The Impact of Nitrogen Fertilization and the Use of Biostimulants on the Yield of Two Maize Varieties (Zea mays L.) Cultivated for Grain

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Abstract: The field experiment was carried out in 2015–2017 on a family farm in Toczyski Czortki, in the Mazovia voivodeship in Poland. The experiment was set up in a split–split–plot system in three replications. The area of one plot was 30 m$^2$. The studied factors were: I—two maize varieties: PR38N86 (medium late hybrid cultivar 280 FAO), P8400 (medium early hybrid cultivar 240 FAO); II—four doses of nitrogen fertilization: 1. control object—without the use of nitrogen (0 kg N·ha$^{-1}$), 2. nitrogen dose—80 kg N·ha$^{-1}$ (applied once before sowing), 3. nitrogen dose—120 kg N·ha$^{-1}$ (applied once before sowing), 4. nitrogen dose—160 kg N·ha$^{-1}$ (applied once before sowing); III—four types of biostimulants used: 1. control object—without using a biostimulant, 2. Asahi®SL biostimulant: I term—four-leaf phase (BBCH 14) at a dose of 0.60 dm$^3$·ha$^{-1}$, II term—eight-leaf phase (BBCH 18) at a dose of 0.60 dm$^3$·ha$^{-1}$, 3. Improver® biostimulant: I term—four-leaf phase (BBCH 14) at a dose of 1.00 dm$^3$·ha$^{-1}$, II term—eight-leaf phase (BBCH 18) at a dose of 0.60 dm$^3$·ha$^{-1}$, 4. Zeal® biostimulant: I term—six-leaf phase (BBCH 16) at a dose of 2.00 dm$^3$·ha$^{-1}$. The aim of the study was to determine the effect of nitrogen fertilization and the use of biostimulants on the size and quality of yield of two varieties of maize grown for grain. Based on the conducted research, it was found that nitrogen doses influenced the amount of maize grain obtained. The highest yields were obtained using 120 kg N·ha$^{-1}$. Nitrogen doses significantly affected the studied maize yield components. The dose of 120 kg N·ha$^{-1}$ increased the number of grains in the cob, while the dose—160 kg N·ha$^{-1}$ exerted the best effect on obtaining the highest values of a thousand seeds. The biostimulants used in the experiment significantly affected the mass of one thousand seeds and the number of grains in the cob. In addition, Asahi®SL, Improver® and Zeal® biostimulants increased maize yield in each growing season studied.

Keywords: maize; nitrogen fertilization; biostimulants; cultivar; grain yield

1. Introduction

It is estimated that the human population will reach 9.6 billion by 2050, which will consequently require at least a doubling of the current agricultural production [1]. Increasing demand for food, including plant origin, stimulates activities aimed at improving the fertility and quality characteristics of the obtained crops. Maize (Zea mays L.) is one such plant, which, along with wheat and rice, is one of the three most important crops in the world because of its many uses. The main producers of maize
are the United States, Brazil and China, where maize production in the USA is almost six times higher than in the European Union [2].

The yield of maize grains depends on the availability of easily digestible nutrients, therefore mineral fertilization is most often used. Nitrogen fertilization is the basic element of agrotechnics shaping the maize grain yield, depending on the habitat conditions [3].

Modern agriculture tends to minimize the use of mineral fertilizers and chemical plant protection products, which are replaced by preparations of natural origin [4]. This group of preparations includes biostimulants, one of which primary tasks is to alleviate environmental stress [5].

The definition of biostimulants is broad and not precise enough. There are two main features that distinguish biostimulants from fertilizers and plant protection products. A biostimulant can be any substance or mixture of substances of natural origin that improves the condition of crops without causing undesirable side effects [6].

The effects of biostimulants can be multifaceted. They differ depending on the type of biostimulant used and the plant cultivar. However, it should be noted that most of them have a beneficial effect on crops [7].

Natural preparations, called biostimulants, increase the efficiency of the use of nutrients by plants, tolerance to abiotic stress and improve the quality of crops [8]. Numerous scientific studies have shown that biostimulants have a positive effect on crop yielding and soil quality improvement [9].

Newly developed biopreparation technologies can make a significant contribution to environmental protection, but above all are closely linked to sustainable agricultural and horticultural production in order to obtain cheap, easily available and high-quality food [10].

The aim of the study was to determine the effect of nitrogen fertilization and the use of biostimulants on the size and quality of the yield of two varieties of maize grown for grain.

2. Materials and Methods

2.1. Arrangement of the Experiment and Research Location

The field experiment was carried out in 2015–2017 on a family farm in Toczyski Czortki (52°30′ N and 22°26′ E, Poland). The experiment was set up in a split–split–plot system in three replications. The area of one plot of land was 30 m². The studied factors were:

I. Two maize cultivars: PR38N86 (medium late hybrid cultivar 280 FAO), P8400 (medium early hybrid cultivar 240 FAO).

II. Four doses of nitrogen fertilization:

1. Control object—without the use of nitrogen (0 kg N·ha⁻¹);
2. Nitrogen dose—80 kg N·ha⁻¹ (applied once before sowing);
3. Nitrogen dose—120 kg N·ha⁻¹ (applied once before sowing);
4. Nitrogen dose—160 kg N·ha⁻¹ (applied once before sowing).

III. Four types of biostimulants:

1. Control object—without using a biostimulant;
2. Biostimulator Asahi®SL (active substances: sodium orto nitrophenol, sodium para nitrophenol, sodium 5-nitroguaiacolate), applied in two doses of 0.60 dm³·ha⁻¹ in the spring (22 May 2015, 23 May 2016, 30 May 2017) at the stage of 4 leaves (BBCH 14) according to the rating of Biologische Bundesantalt, Bundessortenamt and Chemische Industrie (BBCH), and 0.60 dm³·ha⁻¹ at the stage of 8 leaves (BBCH 18) (8 June 2015, 7 June 2016, 26 June 2017);
3. Biostimulator Improver® (active substances: potassium ortho nitrophenol, potassium para nitrophenol, potassium 5-nitroguaiacolate), applied in two doses of 1.00 dm³·ha⁻¹ in the
spring (22 May 2015, 23 May 2016, 30 May 2017) at the stage of 4 leaves (BBCH 14), and 0.60 dm$^3$·ha$^{-1}$ at the stage of 8 leaves (BBCH 18) (8 June 2015, 7 June 2016, 26 June 2017);

4. Biostimulator Zeal® (active substances: GA 142, molybdenum, zinc, P$_2$O$_5$), applied in one dose of 2.00 dm$^3$·ha$^{-1}$ (29 May 2015, 27 May 2016, 13 June 2017) at the stage of 6 leaves (BBCH 16).

The studies were carried out on soil classified as lowland soil, of the luvisols, sandy type, classified as very good rye soil complex, of e IVa grade [11]. Soil chemical analysis was performed by the Actra company using the DGPS system and the MUBU 1.0 Expert (F.H.U. ACTRA, Leżajsk, Poland) soil sampling automat. The soil was characterized by a high content of available forms of potassium, phosphorus and magnesium and an average content of mineral nitrogen in the soil layer 0–30 cm and in the soil layer 30–60 cm (Table 1). The analysis of soil chemical properties was carried out in the laboratory of the Regional Chemical and Agricultural Station in Rzeszów.

| Soil Sampling Depth | Year       |         |         |
|---------------------|------------|---------|---------|
|                     | 2015       | 2016    | 2017    |
| 0–30 cm             | 65.24      | 64.80   | 64.91   |
| 30–60 cm            | 63.35      | 64.50   | 63.97   |

Maize for grain (I, II, III year of research) was the previous crop for maize for grain in individual years. After harvesting the previous crop, in order to shred the crop residues and mix them with soil, a set of post-harvest crops was made using disc harrowing, and then the winter ploughing at a depth of 25.0 cm was applied. In early spring, a tooth harrow was used to compensate and stop evaporation and accelerate the heating of the soil. Then, potassium fertilization was applied in the form of potassium salt at a dose of 250 kg·ha$^{-1}$, i.e., 124.5 kg K·ha$^{-1}$. Before sowing maize, nitrogen fertilization was applied in the form of urea 46%: control object—0 kg·ha$^{-1}$, object 80 kg N·ha$^{-1}$—173.9 kg·ha$^{-1}$, object 120 kg N·ha$^{-1}$—260.9 kg·ha$^{-1}$, object 160 kg N·ha$^{-1}$—347.8 kg·ha$^{-1}$ and it was mixed using a tilling set. Maize sowing was carried out with a pneumatic precision seeder with row fertilization (triple superphosphate 46%—71.8 kg·ha$^{-1}$, i.e., 33 kg P·ha$^{-1}$) in rows with 75 cm spacing, maintaining 8 plants·m$^2$.

The number of maize plants after the emergence was determined when young plants developed 4–5 leaves, counting plants over a length of 10 m, middle rows of maize, at each experimental facility. The actual planting per 1 m$^2$ was determined according to the methodology in the field experiments [12].

Maize plants were collected from an area of 1 m$^2$ from each experimental plot, at full grain maturity stage, and the following biometric characteristics were determined:

1. Number of grains in a cob (pcs.);
2. Mass of one thousand seeds was determined in 2 samples of 1000 pieces of seeds taken from the purified crop from each plot;
3. The amount of seed yield from each plot was determined by weight after threshing, and then, after adjusting to normative humidity (15.0% water content in seeds) was calculated per area for 1 ha.

2.2. Statistical Analysis

The study results were statistically analyzed by means of variance analysis. The significance of sources of variation was tested using the “F” Fischer–Snedecor Test, and the significance of differences at the significance level $p = 0.05$ between the compared means was assessed using multiples of Tukey intervals [13].
2.3. Weather Conditions

Climatic data from 2015 to 2017 were obtained from the Hydrological and Meteorological Station in Siedlce. Weather conditions during the experiment were characterized on the basis of average monthly precipitation sums and average monthly air temperatures. During the growing period of plants, variable weather conditions prevailed (Table 2).

| Specification                        | Year | IV   | V    | VI   | VII  | VIII | IX   | X    |
|--------------------------------------|------|------|------|------|------|------|------|------|
| Average monthly air temperature, °C  | 2015 | 8.2  | 12.3 | 16.5 | 18.7 | 21.0 | 14.5 | 6.5  |
|                                      | 2016 | 9.1  | 15.1 | 18.4 | 19.1 | 18.0 | 14.9 | 7.0  |
|                                      | 2017 | 6.9  | 13.9 | 17.8 | 16.9 | 18.4 | 13.9 | 9.0  |
| Average temperature for many years, °C| 1996–2010 | 8.0 | 13.5 | 17.0 | 19.7 | 18.5 | 13.5 | 7.9  |
| Average monthly rainfall, mm         | 2015 | 30.0 | 100.2| 43.3 | 62.6 | 11.9 | 47.1 | 37.0 |
|                                      | 2016 | 28.7 | 54.8 | 36.9 | 35.2 | 31.7 | 13.6 | 69.8 |
|                                      | 2017 | 59.6 | 49.5 | 57.9 | 23.6 | 54.7 | 80.1 | 53.0 |
| Sielianinov hydrothermal coefficient * | 2015 | 1.35 | 2.91 | 0.84 | 1.21 | 0.20 | 1.20 | 2.15 |
|                                      | 2016 | 1.08 | 1.47 | 0.72 | 0.64 | 0.62 | 0.28 | 3.02 |
|                                      | 2017 | 3.82 | 1.52 | 1.07 | 0.47 | 1.01 | 1.92 | 2.36 |

* Coefficient value (Skowera, 2014): extremely dry K ≤ 0.40, very dry 0.4 < K ≤ 0.7, dry 0.7 < K ≤ 1.0, quite dry 1.0 < K ≤ 1.3, optimal 1.3 < K ≤ 1.6, moderately humid 1.6 < K ≤ 2.0, humid 2.0 < K ≤ 2.5, very humid 2.5 < K ≤ 3.0, extremely humid K > 3.0.

The average temperature in 2016 was higher, and in 2015 and 2017 lower than the average in 1996–2010. The average monthly sum of precipitation in all years of the study was very diversified. In 2015 and 2016, precipitation totals were lower, and in 2017 higher than the average for the long-term period. Based on the calculated Sielianinov hydrothermal coefficient, it was found that in 2015 there were optimal conditions for the growth and development of maize plants (K = 1.41). The year 2016 was quite dry (K = 1.12), while 2017 was moderately humid (K = 1.74).

3. Results and Discussion

Studies have shown that the nitrogen dose determined the amount of maize grain yield (Table 3). A significant increase in grain yield, compared to the control object, occurred at a dose of 80 kg N·ha⁻¹, 120 kg N·ha⁻¹ and 160 kg N·ha⁻¹, respectively, by 1.86 t·ha⁻¹, 2.65 t·ha⁻¹ and 2.5 t·ha⁻¹. As a result of increasing the dose to 160 kg N·ha⁻¹, there was a significant reduction in grain yield by an average of 0.15 t·ha⁻¹ compared to the object where 120 kg N·ha⁻¹ was used. The PR38N86 cultivar, after applying 120 kg N·ha⁻¹, obtained significantly the highest value of the discussed feature in comparison with the control object. The P8400 cultivar obtained the highest grain yield after the application of 120 kg N·ha⁻¹ and 160 kg N·ha⁻¹.

Research conducted by Bogucka et al. [14] shows that the optimum dose for the crop of maize grain of the Junak cultivar was 150 kg N·ha⁻¹, and for the Boruta cultivar 180 kg N·ha⁻¹. Higher doses of nitrogen fertilization did not significantly increase the grain yield. Gołębiewska and Wróbel [15] proved in their research that a regular increase in maize grain yield can be obtained by increasing nitrogen fertilization to a dose of 150 kg N·ha⁻¹. Further increase in fertilization did not significantly differentiate maize yield. A similar relation was observed by Pizolato Neto et al. [16] and Silva et al. [17].

Biostimulants increased the maize grain yield from 0.14 to 0.68 t·ha⁻¹ compared to the control object (Table 4). The highest value of the discussed feature—on average 8.42 t·ha⁻¹—was recorded on the object where the Asahi SL biostimulant was used. The PR38N86 and P8400 cultivars had a higher value of the
discussed feature after using all biostimulants compared to the control object. In the PR38N86 cultivar, the highest grain yield was found after using the Asahi SL biostimulant—7.92 t·ha⁻¹. There were no statistically significant differences in grain yield after using Zeal and Improver biostimulants and the control object. While in the P8400 cultivar, the smallest grain yield was significantly observed after application with the Improver biostimulant—8.20 t·ha⁻¹.

Table 3. Maize grain yield (t·ha⁻¹) depending on the growing season, cultivars and nitrogen dose.

| Cultivars | Research Years | Nitrogen Doses, kg·ha⁻¹ | Objects | Average |
|-----------|----------------|--------------------------|---------|---------|
|           | 2015 | 2016 | 2017 | Control Object | 80 | 120 | 160 |
| PR38N86   | 7.71 B | 8.30 B | 6.96 B | 5.98 B | 7.73 B | 8.61 B | 8.31 B | 7.66 b |
| P8400     | 8.60 | 9.15 | 7.47 | 6.57 A | 8.55 | 9.26 | 9.25 | 8.41 a |
| Average   | 8.16 b | 8.72 a | 7.22 c | 6.28 d | 8.14 c | 8.93 a | 8.78 b | - |

Means followed by the same letters do not differ significantly at \( p = 0.05 \). Means in columns marked with capital letters refer to interactions between the factors. Means in the last column and means in the last row (followed by lowercase) are for cultivars, years and nitrogen doses.

Table 4. Maize grain yield (t·ha⁻¹) depending on the growing season, cultivars and type of biostimulants used.

| Type of Biostimulant Used | Research Years | Cultivars | Average |
|---------------------------|----------------|----------|---------|
|                           | 2015 | 2016 | 2017 | PR38N86 | P8400 |       |
| Control object            | 7.70 C | 8.42 C | 7.10 B | 7.52 C | 7.96 D | 7.74 a |
| Asahi SL®                 | 8.69 A | 9.02 A | 7.56 A | 7.92 A | 8.93 A | 8.42 b |
| Zeal®                     | 8.36 B | 8.78 B | 7.10 B | 7.62 B | 8.54 B | 8.08 c |
| Improver®                 | 7.88 C | 8.68 B | 7.10 B | 7.57 C | 8.20 C | 7.89 d |
| Average                   | 8.16 b | 8.72 a | 7.22 c | 7.66 b | 8.41 a | -     |

Means followed by the same letters do not differ significantly at \( p = 0.05 \). Means in columns marked with capital letters refer to interactions between the factors. Means in the last column and means in the last row (followed by lowercase) are for biostimulants, years and cultivars.

Similar research results were obtained by Michalski et al. [18]. These authors reported that the Asahi SL bioregulator, applied once in the BBCH 14–15 phase and once in the BBCH 18–19 phase, increased the yield by an average of 0.46 t·ha⁻¹. In addition, Sulewska and Kruczek [19] and Kowalik and Michalski [20], depending on the growing season, stated an increase in the discussed feature to 15.0% compared to an object without natural growth stimulants.

The number of grains in the cob depended on increasing nitrogen doses (Table 5). A significantly higher number of grains in the cob, by 105.56 pcs. on average compared to the control object, were obtained after the application of 120 kg N·ha⁻¹. A higher nitrogen dose (160 kg N·ha⁻¹) caused a reduction in the number of grains in the cob.

Research conducted by Lepiarczyk et al. [21] do not confirm the effect of nitrogen fertilization doses on the number of grains in a corn cob. However, the existence of such a relation is indicated by Bakht et al. [22].

The biostimulants used in the experiment resulted in an increase in the number of grains in a corn cob on average from 25.04 to 54.36 pcs. compared to the control object (Table 6). The highest value of the discussed feature—on average 508.37 pcs. was found under the influence of the Asahi SL biostimulant, while the lowest—on average 454.01 pcs., on the control object. Research conducted by Księżak [23] does not confirm the significant effect of biostimulants on the amount of grain in a cob.
Table 5. Number of grains in a cob (pcs.) depending on the growing season, cultivars and nitrogen dose.

| Cultivars | Research Years | Nitrogen Doses, kg·ha⁻¹ | Average | Objects |
|-----------|----------------|--------------------------|---------|---------|
|           | 2015           | 2016                     | 2017    | Control Object | 80 | 120 | 160 |
| PR38N86   | 401.77 B       | 510.27 B                 | 452.39 B| 382.11 B       | 469.01 B | 496.63 B | 471.49 B | 454.81 b |
| P8400     | 495.56 A       | 554.86 A                 | 492.61 A| 449.80 A       | 526.46 A | 546.38 A | 534.73 A | 514.34 a |
| Average   | 448.66 c       | 532.57 a                 | 472.50 b| 415.95 d       | 497.73 c | 521.51 a | 503.11 b | - |

Means followed by the same letters do not differ significantly at $p = 0.05$. Means in columns marked with capital letters refer to interactions between the factors. Means in the last column and means in the last row (followed by lowercase) are for cultivars, years and nitrogen doses.

Table 6. Average number of grains in a cob (pcs.) depending on the growing season, cultivar and type of biostimulants used.

| Type of Biostimulant Used | Research Years | Cultivars | Average |
|---------------------------|----------------|-----------|---------|
|                           | 2015           | 2016      | 2017    | PR38N86 | P8400 |
| Control object            | 414.83 C       | 517.27 C  | 429.94 D| 424.11 D| 483.91 C| 454.01 d |
| Asahi SL®                 | 466.57 A       | 548.99 A  | 509.54 A| 484.30 A| 532.44 A| 508.37 a |
| Zeal®                     | 466.69 A       | 533.11 B  | 490.84 B| 463.19 B| 530.56 A| 496.88 b |
| Improver®                 | 446.57 B       | 530.89 B  | 459.69 C| 447.64 C| 510.46 B| 479.05 c |
| Average                   | 448.66 c       | 532.57 a  | 472.50 b| 454.81 b| 514.34 a | - |

Means followed by the same letters do not differ significantly at $p = 0.05$. Means in columns marked with capital letters refer to interactions between the factors. Means in the last column and means in the last row (followed by lowercase) are for biostimulants, years and cultivars.

The impact of biostimulants on the value of the discussed feature depended on the genotype of the plants. An increase in the number of grains in the cob in both studied cultivars was found using the Asahi SL biostimulant.

Studies have shown that the highest mass of one thousand seeds was obtained after the application of 160 kg N·ha⁻¹ (Table 7). The value of this feature at this nitrogen dose was higher by 30.40 g on average compared to the control object, while the smallest mass of one thousand seeds was obtained after the application of 80 kg N·ha⁻¹. A similar tendency was confirmed in the research by Khaliq et al. [24] and Sharar et al. [25].

Table 7. Mass of one thousand seeds (g) depending on the growing season, cultivars and nitrogen dose.

| Cultivars | Research Years | Nitrogen Doses, kg·ha⁻¹ | Average |
|-----------|----------------|--------------------------|---------|
|           | 2015           | 2016                     | 2017    | Control Object | 80 | 120 | 160 |
| PR38N86   | 273.96 A       | 299.26 B                 | 229.02 A| 253.55 A       | 261.98 A | 273.09 A | 281.03 A | 267.41 a |
| P8400     | 261.98 B       | 308.57 A                 | 228.65 A| 249.15 A       | 261.96 A | 272.03 A | 282.48 A | 266.40 a |
| Average   | 267.97 b       | 303.91 a                 | 228.84 c| 251.35 d       | 261.97 c | 272.56 b | 281.75 a | - |

Means followed by the same letters do not differ significantly at $p = 0.05$. Means in columns marked with capital letters refer to interactions between the factors. Means in the last column and means in the last row (followed by lowercase) are for cultivars, years and nitrogen doses.

The biostimulants used in the study significantly increased the mass of a thousand seeds compared to the control object (without a biostimulant). The highest value of the discussed feature—on average 272.45 g, was found on the object that was sprayed with the Asahi SL biostimulant, while the lowest was found after the application of the Zeal and Improver preparations and on the control object. A similar
relation was proven in the research by Michalski et al. [18] and Księżak [23]; however, the differences in the mass of one thousand seeds were not statistically significant.

The interaction of the genetic factor with the types of biostimulants used has also been proven. The bioregulators used in the studied cultivars caused a significant increase in the mass of one thousand seeds compared to the control object. In the PR38N86 cultivar, the differences between the biostimulants used were not statistically significant, while in P8400 there were no significant differences in the mass of one thousand seeds only between Zeal and Improver (Table 8).

Table 8. Mass of one thousand seeds (g) depending on the growing season, cultivars and type of biostimulants used.

| Type of Biostimulant Used | Research Years | Cultivars | Average |
|----------------------------|----------------|-----------------|---------|
|                            | 2015           | 2016           | 2017    | PR38N86 | P8400 | Average |
| Control object             | 252.72 C       | 297.98 BC      | 227.07 A| 261.16 B| 257.36 C | 259.26 c |
| Asahi SL®                  | 276.95 A       | 307.98 A       | 232.43 A| 269.62 A| 275.29 A | 272.45 a |
| Zeal®                      | 272.06 AB      | 306.78 A       | 228.73 A| 270.53 A| 267.85 B | 269.19 b |
| Improver®                  | 270.14 B       | 302.93 AB      | 227.11 A| 268.34 A| 265.12 B | 266.73 b |
| Average                    | 267.97 c       | 303.91 a       | 228.84 b| 267.41 a| 266.40 a | - |

Means followed by the same letters do not differ significantly at p = 0.05. Means in columns marked with capital letters refer to interactions between the factors. Means in the last column and means in the last row (followed by lowercase) are for biostimulants, years and cultivars.

4. Conclusions

The nitrogen doses used in the experiment determined the amount of maize grain obtained. The highest yield was obtained using a dose of 120 kg N·ha⁻¹.

The nitrogen doses studied in the experiment significantly affected the studied yield components. The nitrogen dose of 120 kg N·ha⁻¹ increased the number of grains in the cob, while the dose of 160 kg N·ha⁻¹ had the best effect on obtaining the highest mass values of one thousand seeds.

The biostimulants used in the experiment significantly affected the mass of one thousand seeds and the number of grains in the cob.

The Asahi SL®, Improver® and Zeal® biostimulants increased maize yield in each studied growing season.

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