The effects of indole-3-butyric acid and 6-benzyloaminopuryn on *Fabaceae* plants morphometrics

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**ABSTRACT**

The experiment was conducted to study the impact of indole-3-butyric acid (synthetic auxin) and 6-benzyloaminopuryn (synthetic cytokinin), and the mixture of these compounds on fresh and dry matter production and the number of leaves, stems, and inflorescences *Medicago x varia* T. Martyn and *Trifolium pratense* L. For the plants to be thoroughly covered, 0.2 dm$^3$ of spraying liquid was used per one cylinder with growth regulator concentration of 30 mg dm$^{-3}$. Synthetic auxin in the form of IBA, cytokinin in the form of BAP, and their mixture significantly affected biometric parameters of hybrid alfalfa and red clover. However, auxin was more effective than cytokinin. Plants treated with it had more leaves, stems, and inflorescences, which caused an increase in fresh and dry matter weight. Hybrid alfalfa produced more fresh and dry matter than red clover. Plants of the first harvest had the highest values of most biometric features.

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1. Introduction

Mineral fertilizer and plant protection products no longer provide a possibility of a significant increase in crop yields so more and more attention is drawn to the use of synthetic growth regulators (Nogalska et al. 2008; Nowak and Wróbel 2010a; Kang et al. 2017). The most important advantage of applying growth regulators is an increase in the economic value of crops as well as an increase in plant disease resistance. According to some authors (Sivakumar et al. 2001; Erekul and Köhn 2006; Zhang et al. 2016), the purpose of such products should be to increase agricultural productivity in adverse climatic conditions, or conditions not favorable to a given plant. Von Richthofen (2006) argued that this was important in the cultivation of *Fabaceae* plants, with their unstable yields and sensitivity to weather conditions. Synthetic hormones have been used as exogenous growth regulators in agriculture and horticulture for several years now (Harms and Nowak 1990; Wójcik and Majewski 1991; Czapla et al. 2005; Nowak and Wróbel 2010a, 2010b).

In the present experiment synthetic organic compounds with hormonal properties, belonging to auxins and cytokinins, were used. According to available knowledge these substances are involved in plant development at all stages, from seed germination through vegetative growth, differentiation and organogenesis, induction and formation of flowers, embryogenesis, bearing fruit, ripening, to aging and death. It has also been discovered that they participate in the activation of enzymes and some metabolites. It is justified then to assume that this hormonal regulation is essential in all processes determining the development of plants and their response to environmental stimuli. Frimal et al. (2003) and Jenik and Barton (2005) claimed that in the physiological regulation of plant processes hormones of these groups are responsible for the transportation of food substances. In addition, according to many authors (Frimal et al. 2003; Jenik and Barton 2005; Cheng et al. 2006, 2007; Hwang and Sakakibara 2006), plant hormonal substances are involved in the regulation of nutrient distribution by affecting root elongation. In turn, it was found that with the development of the root system after applying plant hormones nutrient uptake is increasing. Aldesuquy (2000) and Ali et al. (2008) reported that auxins had the greatest impact here, functioning as signals indicating about physiological processes in cells and about an increasing demand for nutrients, attracting them. Among the huge amount of nutrients absorbed from the soil by the root system, it is very important that a large amount of them should be transported from vegetative organs to generative organs.

Because of economic and environmental aspects, hybrid alfalfa and red clover are popular crops. They limit the use of nitrogen fertilizer and help to activate the transport of many nutrients in the soil. In addition, *Fabaceae* plants grown in mixtures with grasses positively affect the structure, fertility, physical and chemical properties of the soil, biological activity of micro-organisms, and nutrient movement from lower to the upper layers of the soil. Wild-growing red clover (*Trifolium pratense*) has been known for a long time, mainly as a medicinal plant (Booth et al. 2006; Von Richthofen 2006; Ramos et al. 2008).

The aim of the paper is to assess the impact of synthetic growth regulators from the group of auxins and cytokinins on the aboveground biomass of *Medicago x varia* T. Martyn and *T. pratense* L. The experiment was conducted to study the impact of indole-3-butyric acid (synthetic auxin) and 6-benzyloaminopuryn (synthetic cytokinin), and the mixture of these compounds on fresh and dry matter production and the number of leaves, stems, and inflorescences.
2. Material and methods

2.1. Experimental conditions

2.1.1. Location of the experiment
The experiment was conducted at the experimental facility of Siedlce University of Natural Sciences and Humanities (52°10’03″N; 22°17’24″E; Poland) between 2014 and 2016. In the autumn of 2014 plastic cylinders with no bottom, 40 cm high and with the diameter of 36 cm, were sunk 30 cm deep into the ground, with a spacing of 0.8 × 0.8 m. They were filled with the soil of the anthropogenic order, culture earth type, hortiolse subtype, and the granulometric composition of loamy sand. Before the experiment was set up, organic carbon (Corg) concentration in the soil was 13.50 g kg\(^{-1}\) DM, with a total nitrogen concentration of 1.30 g kg\(^{-1}\) DM. The ratio of C:N was 10.4:1, and a soil pH of 6.8 was close to neutral. The soil had a high content of phosphorus and magnesium, while available forms of potassium were of medium quantities. Because of relatively high content of nutrients mineral fertilizers were not used.

2.1.2. Plant material
Plastic sheeting was spread between the plants to prevent weeds from sprouting. Next, 10 seeds were planted into each cylinder. After germination, when seedlings had 3 leaves, three best-looking plants were selected for further each cylinder. After germination, when seedlings had 3 leaves, three best-looking plants were selected for further research, while the others were removed. In the growing seasons of 2015 and 2016 plants reached their full potential to be harvested three times when 30–40% alfalfa and clover plants were in the bloom stage. Harvest dates: harvest 1 – carried out in spring in the first half of June; harvest 2 – carried out in summer in the second half of August; harvest 3 – carried out in autumn in the first half of October. The experiment was set up in a split-plot design with three replications.

Experimental factors:
- plant species: hybrid alfalfa (Medicago x varia T. Martyn) var. Kometa and red clover (T. pratense L.) var. Krynia;
- growth regulator (applied as a spray): no regulator – distilled water (control series), IBA – indole-3-buetic acid (synthetic auxin), BAP – 6-benza loaminopuryn (synthetic cytokinin), IBA + BAP – indole-3-buetric acid + 6-benza loaminopuryn (at a rate of 1:1);

Preparations were purchased from the company Sigma Life Science.

Plants were treated twice during each growing cycle:
- hybrid alfalfa – the first treatment after the development of the first internode, the other when the first flower buds were visible outside leaves;
- red clover – the first treatment after the appearance of the first branches, the other when the first fluorescence emerged.

For the plants to be thoroughly covered, 0.2 dm\(^3\) of spraying liquid was used per one cylinder with growth regulator concentration of 30 mg dm\(^{-3}\). The control unit was treated with the same amount of distilled water. Analyzed features: fresh and dry matter of the plants (g per pot), the number of stems, leaves and inflorescences per pot.

During each harvest, plants from pots were weighed to determine the fresh mass yield, and stems, leaves and inflorescences were counted. Dry matter was determined with the oven drying method, i.e. drying plant material at 105°C to a constant weight.

2.2. Weather conditions
Sielianinov’s hydrothermal coefficient was calculated in order to determine the temporal variation of meteorological conditions and their effects on vegetation. The hydrothermal coefficient (K) was calculated on the basis of monthly sums of precipitation (P) and a sum of monthly air temperatures (T), using the following formula (Radzka et al. 2015):

\[ K = P/0.1 \sum T \]

Values of Sielianinov’s hydrothermal coefficient (K) are presented in Table 1. It was assumed that extreme conditions took place when its value was below 0.7 and above 2.5 (Radzka et al. 2015).

Optimal temperature and moisture conditions were only in April 2014 and in September 2015. In the remaining months of the growing periods, they were not as favorable, varying from extremely dry in August 2015 to extremely wet in May 2014. Throughout the experiment, the best conditions were at the beginning of each growing season. It can be concluded that the most difficult situation for plants was in 2015, when, apart from May and the end of the growing season, the weather ranged from moderately dry to extremely dry. The growing season of 2016 was characterized by a lack of a period with optimal conditions for plant growth. A high level of precipitation in July (K = 2.15) and drought in most of the remaining months hindered proper growth and development of plants.

2.3. Statistical analysis
The results of the multi-factor experiment with 24 experimental units were processed statistically using repeated measures analysis of variance; the same forage parameters were measured during each growing cycle. The Fisher–Snedecor test was done to determine the significance of the effect of experimental factors on the parameters. Tukey’s test was used to compare differences between means at the HSD\(_{0.05}\) significance level. All the calculations were made with the Statistica 13 – 2017 program. In tables, homogenous groups are marked with letters. Means in lines and columns marked with the same letters are not significantly different.

Table 1. The value of Sielianinov’s hydrothermal coefficient (K) in the growing season.

| Years | Months        | April | May | June | July | August | September | October |
|-------|---------------|-------|-----|------|------|--------|-----------|---------|
| 2014  | (o)           | 1.36  | 1.87| 1.64 | 0.59 | 1.92   | 0.64      | 0.12    |
|       | (mw)          | (md) | (sw) | (d)  | (ed) | (rmw)  | (sd)      | (ed)    |
| 2015  | (o)           | 1.22  | 2.63| 0.87 | 1.08 | 0.18   | 1.46      | 1.94    |
|       | (md)          | (sw) | (d)  | (md) | (ed) | (rmw)  | (sd)      | (ed)    |
| 2016  | (o)           | 1.89  | 0.82| 1.02 | 2.15 | 1.05   | 0.36      | 7.65    |
|       | (md)          | (d)  | (md) | (w)  | (w)  | (d)    | (w)       | (ew)    |

Notes: K ≤ 0.4 extreme drought (ed); 0.4 < K ≤ 0.7 severe drought (sd); 0.7 < K ≤ 1.0 drought (d); 1.0 < K ≤ 1.3 moderate drought (md); 1.3 < K ≤ 1.6 optimal (o); 1.6 < K ≤ 2.0 moderately wet (mw); 2.0 < K ≤ 2.5 wet (w); 2.5 < K ≤ 3.0 severely wet (sw); K > 3.0 extremely wet (ew) 2014 – the year in which the experiment was established, 2015 and 2016 – years of full use of experience.
3. Results and discussion

3.1. Fresh and dry matter

According to Nowak and Wróbel (2010a) mineral fertilizers and plant protection products no longer provide a possibility of a significant increase in crop yields, so more and more attention is drawn to the use of different growth substances in crop production. According to the authors, the purpose of this type of products should be to increase the yield potential in adverse climatic conditions, or conditions not favorable to a given plant. In addition, Von Richthofen (2006) pointed out that such substances are important in the cultivation of Fabaceae plants, with their unstable yields and sensitivity to weather conditions. Such growth substances, including exogenous growth regulators, have been used in practice in agriculture and horticulture (Harms and Nowak 1990; Wojcik and Majewski 1991; Aufhammer and Fedorolf 1992; Czapla et al. 2005; Nowak and Wróbel 2010a, 2010b).

Results of the present experiment indicated that on average the fresh matter yield (Table 2) was statistically significantly higher for hybrid alfalfa than for red clover by 44.7%. The yield of hybrid alfalfa was also higher in the second year of the research than in the first (by 82.9%), while yields of red clover during both growing seasons were at a similar level and did not differ significantly. However, there were statistically significant differences between the yields of plants treated with different growth regulators. The highest fresh matter yield was recorded on units with auxin (76.5% in comparison to control), while plants treated with cytokinin responded with a lower value (an increase by 38.7% in comparison to control). The impact of this kind of growth regulators on the fresh matter was also reported by other authors. In their research on the effect of synthetic auxin, cytokinin, and their mixture Nowak and Wróbel (2010a, 2010b) found a significant increase in the soybean (Glycine max) yield. According to them, auxin was the most effective, with the yield being lower in response to cytokinin and the mixture of regulators. This increase in relation to the control was 34%, 32%, and 29%, respectively.

An increase of pea (Pisum sativum) yield after the application of indole-3-butyric acid was also recorded by Reinecke (1999). Other authors like Barclay and McDavid (1998), and Czapla et al. (2005) confirmed beneficial effects of synthetic auxins and cytokinins on yields of other plants. Czapla et al. (2003) treating soybean (Glycine max) with auxin (IBA), cytokinin (NAA), and their mixture found the highest number of pods and a higher seed yield in plants treated with auxin. In the literature on the subject, other authors have not found any significant effect of exogenous synthetic growth hormones on cultivated plants. A lack of significant differences between yields of Triticale treated with cytokinin was reported by Czapla et al. (2005).

In the present experiment, the fresh matter yield varied between the growing season. On all units considerably more fresh matter, on average by 48.7%, was recorded in the second year. The yields also varied between harvests in the same growing season (Table 3). The highest average fresh matter weight was in the first harvest, and it was 7.5% higher than in the second, and 33% higher than in the third one. According to some authors (Simon 1994; Michalek and Borowski 2006) plant growth, development, and yields result from the activity of basic physiological processes such as assimilation and transpiration. Reinecke (1999) maintained that the use of auxins as exogenous growth hormones could raise the physiological activity of plants and thus influence their productivity.

Statistical analysis of research results (Table 4) showed significant differentiation of dry matter weight as a reaction to experimental factors. Similarly to fresh matter, the highest dry matter weight, an average for both species and growing seasons, was obtained from pots treated with auxin. This value was more than twice as high as from the control. Significantly higher values, by 60% in relation to the control, were also on units treated with cytokinin and with the mixture of auxin and cytokinin (by 70%). In the second year, dry matter weight per pot was 58.4% higher than in the first year.

Growth regulators did not minimize differences between the dry matter yield in individual harvests throughout the growing season (Table 5). Analysis of the interaction of the regulators and harvests showed that plants produced significantly higher amounts of dry matter in the first harvest. Application of auxin leveled off values of these features in the second and third harvests, with no statistically significant differences between them. In turn, cytokinin or with the hormone mixture did not increase dry matter weight as much as auxin. In all three harvests statistically significantly lowest value was in the control. Kuang et al. (1991) and Peterson et al. (1990) suggested that the cause of a positive response to synthetic hormones are physiological changes in plants, especially the earlier increase in tissue vascularity, which manifests itself in thickening of some organs, such as stems, leaves, and inflorescences. According to Rylott and Smith (1990), a higher yield after synthetic auxin and cytokinin application caused at the same time the dominance of generative organs over vegetative. It was confirmed by Pandey et al. (2003), who, using synthetic auxin on cotton plants, noted a significant increase in the size and weight of flowers.

**Table 2. The effect of growth regulators on fresh matter yield in consecutive growing seasons (g per pot).**

| Regulator | Year         | Treatments | 2015 | 2016 | Means |
|-----------|--------------|------------|------|------|-------|
| No regulator |             |            | 184.4a | 356.3bc | 270.7c |
| IBA       |              |            | 402.4ab | 552.3ab | 477.9b |
| BAP       |              |            | 303.4bc | 447.4ab | 375.4ab |
| IBA + BAP |              |            | 325.4ab | 452.4ab | 388.9b |
| Hybrid alfalfa |            |            | 316.3a | 578.4a | 447.3a |
| Red clover |              |            | 291.3ab | 326.3ab | 309.3b |

**Table 3. The effect of growth regulators on fresh matter yield in consecutive harvests (g per pot).**

| Regulator | Harvest | Treatments | 1   | 2   | 3    |
|-----------|---------|------------|-----|-----|------|
| No regulator |         |            | 315.3ab | 276.4ab | 220.3c- |
| IBA       |         |            | 573.7ab | 493.9ab | 366.6ab |
| BAP       |         |            | 452.5ab | 393.9ab | 285.3Ch |
| IBA + BAP |         |            | 408.4ab | 454.9ab | 303.2CCh |
| Hybrid alfalfa |       |            | 527.4ab | 475.9ab | 339.3Ca |
| Red clover |         |            | 347.4ab | 333.9ab | 245.7ab |

**Table Notes:** Means followed by the same lowercase letters in columns are not significantly different. Means followed by the same capital letters in lines are not significantly different.
Table 4. The effect of growth regulators on dry matter yield in consecutive growing seasons (g per pot).

| Treatments | 2015       | 2016       | Means  |
|------------|------------|------------|--------|
| Regulator effect |            |            |        |
| No regulator | 35.0 Bc    | 76.0 Ac    | 55.5b  |
| IBA | 92.7 Bca   | 141.7 Ba   | 117.2b |
| BAP  | 70.7 Ab    | 107.1 Ab   | 88.9a  |
| IBA + BAP | 76.8 Bb    | 112.1 Ab   | 94.3a  |
| Species effect |          |            |        |
| Hybrid alfalfa | 70.6 Ba    | 161.2 Aa   | 115.9a |
| Red clover | 67.0 Aa    | 57.3 Ab    | 62.1b  |
| Means | 68.8 Bb    | 109.2 Ab   |        |

Notes: Means followed by the same lowercase letters in columns are not significantly different. Means followed by the same capital letters in lines are not significantly different.

Table 5. The effect of growth regulators on dry matter yield in consecutive harvests (g per pot).

| Treatments | 1         | 2         | 3         |
|------------|-----------|-----------|-----------|
| Regulator effect |          |           |           |
| No regulator | 68.1 Ac    | 61.5 Ac    | 36.8 Ac   |
| IBA | 151.8 Ac   | 105.8 Ac   | 94.3 Ac   |
| BAP  | 111.6 Ab   | 91.9 Ab    | 63.2 Ab   |
| IBA + BAP | 122.0 Ab   | 108.1 Ab   | 53.3 Ab   |
| Species effect |          |           |           |
| Hybrid alfalfa | 165.6 Ac   | 109.6 Ac   | 72.5 Ac   |
| Red clover | 61.2 Ab    | 73.9 Ab    | 51.3 Ab   |
| Means | 113.4 Ab   | 91.7 Ab    | 61.9 Ab   |

Notes: Means followed by the same lowercase letters in columns are not significantly different. Means followed by the same capital letters in lines are not significantly different.

3.2. The number of leaves, stems, and inflorescences

According to Wilczek and Ćwintal (2002) the participation of leaves in Fabaceae plant yields determines its nutritional quality as they contain the greatest amount of protein. In addition, the authors found that alfalfa foliage was affected by weather conditions and harvest time.

In the present experiment, it was discovered (Table 6) that growth regulators affected the number of plant leaves. The highest number was produced by plants treated with auxin, with a 73.5% increase in relation to the control. Application of auxin together with cytokinin resulted in the number of leaves being over 51% higher than in the control. In turn, treatment with cytokinin on its own caused an increase of 35.4%. The average numbers of leaves in individual pots where growth regulators were applied differed from each other in a statistically significant way. They were also significantly different from the control.

Table 6. The effect of growth regulators on the number of leaves per pot in consecutive growing seasons.

| Treatments | 2015       | 2016       | Means  |
|------------|------------|------------|--------|
| Regulator effect |            |            |        |
| No regulator | 946.8 Bc   | 1314.2 Bc  | 1130.5b |
| IBA | 1597.4 Ab   | 2326.7 Ab   | 1961.9b |
| BAP  | 1172.9 Ab   | 1888.9 Ab   | 1530.9b |
| IBA + BAP | 1449.0 Ab   | 1968.4 Ab   | 1708.7b |
| Species effect |          |            |        |
| Hybrid alfalfa | 1748.8 Ab   | 3210.2 Ab   | 2479.9b |
| Red clover | 834.3 Ab    | 538.3 Ab    | 686.5b  |
| Means | 1291.5 Ab   | 1874.4 Ab   |        |

Notes: Means followed by the same lowercase letters in columns are not significantly different. Means followed by the same capital letters in lines are not significantly different.

Table 7. The effect of growth regulators on the number of leaves per pot in consecutive harvests.

| Treatments | 1         | 2         | 3         |
|------------|-----------|-----------|-----------|
| Regulator effect |          |           |           |
| No regulator | 1402.0 Ac  | 1465.9 Ab  | 523.5 Ac  |
| IBA | 2357.4 Ab  | 2155.5 Ab  | 1372.5 Ab |
| BAP  | 1915.3 Ab  | 1695.3 Ab  | 981.7 Ab  |
| IBA + BAP | 2169.8 Ab  | 2036.8 Ab  | 919.4 Ab  |
| Species effect |          |           |           |
| Hybrid alfalfa | 2991.4 Ab  | 3014.2 Ab  | 1433.9 Ab |
| Red clover | 931.7 Ab   | 662.6 Ab   | 465.7 Ab  |
| Means | 1961.3 b   | 1838.4 b   | 949.3 b   |

Notes: Means followed by the same lowercase letters in columns are not significantly different. Means followed by the same capital letters in lines are not significantly different.

Positive effects of the use of synthetic hormones on crop foliage have been confirmed by literature. Aldesuquy (2000) reported an increase in the number and surface of barley (Hordeum) leaf blades after application of auxin (indole-3-acetic acid). Khan et al. (2002) using auxin solution of 100 mg kg\(^{-1}\) in the form of indole-3-butyric acid (IBA) and naphthalene-acetic acid (NAA) on Lilium and Pal and Das (1990) on cabbage (Brassica oleracea) both recorded the growth of leaf surface and number in relation to the control.

In addition, it was found that hybrid alfalfa produced three times more leaves, as an average across regulators and growing seasons, than clover. In the second year plant species treated with regulators grew about 45% more leaves than in the first year.

The average leaf number (Table 7) was also dependent on the harvest, with the highest in the first one, being lower in the second, and the lowest in the third. The above differences were statistically significant. The growth regulators did not change this tendency much. It was found that on the control and on units with the regulators the number of leaves in the spring and summer harvest did not differ significantly, but in the autumn it was statistically significantly lower on all units.

The number of stems (Table 8) was in a statistically significant way higher in hybrid alfalfa than in red clover. This parameter was affected by the growth regulators, with the highest number of stems produced by plants treated with auxin, 24.6% more than on the control. There was also a 10.6% increase in relation to the control on units with cytokinin and with the mixture of the regulators.

The differences between growing seasons were statistically significant only on the control, where in 2016 the number of stems was 51.3% higher than in 2015. The average number of all experimental units was 29.6% higher in the second year.
Table 9. The effect of growth regulators on the number of stems per pot in consecutive harvests.

| Treatments               | Harvest 1 | Harvest 2 | Harvest 3 |
|--------------------------|-----------|-----------|-----------|
| Regulator effect         |           |           |           |
| No regulator             | 24.8ABA   | 26.0Ab    | 21.2Ab    |
| IBA                      | 27.8Ba    | 33.2Ac    | 28.8Ac    |
| BAP                      | 27.3Ac    | 27.3Ab    | 24.3Ac    |
| IBA + BAP                | 24.7Ba    | 32.3Ac    | 23.3Ab    |
| Species effect           |           |           |           |
| Hybrid alfalfa           | 30.5Ab    | 32.9Bb    | 28.2Ba    |
| Red clover               | 21.8Ab    | 26.5Aa    | 20.5Bb    |
| Means                    | 26.2Aa    | 29.7Aa    | 24.4Bb    |

Notes: Means followed by the same lowercase letters in columns are not significantly different. Means followed by the same capital letters in lines are not significantly different.

Table 10. The effect of growth regulators on the number of inflorescences per pot in consecutive growing seasons.

| Treatments               | Growing Season 2015 | Growing Season 2016 | Means |
|--------------------------|---------------------|---------------------|-------|
| Regulator effect         |                     |                     |       |
| No regulator             | 60.2Aa              | 87.1Ac              | 73.3Ab|
| IBA                      | 92.7Ab              | 159.1Aa             | 125.9Ac|
| BAP                      | 69.7Ab              | 119.9Ab             | 94.8Bb|
| IBA + BAP                | 69.8Ab              | 96.3Ac              | 83.1c  |
| Species effect           |                     |                     |       |
| Hybrid alfalfa           | 78.9Bb              | 169.2Aa             | 124.0Aa|
| Red clover               | 67.3Bb              | 62.1Bb              | 64.7b  |
| Means                    | 73.1B               | 115.6A              |       |

Notes: Means followed by the same lowercase letters in columns are not significantly different. Means followed by the same capital letters in lines are not significantly different.

Table 11. The effect of growth regulators on the number of inflorescences per pot in consecutive harvests.

| Treatments               | Harvest 1 | Harvest 2 | Harvest 3 |
|--------------------------|-----------|-----------|-----------|
| Regulator effect         |           |           |           |
| No regulator             | 98.3Ab    | 87.3Ac    | 35.5Ac    |
| IBA                      | 135.4Bb   | 168.8Ab   | 73.5Ac    |
| BAP                      | 103.4Ab   | 126.5Aa   | 54.8Bb    |
| IBA + BAP                | 97.3Ab    | 116.3Ab   | 35.6Ac    |
| Species effect           |           |           |           |
| Hybrid alfalfa           | 170.4Ab   | 164.6Aa   | 37.1Bb    |
| Red clover               | 46.6Bc    | 84.8Ab    | 62.6Bb    |
| Means                    | 108.5Aa   | 124.3Aa   | 49.9Bb    |

Notes: Means followed by the same lowercase letters in columns are not significantly different. Means followed by the same capital letters in lines are not significantly different.

Table 12. General assessment of the impact of regulators on plants and their effects.

| Regulator       | Factor                        | Increase in value | Effect                  |
|-----------------|-------------------------------|-------------------|-------------------------|
| Plant +         | Synthetic auxin (IBA)         | Fresh matter      | 76.5%                   | The most beneficial morphological effects |
|                 |                               | Dry matter        | 51.1%                   | Slight morphological effects              |
|                 |                               | The number of leaves | 28.6%             | Average morphological effects            |
|                 | + Synthetic cytokinin (BAP)   | Fresh matter      | 38.7%                   |                                         |
|                 |                               | Dry matter        | 60.2%                   |                                         |
|                 |                               | The number of leaves | 35.4%             |                                         |
|                 |                               | The number of stems | 9.6%                |                                         |
|                 |                               | The number of inflorescences | 28.6%        |                                         |
|                 | + Synthetic auxin + cytokinin (IBA + BAP) | Fresh matter | 43.7%                   |                                         |
|                 |                               | Dry matter        | 70.3%                   |                                         |
|                 |                               | The number of leaves | 51.1%             |                                         |
|                 |                               | The number of stems | 11.7%              |                                         |
|                 |                               | The number of inflorescences | 12.8%        |                                         |
Growth regulators did not reduce differences between harvests.

A general assessment of the impact of individual regulators on plants and their effects in the form of changes in the values of the analyzed features are presented in Table 12.

4. Conclusions

Hybrid alfalfa produced more fresh and dry matter than red clover. This was related to a large number of leaves, stems and inflorescences developed by the former species. Synthetic auxin in the form of inole-3-butryic acid (IBA), cytokinin in the form of 6-benzylaminopurinpurin (BAP), and their mixture significantly affected biometric parameters of hybrid alfalfa and red clover. However, auxin was more effective than cytokinin. Plants treated with it had more leaves, stems, and inflorescences, which caused an increase in fresh and dry matter weight. Plants of the first harvest had the highest values of most biometric features. In turn, due to the distribution of meteorological conditions, these characteristics were better in the second growing season.

Disclosure statement

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