MODULATION OF PROLINE ACCUMULATION IN CULTIVATED CELERY (*APIUM GRAVEOLENS* L.) INDUCED BY MICROBIAL BIOSTIMULANTS OF BTU BIOTECH COMPANY

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Introduction

Proline is a key osmoprotectant and stress-related amino acid that accumulates in plant tissues in response to various environmental stimuli, including drought, salinity, and microbial interactions [4]. Its accumulation is closely associated with cellular homeostasis, antioxidative defense, and metabolic reprogramming, making it a valuable physiological marker of plant adaptability and health [7]. The enhancement of proline biosynthesis has been reported as one of the beneficial effects of plant growth-promoting rhizobacteria (PGPR) and biostimulants [6].

Celery (*Apium graveolens* L.) is a widely cultivated vegetable crop with high nutritional and pharmacological value. Its physiological responses to microbial biostimulants remain insufficiently characterized, particularly regarding biochemical indicators such as proline content [3].

Recent advances in sustainable agriculture have emphasized the use of microbial-based products, including those developed by BTU-Center (Ukraine), which integrate strains of beneficial bacteria and fungi to enhance plant resilience and productivity. Biopreparations from BTU-Center are widely used in Ukraine and internationally, owing to their multifunctional properties such as nutrient mobilization, phytohormone production, and stress mitigation [2]. In particular, their ability to modulate plant metabolism under abiotic stress conditions, including drought and salinity, makes them promising tools in modern crop management systems.

Despite the growing interest in microbial biostimulants, their specific effects on stress-related metabolic pathways in vegetable crops like celery are still underexplored. There is a critical need to evaluate their biochemical impacts, especially under controlled conditions that simulate stress responses.

The aim of this study was to investigate the influence of microbial biostimulants produced by BTU-Center on the accumulation of free proline in two celery varieties – ‘Diamant’ (root celery) and ‘Tango’ (stalk celery) – under controlled laboratory conditions.

Materials and Methods

The experiment was conducted in the scientific laboratory of the Department of Botany, Bioresources and Biodiversity Conservation, Zhytomyr Ivan Franko State University in the spring of 2025. The study aimed to assess the effect of microbial biopreparations and vitamin B_6 on free proline accumulation in two celery (*Apium graveolens* L.) cultivars:

- 'Diamant' – root celery (*A. graveolens* var. *rapaceum*)
- 'Tango' – stalk celery (*A. graveolens* var. *dulce*)

Experimental design and treatments:

Celery seeds were germinated and grown in pots under controlled conditions (22 ± 2 °C, 60–70% RH, 12-h photoperiod). At the 3–4 true leaf stage, the following treatment variants were applied:

1. Control – untreated plants
2. Vitamin B_6 – aqueous solution of pyridoxine hydrochloride (0.1%)
3. Phytocid® – microbial fungicide based on *Bacillus subtilis*
4. Zhyve Dobryvo® – complex microbial fertilizer containing PGPR (rhizobacteria)
5. Zhyve Dobryvo® + Azotofit® R – combination of microbial fertilizer and nitrogen-fixing *Azospirillum spp.*
6. Zhyve Dobryvo® + Phytocid® – combined microbial consortium (bacteria + fungicide)

All treatments were applied twice: first at the 4-leaf stage and again 14 days later. Preparations were administered both by foliar spraying and root drenching, according to product instructions.

Proline determination:

Thirty days after the first treatment, celery plants were harvested and separated into leaf and stalk tissues. Free proline content was measured using the acidic ninhydrin assay described by Bates et al. (1973) [1]. Briefly, 2 g of fresh plant material was homogenized in 3% aqueous sulfosalicylic acid, reacted with acidic ninhydrin reagent, and incubated in a boiling water bath. Absorbance was measured at 520 nm on a UNICO 1200 spectrophotometer. Proline concentration was calculated against an L-proline standard curve and expressed as mg/100 g fresh weight.

Statistical analysis:

Each treatment was replicated three times ($n = 3$). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test to determine statistically significant differences at $p < 0.05$. Results were processed using Microsoft Excel and Statistica software.

Results and Discussion. The application of microbial biostimulants from BTU-Center had a significant impact on the accumulation of free proline in both root and stalk tissues of two celery varieties — 'Diamant' (root celery) and 'Tango' (stalk celery). The data are presented in Figure 1.

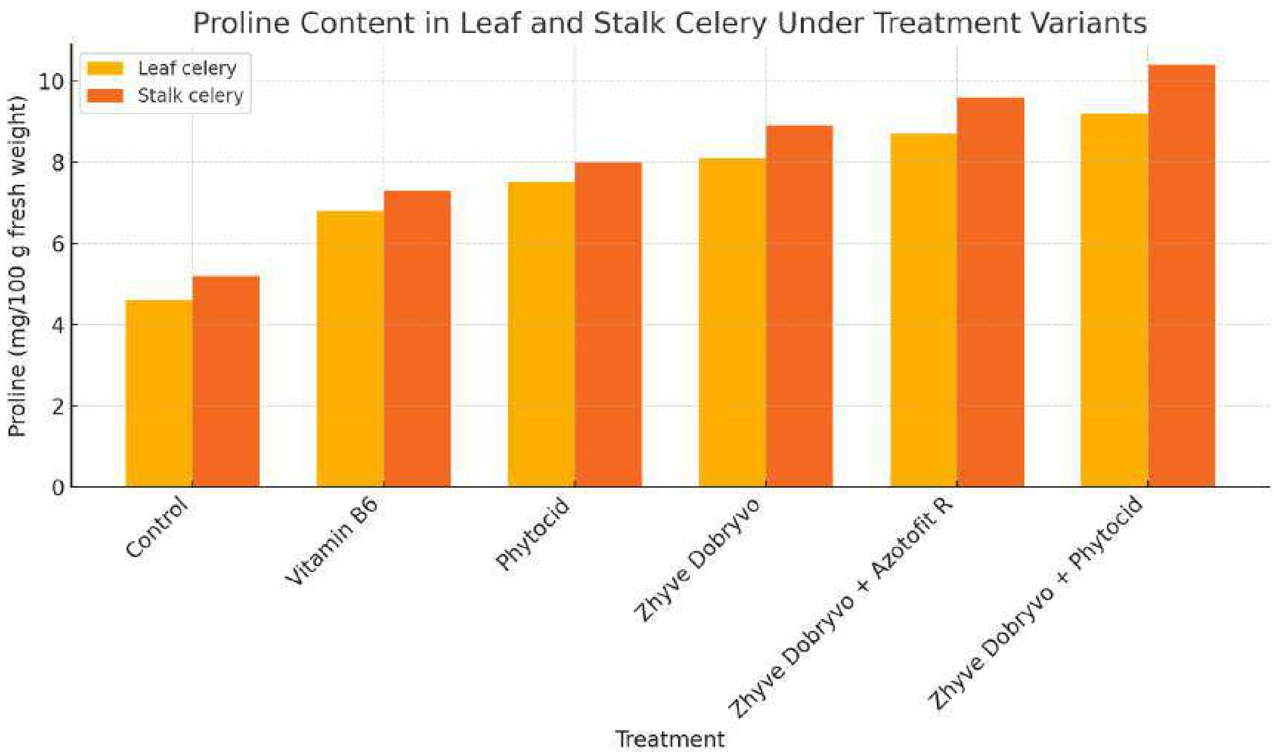

Figure 1. Effect of biopreparations and vitamin B_6 on proline content in leaf and stalk tissues of *Apium graveolens* L.

Quantitative analysis of free proline accumulation revealed significant differences among treatment variants. The lowest proline levels were recorded in the control plants: 4.6 mg/100 g in leaf celery and 5.2 mg/100 g in stalk celery. Application of vitamin B_6 alone moderately increased proline content, confirming its role in amino acid metabolism.

Among the biopreparations, Phytocid® and Zhyve Dobryvo® stimulated proline biosynthesis more strongly, with greater effects observed in stalk tissues. The most pronounced enhancement was detected under combined treatments:

- Zhyve Dobryvo + Phytocid led to the highest accumulation: 9.2 mg/100 g in leaf tissue and 10.4 mg/100 g in stalks.
- Zhyve Dobryvo + Azotofit R also showed elevated values: 8.7 mg and 9.6 mg, respectively.

These results indicate that synergistic formulations combining bacterial consortia and microbial fungicides are more effective in activating stress-related metabolic pathways than single-component treatments. The higher proline content in stalk celery across all treatments suggests varietal specificity in proline regulation and tissue responsiveness.

Among all tested variants, the combined application of Zhyve Dobryvo® + Phytocid® demonstrated the highest efficacy, resulting in maximum proline accumulation in both celery types: 9.2 mg/100 g in leaf celery and 10.4 mg/100 g in stalk celery. This variant significantly outperformed all others ($p < 0.05$) and can be considered the most effective in stimulating stress-associated metabolic responses.

In contrast, the least effective treatment was vitamin B_6 alone, which produced only a moderate increase in proline levels compared to the control. Although pyridoxine participates in transamination reactions and supports amino acid biosynthesis, its effect appears limited when not combined with microbial stimulation.

The hypothesized explanation for the high effectiveness of the Zhyve Dobryvo + Phytocid combination lies in the synergistic interaction between plant growth-promoting rhizobacteria and antagonistic *Bacillus subtilis* strains. These microorganisms likely activate different branches of the plant's stress response system: PGPRs improve nutrient uptake and hormonal signaling, while Phytocid® induces systemic resistance and antimicrobial defense. Their simultaneous action may enhance cellular signaling, antioxidant balance, and nitrogen assimilation, collectively leading to increased proline biosynthesis.

On the other hand, vitamin B_6 monotherapy, lacking microbial elicitation, may be insufficient to trigger the full metabolic cascade required for enhanced proline accumulation under non-stressful conditions.

Throughout all treatment variants, the stalk celery cultivar 'Tango' exhibited consistently higher proline content than the root celery cultivar 'Diamant'. This trend was observed across all treatments, including vitamin B_6, Phytocid®, Zhyve Dobryvo®, and their combinations.

The most notable difference was found in the Zhyve Dobryvo + Phytocid treatment, where proline levels reached 10.4 mg/100 g in 'Tango' stalks compared to 9.2 mg/100 g in 'Diamant' leaves.

These findings align with previous research indicating that microbial biostimulants stimulate proline synthesis as part of the plant's adaptation strategy to environmental and microbial stimuli [5, 7].

These results suggest that 'Tango' possesses greater metabolic responsiveness to microbial biostimulants. Possible explanations include:

- more efficient vascular transport and photosynthetic activity in stalk tissues,
- enhanced enzymatic activity in the proline biosynthetic pathway,
- improved nitrogen assimilation and water uptake under microbial influence.

Conclusion

This study demonstrates that microbial biopreparations developed by Ukrainian manufacturers significantly influence the accumulation of free proline in celery (*Apium graveolens* L.), a key indicator of plant stress adaptation. Among the applied treatments, the combined use of Zhyve Dobryvo® and Phytocid® was the most effective, resulting in the highest proline content in both leaf and stalk tissues.

The stalk celery cultivar 'Tango' consistently showed higher proline levels across all treatments compared to the root cultivar 'Diamant', suggesting cultivar-specific sensitivity and metabolic plasticity in response to microbial stimulation.

The synergistic action of microbial consortia and vitamin B_6 enhances physiological responses by activating stress-related metabolic pathways, particularly proline biosynthesis. These findings highlight the potential of integrated biostimulant-based approaches for improving stress resilience and metabolic performance in vegetable crops under controlled conditions.

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