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ORIGINAL PAPER

INTERACTION EFFECTS OF 6-BENZYLAMINOPURINE (BAP) AND INDOLE-3-BUTYRIC ACID (IBA) ON MICRO- AND MACRONUTRIENT CONTENT IN *MEDICAGO* X *VARIA* T. MARTYN*

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Abstract

The purpose of the research was to determine the effects of synthetic plant hormones in the form of IBA (Indole-3-butyric Acid) and BAP (6-Benzylaminopurine) on the content of selected micro- and macronutrients in alfalfa dry matter (DM). In March 2014, a pot experiment was set up in the experimental room at the Faculty of Natural Sciences of the Siedlce University of Natural Sciences and Humanities (52.169°N, 22.280°E – Poland). The experiment, including the control unit, was completely randomised, with four replications. It was conducted in 40 pots, with four pots in each variant and 3 plants in each pot. Alfalfa seeds were sown in mid-March to a depth of 2-3 cm. After the seeds germinated, 3 representative plants in each pot were left for further research. The experimental factor consisted of two growth regulators: synthetic auxin and synthetic cytokinin. Experimental factors were as follows: K – control; IBA (Indole-3-butyric Acid); BAP (6-Benzylaminopurine); IBA+BAP (Indole-3-butyric Acid + 6-Benzylaminopurine). The application time was dependent on the experimental variant; the plants were treated at the sixth true leaf stage and at the first flower bud stage. The total content of K, Ca, Mg, Fe, Mo, Zn, Cu, and Mn was determined with the ICP-AES method. Furthermore, the ratios of macronutrients Ca:Mg, Ca:P and K:(Ca+Mg) were calculated. The application of a mixture of IBA and BAP in the vegetative stage of alfalfa increased the concentration of K and Mg in plants. The concentration of P, Zn, and Cu in dry matter of plants did not change under the influence of the type and time of application. Treating plants with a mixture of IBA and BAP lowered the Ca:Mg and Ca:P ratios, but they still remained at a very high level. The experiment showed that the application of Indole-3-butyric Acid and 6-Benzylaminopurine in the generative stage of plants did not affect the micro- and macronutrient content in *Medicago x varia* T. Martyn in a statistically significant way.

Keywords: alfalfa, macronutrients rations, synthetic auxin, synthetic cytokinin, chemical composition, growth regulator.

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INTRODUCTION

Nutritional quality of plants depends on the content of chemical elements essential in the human and animal diet. Plant chemical composition is affected by many factors, and it may be modified, among other things, by weather conditions prevailing during the growing season. Plant hormonal substances, according to many authors (Czerpak et al. 1994, Mazur et al. 2001, Friml et al. 2003, Jenik, Barton 2005, Cheng et al. 2006, Xiao-Ping, Xi-Gui 2006, Cheng et al. 2007, Sosnowski et al. 2017), are involved in the regulation of nutrient distribution through their impact on root elongation, which is associated with an increased uptake of soil nutrients. Indole-3--butyric Acid, as a synthetic auxin, and 6-Benzylaminopurine, as a synthetic cytokinin, have an ability to transport food substances in plants (Friml et al. 2003, Jenik, Barton 2005). With the development of the root system as a result of synthetic growth regulator application, the uptake of soil nutrients increases (Hwang, Sakakibara 2006, Sosnowski et al. 2017). Le Bail et al. (2010) reported that auxins play the greatest role here; they are sent as signals informing about physiological processes in plant parts and about an increasing demand for nutrients, at the same time attracting them. With a huge amount of nutrients absorbed from the soil by the root system, it is important that their largest possible quantity should be transported to generative organs (seeds), during the aging process of vegetative parts. Seeds of plants, especially of *Fabaceae*, are a rich source of Ca, P, and K in human nutrition (Griffin et al. 1994, Kocira et al. 2018, Sosnowski et al. 2017). Wierzbowska and Bowszys (2008) provide various, often conflicting, data about the impact of synthetic hormonal substances on the content of mineral elements in legumes, heavily dependent on the growing conditions, weather, species, and variety.

The purpose of the research was to determine the effects of synthetic plant hormones in the form of Indole-3-butyric Acid (IBA) and 6-Benzylaminopurine (BAP) on the content of selected micro- and macronutrients in *Medicago x varia* T. Martyn dry matter (DM). The experiment was to answer the question whether the interaction of these compounds would affect an increase in the concentration of K, Ca, Mg, P, Mo, Zn, Cu, Mn and the ratio of Ca:Mg, Ca:P and K:(Ca + Mg) in plants.

MATERIALS AND METHODS

The conditions of the experiment

In March 2014, a pot experiment with *Medicago* x *varia* T. Martyn cv. Kometa was set up in the experimental room at the Faculty of Natural Sciences of the Siedlce University of Natural Sciences and Humanities, Poland. The experimental conditions were as follows: the temperature of 24 ± 2/16 ± 2°C, the photoperiod of 16/8 h, the light intensity of 200 μmol m^{-2} s^{-1} achieved through the use of high-pressure sodium lamps, and the humidity of 40%. The experiment, including the control unit, was completely randomised, with four replications. It was conducted in 40 pots, with four pots in each variant and 3 plants in each pot. The experimental factor consisted of two growth regulators: synthetic auxin and synthetic cytokinin.

The pots were filled with 5 kg of soil taken from the arable topsoil level, composed of loamy medium sand, class 3 according to the Polish classification system. An analysis carried out at the Regional Chemical Station in Wesoła found that the soil had a very high content of assimilable P and Mg, a high content of K, Cu, and Zn, and a medium content of B, Mn, and Fe (Table 1). Alfalfa seeds were sown in mid-March to a depth of 2-3 cm. After the seeds germinated, 3 representative plants in each pot were left for further research.

Experimental factors (Table 2) were as follows:

Table 1

Soil chemical composition

pH in KCL Humus (%)		C_{org} (g kg^{-1}) Dry matter (%) Humidity (%)		
6.3	3.0	17.1	86	12
Content of mineral N (mg kg^{-1} DM)		Total content of macroelements (g kg^{-1} DM)		
$N-NO_3$	$N-NH_4$	P	K	Mg
1.4	60.9	0.48	0.13	0.10
Total content of microelements (mg kg^{-1} DM)				
B	Mn	Cu	Zn	Fe
2.3	191	8.7	22.3	1570

Table 2

Methodological data

Treatment	Stage	
	F1 (dm^3 pot^{-1})	F2 (dm^3 pot^{-1})
K	water - 0.2	water - 0.2
IBA F_1	IBA - 0.2	water - 0.2
BAP F_1	BAP- 0.2	water - 0.2
IBA+BAP F_1	IBA+BAP - 0.2	water - 0.2
IBA F_2	water - 0.2	IBA - 0.2
BAP F_2	water - 0.2	BAP- 0.2
IBA+BAP F_2	water - 0.2	IBA+BAP - 0.2

K – control (distilled water), IBA – Indole-3-butyric Acid, BAP – 6-Benzylaminopurine, IBA+BAP – Indole-3-butyric Acid+6-Benzylaminopurine, F1 – sixth true leaf stage, F2 – first flower bud stage.

- K – control (distilled water);
- IBA – Indole-3-butyric Acid (synthetic auxin, the concentration of 30 mg dm^{-3});
- BAP – 6-Benzylaminopurine (synthetic cytokinin, the concentration of 30 mg dm^{-3});
- IBA + BAP – Indole-3-butyric Acid + 6-Benzylaminopurine (the quantitative ratio of 1:1).

The application time was dependent on the experimental variant; the plants were treated at the sixth true leaf stage (F1- BBCh 31) and at the first flower bud stage (F2- BBCh 51). The plants were sprayed until they were thoroughly wet, with 0.20 dm^3 of spray liquid per pot. When the flowers had faded, the aerial part of plants was harvested.

Determination of total macro- and micronutrient content

Plant samples intended for further chemical analysis were dried and ground. The total content of P, K, Ca, Mg, Fe, Mo, Zn, Cu, and Mn was determined with the ICP-AES method after the plant material of *Medicago x varia* T. Martyn had been dry-mineralized in a muffle furnace at a temperature of 450°C. Next, 5 ml of diluted HCl (1:1) was added into the crucible, and the solvent was evaporated on a sand bath in order to decompose carbonates and remove silica. Then, 10 ml of 10% HCl was added and the contents were transferred quantitatively into a 100 ml volumetric flask. The content of analyzed elements was determined in samples thus prepared. The concentrations of evaluated macro- and micronutrients were expressed in g kg^{-1} and mg kg^{-1} DM, respectively. Furthermore, the ratios of macronutrients Ca:Mg, Ca:P and K:(Ca+Mg) were calculated.

Data analysis

The results, i.e. the concentration of chemical elements in the biomass, were statistically processed, with an analysis of variance. The Tukey's test was used to determine $HSD_{0.05}$ for means that were significantly different, and the Statistica 12 program was used for all the calculations.

RESULTS

The results of the research (Table 3) showed that the P content in dry matter ranged from 2.45 to 3.10 g kg^{-1}, but neither the growth regulators nor the application time affected its content statistically significantly. However, in the case of K, both the type of regulator and the application time (development stage at the time of treatment) affected the concentration of this chemical element in the dry matter of *Medicago x varia* T. Martyn. The highest

Table 3

The effect of synthetic hormones and the time of application on macronutrient content (g kg^{-1} DM) in *Medicago x varia* T. Martyn dry matter

Treatment	Date of spray		Mean
	F1	F2	
P			
K	2.45^{Aa}	3.04^{Aa}	2.75^{A}
IBA	3.01^{Aa}	3.06^{Aa}	3.04^{A}
BAP	2.97^{Aa}	2.65^{Aa}	2.81^{A}
IBA+BAP	3.02^{Aa}	3.10^{Aa}	3.02^{A}
Mean	2.86^{a}	2.96^{a}	
K			
K	14.9^{Ca}	15.0^{Ab}	15.0^{B}
IBA	19.2^{ABa}	14.8^{Ab}	17.0^{A}
BAP	18.9^{Ba}	15.3^{Ab}	17.1^{A}
IBA+BAP	19.9^{Aa}	14.7^{Ab}	17.3^{A}
Mean	18.2^{a}	15.9^{b}	
Mg			
K	2.18 ^{Ca}	2.20^{Aa}	2.19^{C}
IBA	3.09 ^{Ba}	2.16^{Ab}	2.63^{BA}
BAP	3.02 ^{Ba}	1.98^{Ab}	2.50^{B}
IBA+BAP	3.61 ^{Aa}	2.07^{Ab}	2.84^{A}
Mean	2.98 ^{a}	2.10^{b}	
Ca			
K	12.9^{Ca}	12.9^{Aa}	12.9^{C}
IBA	16.8^{Aa}	13.1^{Ab}	15.0^{A}
BAP	16.6^{Aa}	13.3^{Ab}	15.0^{A}
IBA+BAP	14.9^{Ba}	13.2^{Ab}	14.1^{B}
Mean	15.3^{a}	13.1^{b}	

Means in lines marked with the same small letters do not differ significantly.
Means in columns marked with the same capital letters do not differ significantly.
K – control (distilled water), IBA – Indole-3-butyric Acid, BAP – 6-Benzylaminopurine, IBA+BAP – Indole-3-butyric Acid + 6-Benzylaminopurine.

concentration of K (19.9 g kg^{-1} DM) was when a mixture of 6-Benzylaminopurine and Indole-3-butyric Acid was applied in the vegetative stage (F1) (33.6% increase relative to the control). Similarly, its content grew in relation to the control in the plants treated with Indole-3-butyric Acid on its own (by 28.9%) and in plants treated with 6-Benzylaminopurine on its own (by 26.8%). Treatment during the generative stage (F2) did not alter the content of K, with a similar trend noticed in the case of Mg. The highest content of Mg was in the plant material collected from the units treated with a mixture of IBA and BAP. The Mg content in dry matter, as a mean amount for the vegetative and generative stages, was 2.84 g kg^{-1}, being 29.7% higher than the control. The use of IBA or BAP, both on their own, also increased the Mg content (by 20.1% and 14.2% respectively). It should be noted that the use of these chemicals in the form of spray resulted in a statistically significant increase in the concentration of this chemical element in the dry matter of *Medicago x varia* T. Martyn, but only in the vegetative stage (F1). Interestingly, the average Mg concentration in the dry matter of plants treated during the vegetative phase was 41.9% higher than the content of this macronutrient in plants treated during the generative stage. The research also showed the impact of IBA and BAP on the content of Ca in biomass. The use of the regulator mixture in the vegetative stage resulted in a significant increase in the Ca content in plants (an increase of about 15.5% in respect to controls). However, the highest content of Ca was observed in plants treated with IBA or BAP alone (an increase by 30.0% and 28.7% respectively in comparison to control). Similarly to K and Mg, the use of the regulators in the generative stage (F2) did not affect the Ca content.

The regulators affected highly differently the concentrations of trace elements in the biomass of *Medicago x varia* T. Martyn. As seen in Table 4, the application of the IBA and BAP mixture and IBA on its own contributed to a statistically significant increase in the Mn content in dry matter (by 17.0% and 16.1% respectively higher than the control). The concentration of this chemical element in plants treated this way ranged from 53.4 to 53.8 mg kg^{-1} DM, with the treatment during the generative stage not affecting the Mn content. Additionally, the regulators also affected the Mo content, but the experimental results indicated that only the treatment with IBA or BAP used on their own resulted in an increased content of this element in dry matter relative to control. The increase, as an average response of plants treated twice with those two regulators, was 19%. The combined use of both substances did not bring about any distinct, statistically significant effects. It should be noted that the analysis of the results showed no statistically significant change in the concentrations of Zn and Cu in dry matter of *Medicago x varia* T. Martyn as an effect of different experimental factors, like the type of the applied regulator or the date of its application.

Differences in the content each macroelement under the influence of IBA and BAP application affected the values of the ratio of these chemical elements in plant biomass (Table 5). It turned out that the highest Ca:Mg ratio, as an average of the content of these elements determined at the two development stages, was in plants treated with BAP. This was due to a large increase in the content of Ca, relative to the control, under the influence of this experimental factor and a small increase in the content of Mg. The lowest value of this parameter, 5.25, occurred after an application of the

Table 4

The effect of synthetic hormones and the time of application on trace element content (mg kg^{-1} DM) in *Medicago x varia* T. Martyn dry matter

Treatment	Date of spray		Mean
	F1	F2	
		Mn	
K	44.6^{Ba}	47.3^{Aa}	46.0^{B}
IBA	58.9^{Aa}	47.8^{Ab}	53.4^{A}
BAP	50.2^{Ba}	48.4^{Aa}	49.3^{AB}
IBA+BAP	59.3^{Aa}	48.2^{Ab}	53.8^{A}
Mean	53.3^{a}	47.9^{b}	
		Zn	
K	24.3^{Aa}	22.7^{Aa}	23.5^{A}
IBA	23.2^{Aa}	24.4^{Aa}	23.8^{A}
BAP	24.2^{Aa}	23.9^{Aa}	24.1^{A}
IBA+BAP	25.0^{Aa}	24.1^{Aa}	24.6^{A}
Mean	24.2^{a}	23.8^{a}	
		Cu	
K	6.11^{Aa}	6.20^{Aa}	6.16^{A}
IBA	6.24^{Aa}	6.16^{Aa}	6.20^{A}
BAP	6.16^{Aa}	6.11^{Aa}	6.14^{A}
IBA+BAP	6.20^{Aa}	6.09^{Aa}	6.15^{A}
Mean	6.20^{a}	6.14^{a}	
		Mo	
K	0.43^{Ba}	0.40^{Aa}	0.42^{B}
IBA	0.54^{Aa}	0.46^{Ab}	0.50^{A}
BAP	0.53^{Aa}	0.45^{Ab}	0.49^{A}
IBA+BAP	0.50^{ABa}	0.42^{Ab}	0.46^{AB}
Mean	0.50^{a}	0.43^{b}	

Explanations see Table 3

IBA and BAP mixture. A similar trend was noted in the case of the Ca:P ratio. In turn, the ratio of K ions to the sum of Ca and Mg ions did not substantially vary under the influence of experimental factors.

Table 5

The effect of synthetic hormones and the time of application on chemical element ratio in *Medicago x varia* T. Martyn dry matter

Treatment	Date of spray		Mean
	F1	F2	
Ratio Ca:Mg			
K	5.92 ^{Aa}	5.86 ^{Ba}	5.89 ^{B}
IBA	5.44^{Ab}	6.06^{ABa}	5.75 ^{B}
BAP	5.51^{Ab}	6.72^{Aa}	6.12 ^{A}
IBA+BAP	4.13^{Ba}	6.38^{Aa}	5.25 ^{C}
Mean	5.25^{b}	6.26^{a}	
Ratio Ca:P			
K	5.27^{ABa}	4.24^{Bb}	4.78^{AB}
IBA	5.58^{Aa}	4.28^{Bb}	4.93^{AB}
BAP	5.59^{Aa}	5.02^{Ab}	5.31^{A}
IBA+BAP	4.93^{Ba}	4.26^{Bb}	4.60^{B}
Mean	5.34^{a}	4.45^{b}	
Ratio K:(Ca + Mg)			
K	0.99^{Aa}	0.99^{Aa}	0.99^{A}
IBA	0.97^{Aa}	0.97^{Aa}	0.97^{A}
BAP	0.96^{Aa}	1.00^{Aa}	0.98^{A}
IBA+BAP	1.00^{Aa}	0.96^{Aa}	0.98^{A}
Mean	0.98^{a}	0.98^{a}	

Explanations see Table 3

DISCUSSION

Organic compounds with hormonal properties are used as growth regulators. They have an ability to attract nutrients and they also regulate the distribution and accumulation of substances important for the biological value of plant material. (Panwar et al. 1990, Nowak et al. 1991, 1997, Czapla et al. 2003). In the literature (Meuwly, Pilet 1991, Karcz et al. 1996, Ali et al. 2008) various, often conflicting, opinions can be found about the accumulation of chemical elements in different plant species after an application of exogenous growth regulators. As it is known, the content of minerals in plants can be modified by multiple internal and external factors. In the present experiment, a beneficial effect of the applied substances on the amount of macronutrients accumulated in the biomass plant was demonstrated. However, their content was varied depending on the type of treatment and on the plant development stage, i.e. the date of application. It was found that there was an impact of the interaction between IBA and BAP (synthetic auxin and cytokinin) on the increase in K and Mg content in dry matter of *Medicago x varia* T. Martyn. However, the amount of Ca was the highest in plants treated with IBA – auxin or BAP – cytokinin on their own. The combination of these regulators decreased the content of this chemical element, but it was still significantly higher than its content in the control. Czapla et al. (2003) reported lower K content, on average by 9%, and slightly lower Ca and Mg amounts in soybean after treating plants with synthetic auxins: IBA and NAA, IBA + NAA. The literature reports of highly varied macronutrient content in plants, which may have been caused by different climatic and growing conditions during the course of the experiment (Karcz et al. 1996, Barclay et al. 1998). Prusiński and Borowska, (2002) observed a decrease in the K content in plants, especially in seeds, under the influence of IBA, BAP, and IBA + BAP regulators.

In the present experiment, the growth regulators (IBA and BAP) did not affect the P content. This lack of effect was also confirmed by the studies of Czapla et al. (2003) and Nowak et al. (1997), who found no changes in the concentration of this chemical element in the seeds of spring field bean, soybean, and lupin after application of synthetic auxins and cytokinins. In the literature (Meuwly et al. 1991, Svenson 1991, Ali et al. 2008) it was noted that an increase in the content of certain minerals in the aerial parts after applying synthetic plant hormones may be caused by the stronger development of the root system, especially the elongation of the root hair zone. Consequently, this leads to an increased uptake of soil nutrients. Czapla et al. (2003, 2005) reported that an auxin is very active in this process, sending signals about physiological activities in different parts of the plant, and about an increasing demand for nutrients. In addition, cytokinins and auxins stimulate cambium activities and the formation of conductive tissues, facilitating the transportation of various nutrients. Plant hormones have an impact on the growth of the chemical element content by facilitating ion penetration to the roots, and they accelerate their transport to other parts. They also have a direct impact on the permeability of cell membranes. Synthetic auxins may regulate the transport of ions opening and closing of ion channels in cell membranes (Zhao 2008).

An appropriate balance between Ca and Mg is very important for maintaining normal metabolism in humans and animals, proving the nutritional value of plants. The ratio between Ca and Mg ions that is too high, higher than 3.0, means an insufficient supply of Mg in the diet (Czapla et al. 2003, 2005). The ion ratio of Ca:Mg in pulses is very diverse, depending on the species. In seeds of field bean it can range from 1.85 to 2.57, and it varies from 2.2 to 4.0 in green bean pods. In the present experiment, the ratio of Ca:Mg was higher than quoted above and ranged from 4.13 to 6.72. Such a big broad range of this ratio is the evidence of a low content of Mg and excessive amounts of Ca in plants. Ionic relations between K and divalent ions of Mg and Ca are also very important for maintaining the health of the consumer. This ratio should be close to 1.62 because when it is higher it results in Ca and Mg deficiency. Wierzbowska and Bowszys (2008), studying the effects of hormonal growth regulators on the accumulation of K, Mg, and Ca in spring wheat, found that kinetin and auxin increased the K content in wheat grains, while gibberellin increased it slightly. Gibberellin increased the content of Mg in stalks, chaffs, the oldest leaves, and grains, while kinetin and auxin decreased it. Synthetic plant hormones also increased Mg uptake by wheat. In addition, growth regulators increased the content of Ca in wheat grains. Gibberellin and auxin increased Ca intake by wheat. In addition, the same authors (Wierzbowska, Bowszys 2008) emphasized that the hormones slightly increased the molar ratio of Ca:P in grains, chaffs and stalks, while kinetin and auxin lowered the Ca:P ratio in leaves. Increasing doses of hormones, primarily in the vegetative parts, lowered the Ca:P ratio. Gibberellin reduced, while kinetin extended the K:(Mg + Ca) ratio in grains and vegetative organs. Auxin extended this ratio in grains and leaves, at the same time reducing it in chaffs and stalks. The authors also reported that the lowest and the highest doses of P resulted in the lowering of the K:(Mg + Ca) ratio in grains and chaffs. Different results were obtained in the present experiment. The application of IBA (Indole-3-butyric Acid) and BAP (6-Benzylaminopurine), both on their own and as a mixture, did not affect the ratio of K to the sum of the Ca and Mg ions in *Medicago x varia* T. Martyn. In turn, the lowest value of the Ca:P ratio was in the plant material treated with a mixture of hormones. However, the results did not differ significantly from those obtained from the control and the treatments with IBA-Indole-3-butyric Acid.

CONCLUSIONS

1. The application of a mixture of Indole-3-butyric Acid and 6-Benzylaminopurine (IBA + BAP) in the vegetative stage of *Medicago x Varia* T. Martyn increased the concentration of K and Mg in plants.

2. The concentration of P, Zn, and Cu in dry matter of plants did not change under the influence of the type and time of application of the tested substances.

3. Treating plants with a mixture of IBA and BAP lowered the Ca:Mg and Ca:P ratios, but they still remained at a very high level.

4. The experiment showed that the application of Indole-3-butyric Acid and 6-Benzylaminopurine in the generative stage of plants did not affect the micro- and macronutrient content in *Medicago x varia* T. Martyn in a statistically significant way.

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