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EFFECT OF TWO TECHNOLOGIES OF NITROGEN FERTILIZATION ON CONTENTS OF GLYCOALKALOIDS AND AMINO ACIDS IN POTATO TUBERS

ODDZIAŁYWANIE DWÓCH TECHNOLOGII NAWOŻENIA AZOTEM NA ZAWARTOŚĆ GLIKOALKALOIDÓW ORAZ AMINOKWASÓW W BULWACH ZIEMNIAKA

Abstract: The purpose of this study has been to assess the effect of two technologies of top-dressing nitrogen fertilization on contents of total nitrogen, glycoalkaloids (TGA) and amino acids in tubers of a medium-early potato cultivar called Mila. The study was based on a two-factor experiment in a random block design with four replications and involved four different variants of top-dressing nitrogen fertilization: soil and foliar application. In the first series, 1/2 of the total nitrogen rate was introduced as top-dressing soil fertilization and in the second series as foliar application. The rates of NPK were 107, 214, 321 and 428 kg NPK ha^{-1} at a N : P : K ratio equal to 1.00 : 0.44 : 1.25.

Increasing NPK fertilization caused a rise in the total nitrogen and glycoalkaloids in tubers of the test potato cultivar. No effect of the top-dressing nitrogen fertilization technology on the above characteristics was noticed. The content of glycoalkaloids in tubers was positively correlated with the content of total nitrogen, with the dependence being more evident in tubers from objects fertilized with nitrogen introduced to soil than over leaves. The type of top-dressing nitrogen fertilization had impact on the shape of relationships between the total nitrogen content and the content of amino acids. Regarding exogenous amino acids, significant dependences were verified for phenylalanine, methionine and the sum of these amino acids. In the pool of endogenous amino acids, such dependences occurred in the case of glycine and prolamin. The total content of glycoalkaloids (TGA), depending on the method of top-dressing nitrogen fertilization, was positively correlated with the content of asparagic acid and tyrosine and negatively correlated with the presence of alanine, glycine and lycine.

Keywords: total N, glycoalkaloids, TGA, potato tubers, foliar fertilization, nitrogen

Glycoalkaloids are specific toxic substances present in plants belonging to the nightshade family (*Solanaceae*). In potato (*Solanum tuberosum* L.), very high levels of glycoalkaloids, commonly known as solanine, appear in the organs in which metabolism

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is very intense, ie in leaves and stem apexes, as well as in places where tissues have been damaged; less solanine is accumulated in tubers. Potatoes with elevated amounts of glycoalkaloids are characterized by a bitter, burning taste and the content of glycoalkaloids above 20 mg · 100 g^{-1} can be harmful to consumers' health [1–3]. Currently grown cultivars of potatoes contain between 3 to 10 mg TGA 100 · g^{-1} [4, 5]. The quantity of TGA (total glycoalkaloids) is changeable depending on the cultivar, weather conditions during the growing season, disease incidence and occurrence of pests which damage leaves and tubers, rates and types of fertilizers [6–9] Many authors found out that TGA grew steadily under the influence of increasing rates of nitrogen [10–12]. However, in the literature references lack any update information on how the content of TGA is shaped in potato tubers depending on the nitrogen fertilization technology applied.

This paper discusses the effect of two methods of nitrogen fertilization against a background of increasing NPK rates on the content of glycoalkaloids and the content of some exo- and endogenous amino acids in potato tubers.

Material and methods

The study was based on a two-factor field experiment, set up according to the random block method with four replications. The material consisted of an edible, medium-early potato cultivar called Mila. This cultivar produces moderately high yields, contains an average amount of starch, ie 2.4 mg 100 g^{-1} of potato flesh and is very suitable for food processing, including production of crisps, chips as well as dried and frozen potato products.

In a three-year, field experiment, conducted at the Experimental Station in Tomaszkowo near Olsztyn, two top-dressing nitrogen fertilization technologies were compared against a background of increasing NPK fertilization rates. The first-order factor consisted of two series of nitrogen top-dressing: soil (conventional) and foliar (sprays) methods. Top-dressing involved 50 % of the nitrogen rate applied. The nitrogen fertilizer used in the experiment was 46 % urea. The second-order factor comprised increasing mineral fertilization, which consisted of the following rates: 107, 214, 321 and 428 kg NPK ha^{-1} at an N:P:K ratio equal 1.00 : 0.44 : 1.25. The rates of nitrogen applied as top-dressing fertilization treatments were, respectively, 20, 40, 60 and 80 kg N ha^{-1}. Foliar application of this element was performed six times during the whole growing season, using the concentrations of urea equal 2.4, 4.8, 7.2 and 9.6 %. The first spraying was performed a week after the full emergence of potato plants; the following treatments took place at seven-day intervals. The remaining mineral fertilizers, phosphorus and potassium, were introduced in the form of triple granulated superphosphate 45 % P_2O_5 and potassium salt 57 % K_2O.

During the growth of potato plants, ie in the early and after the flowering phase as well as after the vegetative growth, samples of tubers were taken for determination of glycoalkaloids with the colorimetric method according to Bergers [13], total nitrogen content with Kjeldahl's method after open sulfuric acid digestion of plant samples [14] and the amino acid content on an amino acid analyzer of Beckmann Instruments (Beckmann Automatic Amino Acid Analyser).

The results underwent statistical processing with ANOVA at the significance level of $\alpha = 0.05$, using Statistica v. 8.0 software [15].

Results and discussion

Nitrogen fertilization plays a decisive role in shaping the content of all forms of nitrogen substances found in crops. This basic element can modify the chemical composition of plants by increasing the percentage of protein and non-protein nitrogen compounds. High nitrogen fertilization may raise the content of total N up to two-fold compared with the values recorded in potato tubers from objects not fertilized with this component [16]. In a study completed by Ciecko et al [17], the total N content was 0.39 to 0.48 % higher than the control, depending on a cultivar, under the influence of a rate of nitrogen equal 200 kg $N \cdot ha^{-1}$. These results have been confirmed by Wyszkowski [18] who found out that total N increased considerably under the effect of increasing rates of nitrogen introduced as a fertilizer. This author completed a study on four potato cultivars and reported that the content of total nitrogen under the influence of 200 kg $N \cdot ha^{-1}$ rose from 0.34 to 0.61 % versus the concentrations of nitrogen in potato tubers from objects not fertilized with nitrogen. Significant correlation between nitrogen fertilization and total nitrogen content in potato tubers has also been described by Roztropowicz [19]. By raising rates of nitrogen from 40 to 200 kg $N \cdot ha^{-1}$, it was possible to observe a parallel increase in the total and protein nitrogen in potato tubers.

In the experiment presented in this paper, the increasing NPK fertilization caused significant increase in the content of total N determined in harvested potato tubers (Table 1). The maximum quantities of this component, about 17 % higher in the soil top-dressed objects and 23 % in the foliar top-dressed series, were observed under the influence of the highest rate of the fertilizers, ie $N_{160}P_{160}K_{240}$. However, the differences were not confirmed statistically, which means that the technology of fertilization with the main rate of nitrogen applied in the form of top-dressing treatments was not a decisive factor in shaping the content of total nitrogen. The mean amounts of total N in the analyzed tubers were similar and reached 1.47 % in the series where nitrogen was introduced to soil and 1.53 % in the series involving foliar application of nitrogen.

In the present study, NPK fertilization had a significant influence on the synthesis of glycoalkaloids in the potato. This dependence, however, occurred in forming tubers after the flowering phase. Also at that time, the content of TGA in tubers was the highest, on average 4.79 $mg \cdot 100g^{-1}$ in the series top-dressed with nitrogen introduced to soil and 4.88 $mg \cdot 100\ g^{-1}$ in the series where nitrogen was sprayed over leaves. Afterwards, the content of glycoalkaloids in tubers began to decline. At harvest, it was 3.64 and 4.14 $mg \cdot 100\ g^{-1}$ for the respective series. Although no significant differences were found in the content of TGA between the two series, the results suggest that more intense synthesis of glycoalkaloids took place in plants fertilized with nitrogen sprayed over leaves than introduced to soil.

Table 1

The effect of NPK fertilization at two top-dressing nitrogen fertilization technologies on the content of total N and glycoalkaloids in potato tubers

Treatments	Total-N [%] after harvest time	TGA [mg · 100 g^{-1}] at the early flowering phase	TGA [mg · 100 g^{-1}] after flowering phase	TGA [mg · 100 g^{-1}] after harvest time
1. Control (without fertilizers)	1.36	2.82	3.40	2.24
1/2 of nitrogen rate introduced as a top-dressing soil application				
2. $N_{40}P_{17}K_{50}$	1.36	3.83	4.10	2.59
3. $N_{80}P_{34}K_{100}$	1.40	4.62	4.89	2.92
4. $N_{120}P_{51}K_{150}$	1.50	5.05	5.15	4.50
5. $N_{160}P_{68}K_{200}$	1.60	4.77	5.03	4.56
Average	1.47	4.56	4.79	3.64
Correlation coefficient between nitrogen fertilization and TGA content r =		0.89*	0.91*	0.95**
1/2 of nitrogen rate introduced as a top-dressing foliar application				
6. $N_{40}P_{17}K_{50}$	1.36	5.19	3.49	3.50
7. $N_{80}P_{34}K_{100}$	1.43	4.52	4.65	4.43
8. $N_{120}P_{51}K_{150}$	1.65	4.36	5.51	5.31
9. $N_{160}P_{68}K_{200}$	1.68	4.46	5.89	3.32
Average	1.53	4.63	4.88	4.14
Correlation coefficient between nitrogen fertilization and TGA content r =		0.44	0.97**	0.54
LSD (p = 0.05) for: technique of nitrogen fertilization	n.s.	n.s.	n.s.	n.s.
increasing NPK fertilization	0.06	n.s.	1.34	0.97
interaction	n.s.	n.s.	n.s.	n.s.

n.s. – not significant; * – correlation coefficient significant at p = 0.05, ** – correlation coefficient significant at p = 0.01.

Results of determinations from three sampling dates were used for calculating correlation coefficients, which suggested that there was a clear positive correlation between the rate of applied nitrogen and the amount of TGA in tubers. This dependence occurred most evidently in the series with soil top-dressing nitrogen fertilization. On each of the examined dates, a high correlation coefficient in the early flowering phase (r = 0.89), after flowering (r = 0.91) and after harvest (r = 0.95) suggested significant dependence between the rate of nitrogen fertilization and the content of glycoalkaloids in tubers.

According to reported data [20] glycoalkaloids gathered in tubers can be indirectly dependent on nitrogen fertilization. Potato glycoalkaloids are a by-product (waste) occurring during metabolic transformations in potato plants, which may explain their larger accumulation in tubers from objects receiving more nitrogen with fertilizers [21].

Besides shaping the total N content in plants, nitrogen fertilization can also modify the amino acid composition of protein and therefore affect protein content and quality. In the present experiment, concentrations of some exogenous and endogenous amino acids were determined, which enabled the author to trace dependences between their quantities and the volume of an NPK rate or the content of solanine in tubers (Tables 2 and 3).

Having analyzed the composition of amino acids in potato tubers, it was demonstrated that only one endogenous amino acid, namely glycine, was highly significantly correlated (r = –0.92) with the content of total N in tubers of potatoes top-dressed with nitrogen introduced to soil, whereas in the series with nitrogen sprayed over leaves such a dependence occurred in the case of two amino acids: glycine (r = –0.85) and, positively correlated, prolamin (r = 0.95). Regarding exogenous amino acids (Table 3), the following were dependent on the content of total N in the series fertilized by soil application of nitrogen: phenylalanine (r = –0.95), methionine (r = –0.88) and sum of exogenous amino acids (r = –0.85). In the series in which nitrogen was sprayed over leaves, only methionine proved to be dependent (r = –0.87).

The content of solanine determined in potato tubers proved to be associated with certain amino acids. Glycoalkaloids are a product appearing during secondary metabolism in potato plants. Should toxic substances, a waste product in potato tubers, be produced in excessive amounts, the secondary metabolism mechanism is activated, which aids synthesis of solanine. This situation occurs when nitrogen fertilization is high. When the level of available nitrogen rises, plants take advantage of sugars and nitrogen-free compounds to synthesize nitrogen acceptors, especially asparagic and glutamic acids. In general, these amino acids are not used up for synthesis of protein but for production of glycoalkaloids during secondary metabolism. The substrates used for their synthesis are not tolerated by plants, therefore they have to be detoxicated via their conversion into less toxic solanine [21], so significant correlations between the content of solanine and the amounts of such amino acids as aspartic acid and glutamic acid are justify [20].

In the present experiment, the content of glycoalkaloids in tubers was correlated with the content of such endogenous amino acids as alanine, aspartic acid and glycine in the series with top-dressed nitrogen applied to soil (Table 2) as well as tyrosine in the series

Table 2

The effect of NPK fertilization at two top-dressing nitrogen fertilization technologies on the content of some endogenous amino acids in potato tubers, in g · 100 g^{-1} of total protein

Treatments	Ala	Asp	Cys-Cys	Glu	Gly	Pro	Ser	Tyr	Sum of endogenous amino acids
1. Control (without fertilizers)	3.84	11.32	0.76	14.06	3.17	3.39	3.46	3.08	43.08
1/2 of nitrogen rate introduced as a top-dressing soil application									
2. $N_{40}P_{17}K_{50}$	3.81	13.00	0.79	13.58	3.21	3.89	3.55	3.16	44.99
3. $N_{80}P_{34}K_{100}$	3.65	12.87	0.70	13.60	3.19	4.19	3.32	3.10	44.62
4. $N_{120}P_{51}K_{150}$	3.63	15.69	0.63	13.48	2.91	4.05	3.33	3.11	46.83
5. $N_{160}P_{68}K_{200}$	3.62	14.14	0.69	13.35	2.91	4.04	3.33	3.14	45.22
Average	3.68	13.93	0.70	13.50	3.06	4.04	3.38	3.13	45.42
correlation coefficient between amino acids content and total N content *r* =	–0.80	0.70	–0.69	–0.74	–0.92**	0.47	–0.68	0.26	0.56
correlation coefficient between amino acids content and TGA content *r* =	–0.85*	0.90*	–0.83	–0.80	–0.96**	0.58	–0.69	0.25	0.80
1/2 of nitrogen rate introduced as a top-dressing foliar application									
6. $N_{40}P_{17}K_{50}$	3.73	14.61	0.71	13.33	3.06	3.55	3.47	3.23	45.69
7. $N_{80}P_{34}K_{100}$	3.73	14.49	0.64	12.86	2.97	3.76	3.38	3.22	45.05
8. $N_{120}P_{51}K_{150}$	3.77	15.16	0.64	13.92	2.85	3.84	3.30	3.25	46.73
9. $N_{160}P_{68}K_{200}$	3.56	15.33	0.65	13.54	2.86	4.35	3.35	3.19	46.83
Average	3.70	14.90	0.66	13.41	2.94	3.88	3.38	3.22	46.08
correlation coefficient between amino acids content and total N content *r* =	–0.78	0.62	–0.65	0.13	–0.85*	0.95**	–0.77	0.28	0.76
correlation coefficient between amino acids content and TGA content *r* =	–0.06	0.71	–0.82	–0.27	–0.73	0.28	–0.76	0.86*	0.65

* – correlation coefficient significant at p = 0.05, * – correlation coefficient significant at p = 0.01; Ala – alanine, Glu – glutamic acid, Ser – serine, Asp – aspartic acid, Gly – glycine, Tyr – tyrosine, Cys-Cys – cystine + cysteine, Pro – proline.

Tabela 3

The effect of NPK fertilization at two top-dressing nitrogen fertilization technologies on the content of some exogenous amino acids in potato tubers, in g · 100 g^{-1} of total protein

Treatments	Arg	Phe	His	Ile	Leu	Lys	Met	Thr	Val	Sum of exogenous amino acids
1. Control (without fertilizers)	3.12	3.75	1.57	3.43	5.84	4.91	1.24	3.15	4.34	31.35
1/2 of nitrogen rate introduced as a top-dressing soil application										
2. $N_{40}P_{17}K_{50}$	3.44	3.71	1.60	3.59	6.02	4.75	1.35	3.24	4.31	32.01
3. $N_{80}P_{34}K_{100}$	3.29	3.67	1.56	3.33	5.73	4.73	1.20	3.02	4.20	30.73
4. $N_{120}P_{51}K_{150}$	3.36	3.65	1.50	3.29	5.83	4.58	1.20	3.02	4.30	30.73
5. $N_{160}P_{68}K_{200}$	3.30	3.70	1.61	3.30	5.45	4.59	1.06	3.03	4.50	30.54
Average	3.35	3.68	1.57	3.38	5.76	4.66	1.20	3.08	4.33	31.00
correlation coefficient between amino acids content and total N content *r* =	0.53	–0.95**	–0.35	–0.78	–0.72	–0.76	–0.88*	–0.75	–0.45	–0.85*
correlation coefficient between amino acids content and TGA content *r* =	0.32	–0.64	–0.27	–0.72	–0.62	–0.94*	–0.72	–0.70	0.49	–0.72
1/2 of nitrogen rate introduced as a top-dressing foliar application										
6. $N_{40}P_{17}K_{50}$	3.54	3.73	1.43	3.31	5.55	4.74	1.14	3.16	4.40	31.00
7. $N_{80}P_{34}K_{100}$	3.28	3.66	1.42	3.23	5.36	4.77	1.02	3.08	4.32	30.14
8. $N_{120}P_{51}K_{150}$	3.52	3.62	1.49	3.24	5.30	4.70	1.06	3.01	4.26	30.20
9. $N_{160}P_{68}K_{200}$	3.51	3.57	1.42	3.17	5.30	4.66	0.82	3.06	4.15	29.66
Average	3.46	3.65	1.44	3.24	5.38	4.72	1.01	3.08	4.28	30.25
correlation coefficient between amino acids content and total N content *r* =	0.15	–0.41	0.01	–0.71	–0.82	–0.83	–0.87*	–0.67	–0.43	–0.75
correlation coefficient between amino acids content and TGA content *r* =	0.51	–0.51	–0.40	–0.61	–0.79	–0.60	–0.30	–0.77	–0.16	–0.72

* – correlation coefficient significant at p = 0.05, ** – correlation coefficient significant at p = 0.01; Arg – arginine, Ile – isoleucine, Met – methionine, Phe –phenylanine, Leu – leucine, Thr – threonine, His – histidine, Lys – lysine, Val – valine.

in which nitrogen was sprayed over leaves. Among the exogenous amino acids (Table 3), it was only tubers from objects receiving soil top-dressing nitrogen fertilization that the content of lysine proved to be closely correlated with the content of total N (r = –0.94). In the series with foliar application of nitrogen, no significant dependences were observed between the content of amino acids and the content of total nitrogen in tubers.

Conclusions

1. Application of increasing NPK fertilization rates caused significant increase in the content of total N in potato tubers. The technology used for top-dressing fertilization with nitrogen did not play a decisive role in shaping the content of this component in tubers.

2. Under the influence of rising NPK rates, the content of glycoalkaloids was observed to have increased in tubers of the examined potato cultivar. Correlation coefficients suggest that the level of TGA in tubers was much more dependent on the volume of NPK rates in the series with top-dressing nitrogen fertilization applied to soil than in the series with foliar application of nitrogen.

3. The content of total N, which was shaped in tubers under the effect of the applied NPK rates, in general was negatively correlated with the content of exogenous amino acids, especially phenylalanine, methionine and sum of exogenous amino acids, in the series with the top-dressing nitrogen fertilization applied as a soil treatment and methionine in the series of top-dressing nitrogen fertilization applied as sprays over leaves. Regarding endogenous amino acids, significant correlation was determined for the content of glycine and prolamin.

4. The content of TGA in the analyzed tubers was negatively correlated with the content of alanine, glycine and lysine, being positively correlated with the content of asparagine acid in the series with nitrogen introduced as a foliar treatment, and positively correlated with the content of tyrosine in the series with nitrogen introduced to soil.

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ODDZIAŁYWANIE DWÓCH TECHNOLOGII NAWOŻENIA AZOTEM NA ZAWARTOŚĆ GLIKOALKALOIDÓW ORAZ AMINOKWASÓW W BULWACH ZIEMNIAKA

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Abstrakt: Celem pracy było określenie wpływu dwóch technologii pogłównego nawożenia azotem na zawartość azotu ogólnego, glikoalkaloidów (TGA) i aminokwasów w bulwach ziemniaka średnio-wczesnego odmiany Mila. Badania oparto na doświadczeniu dwuczynnikowym prowadzonym w układzie losowanych bloków w czterech powtórzeniach, z różnymi wariantami pogłównego nawożenia azotem – doglebowo i dolistnie. W pierwszej serii 1/2 ogólnej dawki N stosowano pogłównie w formie doglebowej, a w drugiej w postaci oprysku nalistnego. Dawki NPK wynosiły kolejno 107, 214, 321 i 428 kg NPK · ha^{-1} przy stosunku N : P : K – 1.00 : 0.44 : 1.25.

Wzrastające nawożenie NPK spowodowało wzrost zawartości N-ogółem oraz glikoalkaloidów w bulwach uprawianej odmiany ziemniaka. Nie stwierdzono wpływu technologii stosowania azotu aplikowanego pogłównie na wymienione cechy. Zawartość glikoalkaloidów w bulwach była dodatnio skorelowana z zawartością N-ogółem, przy czym zależność ta była bardziej widoczna w przypadku bulw pochodzących z obiektów nawożonych azotem pogłównie doglebowo niż dolistnie. Sposób pogłównego nawożenia azotem wpłynął na kształtowanie się zależności pomiędzy zawartością N-ogółem a zawartością aminokwasów. W odniesieniu do aminokwasów egzogennych zależności istotne stwierdzono w przypadku fenyloalaniny, metioniny i sumy tych aminokwasów, a w puli aminokwasów endogennych w odniesieniu do glicyny i prolaminy. Zawartość glikoalkaloidów (TGA), w zależności od sposobu nawożenia pogłównego azotem była dodatnio skorelowana z zawartością kwasu asparaginowego i tyrozyny oraz ujemnie z zawartością alaniny, glicyny i lizyny.

Słowa kluczowe: N-ogólny, glikoalkaloidy, TGA, bulwy ziemniaka, nawożenie dolistne, azot