–2018), Table S2: Soil conditions after harvesting maize for grain (2017–2019), Table S3: Weather conditions during the growing season of maize (2017–2019).

Author Contributions: Conceptualization, A.A.; formal analysis, A.A.; investigation, A.A.; methodology, A.A.; supervision, A.A.; visualization, D.G.; writing—original draft, A.A. and D.G.; writing—review and editing, A.A. and D.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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