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RESEARCH ARTICLE

Effects of growth stimulants on the morphological and anatomical characteristics of feed bean plants

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Abstract

Treatment of 'Pikantni' feed bean plants at the 10 cm shoot stage with the broad-spectrum growth stimulant Biolane and the semi-synthetic film-forming plant growth regulator N¹-Acetyl-5-Methoxykynuramine (AMK) with antioxidant activity induced significant morphogenetic changes. The agents substantially accelerated the growth of the above-ground biomass, particularly from the branching stage onwards, peaking prior to pod formation. The strongest effects on plant height and the fresh weight of leaves, stems, and roots were observed following Biolane treatment. The treatments promoted taproot elongation and improved leaf mesostructure: an increase in the number of epidermal cells, stomata, their area and the thickness of the leaf blade through the development of chlorenchyma, demonstrating an increase in the crop's productive capacity.

Keywords: Plant growth regulators, Morphogenesis, Growth and development, Mesostructural organisation, Stomatal apparatus, Feed beans

Introduction

Against the context of the transformation of domestic agricultural production and the increasing demand for sources of plant protein, there is a growing need to expand the cultivation of high-yielding leguminous crops (Nebaba, et al. 2025). Of particular importance are feed beans (*Vicia faba* L.), which are distinguished by a high protein content, considerable yield capacity and agro-ecological value (Barvinchenko and Aralova, 2024, Różewicz 2020). With their ability to fix atmospheric nitrogen through symbiosis, feed beans help to enhance soil fertility and cut down on the need for mineral fertilisers, in line with the tenets of sustainable agriculture. Feed beans are a valuable predecessor in crop rotation, as they improve soil structure, replenish it with organic matter and help boost the yield of subsequent crops (Sichkar, et al., 2023). At the same time, enhancing the efficiency of their cultivation necessitates the optimisation of cultivation techniques, particularly through the use of plant growth regulators (Shevchuk, et al. 2020, Hadzalo, et al. 2025). Recent advances in biotechnology highlight the role of exogenous biostimulants as key tools for improving seed germination and plant resilience under fluctuating environmental conditions (Bashir, et al. 2021, Liang, et al. 2026). These compounds activate physiological and biochemical processes at the molecular level (Baltaza, et al. 2021), increase seed germination energy, the intensity of photosynthesis, root system development and resistance to stress factors, and ensure the realisation of the crop's genetic capacity.

Materials and Methods

The plants were treated when the shoots reached a height of 10 cm using aqueous solutions of Biolane (15 ml/ha) and N¹-Acetyl-5-Methoxykynuramine (AMK) (0.5 l/ha) until the leaves were thoroughly moistened. The plants in the reference group were treated with tap water. Morphometric parameters were determined every 10 days (including the day of treatment) using linear measurements. Organ weight was calculated using the weighing method. To preserve the leaf samples, a fixing solution was prepared by blending ethanol, glycerine, and distilled water in a 1:1:1 ratio, supplemented with 1% formalin. For the maceration process, a mixture of 5% acetic acid in 2 mol/L hydrochloric acid was employed. Structural characteristics and the quantity of epidermal cells were evaluated through the partial tissue maceration technique. The surface area of the epidermis was quantified utilizing an Microscope Eyepiece Micrometer (MOB-1-15×) × integrated with a light microscope. By calculating cell density relative to the field of view, we subsequently derived the specific area and volume of individual cells. Experimental reproducibility was ensured using five replicates in accordance with AOAC guidelines (AOAC, 2010). The experimental data were analysed using one-way ANOVA (Analysis Of Variance).

Results and Discussion

The results showed that treatment of 'Pikantni' feed bean plants with aqueous solutions of the growth stimulants Biolane and N¹-Acetyl-5-Methoxykynuramine (AMK) led to considerable morphogenetic changes in the crop. The treatments significantly enhanced above-ground biomass accumulation, starting from the branching phase. The greatest increase in plant height was observed in plants treated with Biolane (Tab. 1).

Table 1. The height of 'Pikantni' feed bean plants, depending on the use of growth stimulants.

Development stages	Variant		
	Reference group	AMK	Biolane
5-6 leaves	20.0 ± 0.12	21.1 ± 0.46*	22.1 ± 0.51*
Branching	42.1 ± 1.54	43.3 ± 1.14*	45.7 ± 1.84*
Flowering	61.8 ± 2.32	65.6 ± 2.24*	67.0 ± 2.12*
Bean formation	78.2 ± 2.10	100.5 ± 2.28*	115.7 ± 2.14*
Seed filling	82.0 ± 2.12	103.6 ± 2.62*	120.4 ± 3.02*
Ripening	85.0 ± 1.09	109.6 ± 1.96*	125.5 ± 4.14*

Note: *Difference is significant at $p<0.05$.

During the branching phase, the N¹-Acetyl-5-Methoxykynuramine (AMK) formulation increased plant growth by 2.9%, whilst Biolane increased it by 8.6%. Analogous results were obtained during the flowering phase. In this phase, the semi-synthetic wrap-forming plant growth regulator with antioxidant action, N¹-Acetyl-5-Methoxykynuramine (AMK), increased plant growth by 6.1%, whilst Biolane increased it by 8.4%. The highest plant height of feed beans was observed during the ripening phase, although growth rates varied throughout the growing season. Intensive accumulation of above-ground biomass was observed up to the pod formation phase, after which there was a marked slowdown. These trends likely resulted from preferential assimilate allocation to reproductive organs, a mechanism consistent with the regulation of morphogenesis and trophic supply optimization through growth stimulators (Rogach, et al. 2025, Skliar, et al. 2024). During the pod formation stage, the N¹-Acetyl-5-Methoxykynuramine (AMK) treatment enhanced plant growth by 28.5%, whilst Biolane increased it by 48%. The same results were recorded during the seed filling stage. In this stage, the N¹-Acetyl-5-Methoxykynuramine (AMK) plant growth regulator boosted plant growth by 26.3%, whilst Biolane increased it by 46.8%. During the ripening phase, the N¹-Acetyl-5-Methoxykynuramine (AMK) agent increased plant growth by 28.9%, whilst Biolane did so by 47.6%.

It was noted that the average per-day growth of feed beans varied across the phases of vegetation, depending on the experimental factors. The maximum increase in plant height was observed during the 5-6 leaf-branching, branching-flowering and flowering-pod formation stages, whilst during the pod formation-seed filling and seed filling-ripening stages, the increase in height was markedly lower.

Application of the treatments increased leaf mass per plant. Specifically, when the growth stimulant Biolane was used, leaf mass increased by 73% compared with the reference group, whereas when the experimental plants were treated with the growth regulator N¹-Acetyl-5-Methoxykynuramine (AMK), this figure was somewhat lower, with an increase of 15% (Tab. 2). The action of the studied agents also resulted in an increase in stem weight in feed bean plants. A statistically significant increase in stem weight was observed with the use of the N¹-Acetyl-5-Methoxykynuramine (AMK) agent (by 11%). The maximal increase in stem weight was found after the use of the aqueous solution of Biolane (92%).

Table 2. Morphometric characteristics of 'Pikantni' feed bean plants during the pod formation stage, depending on the use of growth-promoting agents.

Indicators	Fresh weight of leaves from a single plant, g	Fresh weight of the stem, g	Fresh root weight, g	Length of the taproot, cm
Reference group	386.2 ± 8.24	177.3 ± 5.30	54.2 ± 1.24	28.9 ± 0.90
AMK	443.7 ± 14.30*	201.9 ± 6.38*	98.3 ± 2.30*	35.7 ± 1.02*
Biolane	668.2 ± 21.28*	340.6 ± 9.24*	62.3 ± 1.12*	44.8 ± 1.24*

Note: *Difference is significant at $p<0.05$.

The use of these agents led to changes in the root system of feed beans: the roots became longer and the fresh root weight increased. In the case of N^1-Acetyl-5-Methoxykynuramine (AMK), the taproot length and root mass increased by 23% and 81%, respectively, whilst treatment with Biolane increased these figures by 55% and 15% respectively. N^1-Acetyl-5-Methoxykynuramine (AMK) treatment also promoted lateral root development in feed bean plants. Photosynthesis plays a vital role in determining the yield of all crops. Its intensity largely depends on the total leaf area and the duration of its activity during the growing season (Stasik, et al. 2021).

The photosynthetic activity of a plant is largely dependent on the mesostructural organisation of the leaf. Investigations into the anatomical structure of feed bean leaves following the application of growth-promoting agents indicate a noticeable thickening of the leaf blade in the test plants, due to the proliferation of the columnar and spongy parenchyma (Tab. 3).

Table 3. Mesostructural features of leaves of the 'Pikantni' feed bean variety during the pod formation stage, depending on the use of growth-promoting agents.

Variant	Reference group	AMK	Biolane
Leaf thickness, μm	229.62 ± 5.17	242.44 ± 8.35*	259.13 ± 8.24*
Number of epidermal cell	26.41 ± 0.37	18.41 ± 0.42*	33.14 ± 0.21*
Stomatal density	11.03 ± 0.43	8.61 ± 0.22*	13.34 ± 0.24*
Cross-sectional area of a single stoma, μm^2	419.94 ± 7.24	439.02 ± 8.87*	465.66 ± 6.92*
Volume of palisade parenchyma cells, μm^3	3986.89 ± 72.03	4586.62 ± 102.61*	5414.41 ± 94.63*
Spongy cell length, μm	24.90 ± 1.16	28.67 ± 1.12	28.16 ± 0.62
Spongy cell width, μm	16.97 ± 0.72	18.29 ± 0.46	19.13 ± 0.78

Note: *Statistically significant at $p<0.05$ compared to the reference.

It is widely known that the main assimilative tissue of the leaf is the columnar chlorenchyma. It has been established that treatment with N^1-Acetyl-5-Methoxykynuramine (AMK) resulted in a 15% increase in the volume of the palisade parenchyma, whereas treatment with Biolane led to a 36% increase, indicating an improvement in the plant's photosynthetic process and overall productivity, as previously observed in studies on other industrial crops under growth regulator treatment (Khodanitska, et al. 2023). Leaf thickening in treated plants was also associated with increased spongy parenchyma development. Treatment of the plants with the agents led to a slight increase in the length and width of the spongy parenchyma. Examination of the lower epidermis revealed an increase in stomatal area under the influence of the stimulants compared to the control. Thus, when using the N^1-Acetyl-5-Methoxykynuramine (AMK) compound, the area increased by 4.5%, and when treated with Biolane, by 11%.

Evaluation of leaf mesostructural parameters demonstrated that the application of N^1-Acetyl-5-Methoxykynuramine (AMK) and Biolane enhanced photosynthetic apparatus development in *Vicia faba* L., confirming that these growth regulators promote a more robust photosynthetic system. This enhancement is characterized by a significant increase in epidermal cell density and stomatal frequency, alongside expanded individual stomatal areas. Furthermore, the overall leaf thickness was found to increase, primarily driven by the proliferation of chlorenchyma tissue.

Conclusion

Application of the growth regulators Biolane and N^1-Acetyl-5-Methoxykynuramine (AMK) induced significant changes in morphogenesis, growth processes, and photosynthetic apparatus development in 'Pikantni' feed bean plants. The use of the agents contributed to enhanced growth of above-ground and root mass, elongation of the taproot and stimulation of plant development throughout the growing season, with the greatest effect observed up to the pod formation stage. The highest efficacy was observed following Biolane treatment. The application of stimulatory agents exerted a positive influence on the leaf mesostructural organization. This effect was associated with increased epidermal cell density, stomatal development, and leaf blade thickening. Such changes, driven by chlorenchyma development, indicate enhanced photosynthetic apparatus development and increased productive potential of the crop.

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