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Zinc-Paba Complex: Synthesis, Structure and Biostimulatory Effect on Gisela-6 in Vitro

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Abstract. In this study, a novel zinc-based coordination complex derived from para-aminobenzoic acid was synthesized, and its structural properties were characterized using various physicochemical and spectroscopic techniques. Additionally, the biological activity of the complex was evaluated to assess its potential applicability in biomedical or pharmaceutical fields. During the synthesis process, various chemical reagents were used while maintaining stoichiometric ratios, and the crystalline structure of the obtained product was examined under a microscope. The complex compound was characterized using several analytical methods, including FT-IR spectroscopy, thermogravimetric analysis, and crystallography. The absorption frequencies of carboxyl groups in the IR spectrum were analyzed, and it was determined that the coordination type is ionic in nature. The thermolysis process occurred in four main stages, and the structural changes were monitored throughout. The synthesized compound was also applied to “Gisela-6” plants under in vitro conditions at various concentrations. Significant improvements in plant development and proliferation parameters were observed in the nutrient medium enriched with the complex compound. In particular, at a concentration of 700 mg/L, a high initiation and proliferation rate (90%) along with rapid growth was recorded. The conducted study demonstrated that the synthesized complex compound can serve as an effective biologically active substance for stimulating plant development.

Keywords: para-aminobenzoic acid, FT-IR spectroscopy, thermogravimetric analysis, in vitro, plant development, biologically active substance, initiation, proliferation

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Sink-PABA Kompleksi: Sintezi, Quruluşu və Gisela-6 üzərində invitro biostimullaşdırıcı təsiri

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Xülasə. Bu tədqiqatda para-aminobenzoy turşusundan əldə olunmuş yeni sink əsaslı koordinasiya kompleksi sintez edilmiş və onun struktur xüsusiyyətləri müxtəlif fiziki-kimyəvi və spektroskopik metodlarla xarakterizə olunmuşdur. Eyni zamanda, kompleksin bioloji aktivliyi onun biotibbi və ya farmasevtik sahələrdə potensial tətbiq imkanlarını qiymətləndirmək məqsədilə tədqiq edilmişdir. Sintez prosesi zamanı stexiometrik nisbətlər saxlanılmaqla müxtəlif kimyəvi reagentlərdən istifadə olunmuş və alınan məhsulun kristal strukturu mikroskop altında analiz edilmişdir. Kompleks FT-IR spektroskopiyası, termogravimetrik analiz (TGA) və kristalloqrafiya daxil olmaqla bir neçə analitik metodla xarakterizə edilmişdir. İnfraqırmızı (IR) spektrdə karboksil qruplarının udulma tezlikləri analiz olunmuş və koordinasiya tipinin ion xarakterli olduğu müəyyən edilmişdir. Termal parçalanma prosesi dörd əsas mərhələdə baş vermiş və struktur dəyişiklikləri proses boyunca izlənilmişdir. Sintez edilmiş birləşmə müxtəlif konsentrasiyalarda “Gisela-6” bitkilərinə in vitro şəraitdə tətbiq edilmişdir. Komplekslə zənginləşdirilmiş qida mühitində bitki inkişafı və proliferasiya göstəricilərində əhəmiyyətli artım müşahidə olunmuşdur. Xüsusilə 700 mg/L konsentrasiyada yüksək başlanğıc və proliferasiya göstəricisi (90%) və sürətli böyümə qeydə alınmışdır. Aparılan tədqiqat göstərmişdir ki, sintez edilmiş kompleks bitki inkişafını stimullaşdıran effektiv bioloji aktiv maddə kimi çıxış edə bilər.

Açar sözlər: para-aminobenzoy turşusu, FT-IR spektroskopiyası, termogravimetrik analiz, in vitro, bitki inkişafı, bioloji aktiv maddə, başlanğıc, proliferasiya

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Introduction

Plant biotechnology has advanced significantly in recent decades, particularly through the development of *in vitro* propagation techniques. These innovations are essential for large-scale plant multiplication and the preservation of genetic fidelity under sterile conditions. *In vitro* culture systems facilitate the rapid breeding of high-yielding, stress-tolerant, and disease-resistant cultivars—traits critical for ensuring global food security and promoting sustainable agriculture (Yadav & Rani, 2015).

A pivotal component in the success of *in vitro* culture is the formulation of the nutrient medium. While the Murashige and Skoog (MS) medium remains a standard choice, recent research highlights the benefits of enriching culture media with various additives, including plant growth regulators, amino acids, and metal complexes (Khan & Sulaiman, 2018; Muthukumar & Jayakumar, 2017). These supplements can significantly enhance plant development by supplying essential nutrients and stimulating physiological processes.

Among these additives, metal–organic complexes—especially those involving zinc (Zn)—have garnered increasing attention for their dual functionality as nutrient sources and biologically active agents. Zinc is an essential micronutrient, playing a key role in numerous enzymatic processes and maintaining cellular homeostasis in plants (Ali & Ghanem, 2020; Fageria & Stone, 2017). Emerging studies have explored novel zinc-based coordination compounds, such as those incorporating para-aminobenzoic acid, to assess their potential applications in plant biotechnology (Bałaban & Górka, 2018; Hanjani & Kazemian, 2017; Sharma & Sharma, 2016).

The biological activity of zinc-based coordination compounds is associated with several physiological and biochemical mechanisms in plants. Zinc functions as a structural or catalytic component of numerous enzymes involved in protein synthesis, carbohydrate metabolism, and nucleic acid regulation. In addition, zinc contributes to the stabilization of cellular membranes and participates in antioxidant defense systems by regulating the activity of enzymes such as superoxide dismutase. Adequate zinc availability has also been linked to enhanced auxin biosynthesis, improved cell division, and increased tolerance to abiotic stress conditions. Consequently, zinc-containing complexes have attracted increasing attention as potential biostimulants in plant tissue culture and controlled propagation systems.

Para-aminobenzoic acid (PABA) is known not only as an important aromatic carboxylic acid but also as a biologically active compound involved in cellular metabolic processes. Previous studies have suggested that PABA may influence plant growth through its participation in folate-related metabolic pathways, regulation of cellular redox balance, and enhancement of stress tolerance mechanisms. Furthermore, aromatic ligands such as PABA can modify the bioavailability and transport properties of coordinated metal ions, potentially increasing their biological effectiveness. These characteristics make PABA a promising ligand for the development of biologically active zinc complexes intended for plant biotechnology applications.

Recent studies have demonstrated that zinc-based coordination compounds regulate plant growth not only through nutritional supplementation but also via modulation of phytohormone signaling pathways, enhancement of auxin biosynthesis, and activation of antioxidant defense systems including superoxide dismutase and catalase enzymes. Moreover, zinc availability has been closely associated with improved reactive oxygen species (ROS) homeostasis under *in vitro* stress conditions. Similarly, para-aminobenzoic acid (PABA) has been reported to participate in folate-mediated metabolic pathways, contributing to redox balance regulation and stress adaptation in plant tissues. These findings from recent literature (2020–2025) further support the multifunctional role of Zn–organic ligand complexes as potential biostimulants in plant tissue culture systems.

The present study focuses on the synthesis and characterization of a novel zinc–para-aminobenzoic acid complex. The compound was synthesized using stoichiometrically balanced reagents, and its structure was elucidated using Fourier-transform infrared (FT-IR) spectroscopy, thermogravimetric analysis (TGA), and single-crystal X-ray diffraction (Gauthier & Dumoulin, 2005; Jomaa & Tröger, 2017; Patil & Sundaresan, 2019; Rizzi & Zoppellaro, 2014). To evaluate its biological activity, the complex was applied to *in vitro* cultures of the “Gisela-6” rootstock. The study examines the effects of varying concentrations of the complex on plant initiation and proliferation, offering valuable insights into its potential as a biostimulant in plant tissue culture (Saha & Rahman, 2019; Zafar & Iqbal, 2017).

To the best of our knowledge, there are limited reports on the application of Zn–PABA coordination complexes as growth-promoting agents in the micropropagation of Gisela-6 rootstock under *in vitro* conditions. Therefore, the novelty of the present study lies not only in the synthesis and structural characterization of a Zn–PABA complex but also in evaluating its biostimulatory effect as a substitute for conventional zinc sources in MS medium.

Materials and Methods

Synthesis of the complex compound

The crystalline zinc complex was synthesized by reacting stoichiometric amounts of para-aminobenzoic acid (2.74 g, 0.01 mol) and sodium bicarbonate (1.68 g, 0.02 mol) in 100 mL of ultrapure distilled water. The mixture was heated to 70–80 °C and stirred for 3 minutes to allow for neutralization of the carboxylic acid. A solution of zinc sulfate heptahydrate (2.87 g, 0.02 mol) was then added slowly under continuous stirring. The combined solution was maintained at 50–60 °C for an additional 3 minutes before being allowed to cool to room temperature. Upon cooling, a white precipitate formed, which was collected by filtration, dried in a desiccator over anhydrous calcium chloride (CaCl₂), and stored in the dark at ambient conditions for 48 hours (Gauthier & Dumoulin, 2005; Hanjani & Kazemian, 2017).

Elemental Analysis and Reaction Yield

The slight deviations between calculated and experimental values are within acceptable limits, confirming the proposed structure of the zinc–para-aminobenzoic acid complex (Bałaban & Górska, 2018; Patil & Sundaresan, 2019).



Spectroscopic Analysis

Figure 1

Microscopic image of the synthesized complex compound

FT-IR spectroscopy of the complex was performed using a Perkin-Elmer 100 FT-IR spectrophotometer in the 4000–250 cm⁻¹ range, employing KBr pellets (Rizzi & Zoppellaro, 2014).

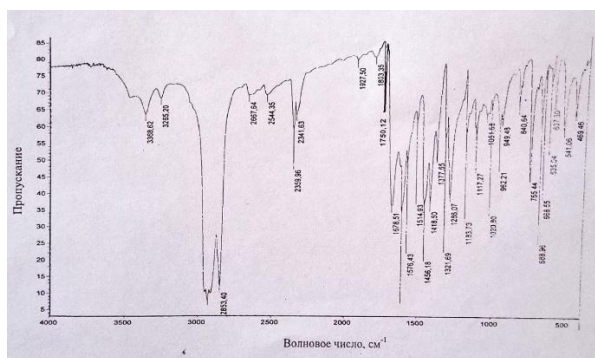


Figure 2

FT-IR spectrum of the synthesized complex compound

An absorption frequency is observed at 1589 cm^{-1} in the IR spectrum, which corresponds to the asymmetric stretching vibration of the carboxylate group ($\nu_{\text{as}}(\text{COO}^-)$). The absorption frequency observed at 1366 cm^{-1} in the recorded spectrum corresponds to the symmetric stretching vibration of the carboxylate group ($\nu_{\text{s}}(\text{COO}^-)$). Based on the difference between the asymmetric (ν_{as}) and symmetric (ν_{s}) stretching vibrations of the carboxylate group, the coordination mode of the carboxyl oxygen atoms to the central metal ion can be determined. If the separation ($\Delta\nu = \nu_{\text{as}} - \nu_{\text{s}}$) is less than 200 cm^{-1} , it suggests that the carboxylate group forms a chelating bond with the metal center, indicating a bidentate or bridging bidentate coordination mode. In contrast, if $\Delta\nu$ exceeds 200 cm^{-1} , the bonding is likely monodentate or predominantly ionic in nature.

Based on the aforementioned rule, when applied to the Zn(II) complex compound, the separation between the asymmetric and symmetric stretching frequencies of the carboxylate group is calculated as $\Delta\nu = \nu_{\text{as}}(\text{COO}^-) - \nu_{\text{s}}(\text{COO}^-) = 1589\text{ cm}^{-1} - 1366\text{ cm}^{-1} = 223\text{ cm}^{-1}$.

This value indicates that the oxygen atoms of the carboxyl group in para-aminobenzoic acid coordinate to the Zn(II) cation in a predominantly ionic or weakly coordinated monodentate mode. The thermogravimetric analysis (TGA) of the synthesized complex compound was performed using a NETZSCH STA-449 FZA-0836 M thermoanalyzer (Patil & Sundaresan, 2019).

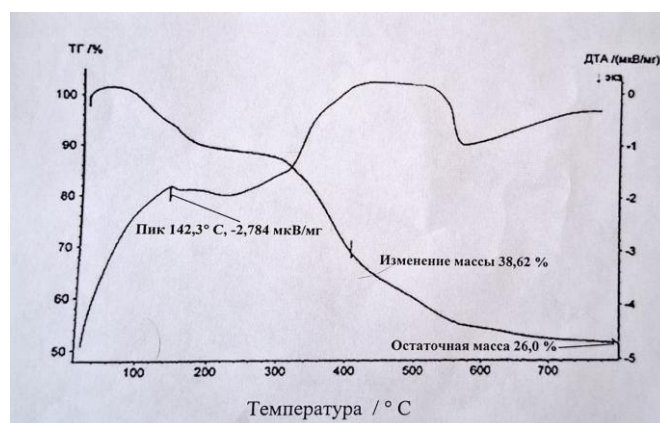


Figure 3
Thermogravimetric analysis result of a complex compound

As seen in Figure 3, the thermolysis process of the coordination compound proceeds in four main stages. The first stage occurs within the temperature range of $160\text{--}210\text{ }^{\circ}\text{C}$. During this stage, four water molecules included in the complex leave the structure. This process is characterized by an endothermic effect observed at around $192\text{ }^{\circ}\text{C}$.

The second stage takes place in the temperature range of $215\text{--}340\text{ }^{\circ}\text{C}$, during which the dehydrated structure transforms into a dimeric form.

The third stage occurs between $380\text{--}440\text{ }^{\circ}\text{C}$. In this stage, the dimeric structure decomposes, resulting in the formation of a hydrocarbon residue and the compound ZnCO_3 . This is confirmed by the exothermic effect observed at $440\text{ }^{\circ}\text{C}$.

The fourth stage occurs in the temperature range of $470\text{--}700\text{ }^{\circ}\text{C}$. During this stage, ZnCO_3 decomposes to form ZnO .

Preparation of Nutrient Medium for Gisela-6 Plant

Stock solution preparation

To prepare the stock solution of the synthesized zinc–para-aminobenzoic acid (Zn–PABA) complex, the complex was weighed using a Kern ABJ220-4NM analytical balance. Amounts of 500 mg, 700 mg, and 900 mg of the synthesized complex were each dissolved in 100 mL of distilled water. The solutions were stirred at 90 °C using a Daihan Scientific Hotplate Stirrer MSH-20A until complete dissolution and clarification were achieved (Ali & Ghanem, 2020).

In place of the standard $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ used in the Murashige and Skoog (MS) medium, the respective amounts of the synthesized Zn–PABA complex were incorporated at different concentrations (500, 700, and 900 mg). The selection of 500, 700 and 900 mg/L concentrations was based on a gradient approach to evaluate dose-dependent biological responses, representing low, medium and high levels of Zn–PABA supplementation.

This substitution was made to evaluate the effects of the complex on the growth of Gisela-6 plants during the initiation stage (Fageria & Stone, 2017).

To solidify the MS medium, 6.4 g of agar-agar was added to the solution. The mixture was boiled at 100 °C for 50 minutes on a magnetic stirrer until it became transparent. The resulting medium was then poured into subculture vessels and sterilized in an autoclave at 121 °C for 15 minutes (Gupta & Sharma, 2014).

Nutrient Medium Preparation

For the purpose of evaluating the effects of the Zn–PABA complex at different concentrations, three separate 200 mL culture media were prepared. Each medium was supplemented with the respective concentrations of the synthesized complex (500 mg, 700 mg, and 900 mg). Additionally, a control medium was prepared with the standard MS medium (Khan & Sulaiman, 2018).

The stock solutions for the standard MS culture medium were prepared by separately dissolving the following components in distilled water:

- Five macronutrient salts
- Seven micronutrient salts
- Iron source

The specific amounts of each salt used for preparing the stock solutions are provided in the table below (Tandon & Kaur, 2020).

Table 1

Composition of stock solutions prepared for the nutrient medium

	Reagents	Weight(mg)	Weight(gr)	Added water(ml)	Using ml/L
Macrosalts	NH_4NO_3	16500	16,5	100	10
	KNO_3	1900	1,9	100	10
	$\text{MgSO}_4 \times 7\text{H}_2\text{O}$	3700	3,7	100	10
	KH_2PO_4	1700	1,7	100	10
	$\text{CaCl} \times 2\text{H}_2\text{O}$	4400	4,4	100	10
Iron	$\text{FeSO}_4 \times 7\text{H}_2\text{O}$	557	5,57	100	5
	$\text{Na}_2\text{EDDT} \times 2\text{H}_2\text{O}$	745	7,45		

Microsalts	MnSO ₄ x 4H ₂ O	2230	2,23		
	ZnSO ₄ x 7H ₂ O	860	0,86		
	H ₃ BO ₃	620	0,62		
	KJ	83	0,083	100	1
	Na ₂ MoO ₄ x 2H ₂ O	25	0,025		
	CuSO ₄ x 5H ₂ O	2,5	0,0025		
	CoCl ₂ x 6H ₂ O	2,5	0,0025		

As shown in Table 1, the macronutrients were prepared separately, while the micronutrients were weighed accordingly and dissolved in 100 mL of water. For the purposes of this study, ZnSO₄·7H₂O was excluded from the micronutrient stock solution, and three separate stock solutions were prepared by adding different amounts of the synthesized complex compound.

In the next stage, four different media (three variants and one standard control) of 200 mL each were prepared using the stock solutions.

The composition of the standard nutrient medium is shown in Table 2.

Table 2

Composition of the standard nutrient medium

Reagents	Unit of measurement	Amount
NH ₄ NO ₃	ml	10
KNO ₃	ml	10
KH ₂ PO ₄	ml	10
CaCl ₂	ml	10
MgSO ₄	ml	10
Microsalts	ml	1
Iron	ml	5
B ₁	ml	0.5
B ₆	ml	0.5
PP	ml	0.5
C	ml	1
BAP	ml	0.5
GA ₃	ml	0.01
Sucrose	qr	30
Agar	qr	6

After the standard nutrient medium was prepared, three different nutrient media containing the synthesized complex compound were prepared. As mentioned above, the ZnSO₄·7H₂O salt from the micronutrient stock was replaced with the synthesized complex compound (Table 3).

Table 3

Composition of the nutrient medium supplemented with the complex compound

	Reagents	Weight(mg)	Weight(qr)	Added water(ml)	Using ml/L
Microsalts	MnSO ₄ x 4H ₂ O	2230	2,23		
	ZnSO ₄ x 7H ₂ O	500, 700, 900	0,5;0,7;0,9	100	1
	H ₃ BO ₃	620	0,62		

KJ	83	0,083
Na ₂ MoO ₄ x 2H ₂ O	25	0,025
CuSO ₄ x 5H ₂ O	2,5	0,0025
CoCl ₂ x 6H ₂ O	2,5	0,0025

The selected concentrations of the Zn–PABA complex (500, 700 and 900 mg/L) were chosen to evaluate the biological response of Gisela-6 plantlets under low, moderate and high levels of Zn supplementation and to determine the optimal concentration range for in vitro growth and proliferation.

In Vitro Preparation Stage of Gisela-6 Plant

To initiate the in vitro culture of Gisela-6 plants, 20–30 cm long green annual branches with axillary buds were collected from the donor plant and transferred to the laboratory. The branches were then cleaned of any leaves and cut into 1–2 cm segments, ensuring that each segment contained one axillary bud at a node (Singh & Agrawal, 2019).

The explants were sorted according to their mechanical firmness, as uniform structural integrity is crucial for effective sterilization and successful culture establishment (Yadav & Rani, 2015).

The selected explants were placed in gauze bags and subjected to a surface cleaning procedure. This involved shaking the bags in distilled water three times to remove any contaminants. A total of four bags, each containing 30 plant samples (120 explants in total), were prepared for sterilization.

The sterilization process consisted of the following three steps:

1. Fairy (detergent) wash: The explants were initially cleaned with a detergent solution to remove any dirt or residues (Khan & Sulaiman, 2018).
2. Fungicide treatment: The explants were then immersed in a fungicide preparation to eliminate any fungal contamination (Fageria & Stone, 2017).
3. Sodium hypochlorite (NaOCl) treatment: To finalize the sterilization, the explants were soaked in a 20% sodium hypochlorite solution for 15 minutes. This step proved effective in eliminating microbial contamination while maintaining the explants' viability for further culture.

Sterilization Procedure for Gisela-6 Explants (In Vitro Preparation Stage)

First Sterilization Stage

A 250 mL laboratory beaker was used to prepare the first sterilization solution. Three milliliters of Fairy detergent were added to the beaker and diluted with 200 mL of distilled water until visible foaming occurred. Plant samples wrapped in gauze bags were immersed in this solution, sealed tightly, and placed on a shaker operating at 150 rpm for 6 minutes. Following this treatment, the explants were rinsed thoroughly with distilled water to remove detergent residues.

Second Sterilization Stage

Twenty-five grams of fungicide (exact composition not specified) were weighed using an analytical balance and dissolved in 1 liter of distilled water in a 2-liter beaker. The solution was stirred for 6 minutes using a magnetic stirrer. Then, the solution was transferred to a 300 mL container, and the plant samples were immersed in it. The container was sealed tightly and shaken at 180 rpm for 5–10 minutes, depending on the mechanical firmness of the explants. At the end of this treatment, the

samples were rapidly rinsed with distilled water to remove all fungicide residues and stored in clean water for the next step.

Final Sterilization Stage

Sixty milliliters of the disinfectant Domestos were added to a 500 mL beaker, followed by 340 mL of distilled water. The mixture was stirred and transferred to a 300 mL container containing the explants. The container was sealed and placed on a shaker at 180 rpm for 5 minutes. Finally, under sterile conditions in a laminar flow hood, the container was opened, and the samples were rinsed with distilled water that had been autoclaved at 121 °C for 15 minutes. The explants were kept in this sterile water until transferred to culture media for the planting phase (Smith, 2013).

In Vitro Initiation Stage of Gisela-6 Plant

The explants that had undergone sterilization were stored in sterile distilled water prior to culture initiation. Under sterile conditions in a Laminar Flow Hood, the plant samples were transferred to culture vessels. The culture medium was prepared using 3% sucrose and 5% agar as the basic components. In the core part of the experiment, microelement stock solutions were modified by replacing $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ with a previously synthesized zinc–para-aminobenzoate complex at three different concentrations: 500 mg, 700 mg, and 900 mg.

The inoculated plantlets were placed in a digitally controlled growth chamber, with conditions set to 3000–4500 lux light intensity, 24 °C temperature, and 60% humidity. The plants were maintained under these conditions for 23 days, during which regular observations were conducted. Monitoring results revealed that, in comparison to the control medium, the plantlets grown on media supplemented with the zinc complex showed improved growth and development. Notably, the survival rate also varied between groups: in the control group, 18 out of 30 plantlets (i.e., 60%) successfully completed the initiation phase. In contrast, in the treatment group supplemented with 700 mg of the complex compound, 27 out of 30 plantlets (i.e., 90%) successfully completed the initiation stage. The average height of these plantlets was approximately 3.5 cm (Bałaban & Górska, 2018; Khan & Sulaiman, 2018).



Figure 4

Plantlets cultured on nutrient media supplemented with the synthesized complex compound during the initiation stage

Proliferation Stage of Gisela-6 Plantlets

Plantlets that successfully completed the initiation stage were transferred to the next phase, the proliferation stage. The nutrient medium for this stage was prepared with 3% sucrose and 7% agar. The stock solution of microelements was supplemented with the same synthesized complex compound, as described previously.

The plantlets were subcultured in four passages, each lasting 23 days. The composition of the nutrient medium remained constant throughout all passages. The climate chamber parameters were adjusted to 23 °C and 55% humidity.

Compared to the control group, plantlets grown on media supplemented with the complex compound exhibited a higher growth rate. At the end of each passage, the number of new shoots was assessed. On average, 4 new shoots were observed per plantlet in the experimental groups, while the control group exhibited a maximum of 2 to 3 shoots.

As a result, 216 new plantlets were obtained from 18 plantlets in the control group by the end of the proliferation phase. In contrast, the experimental variant supplemented with 700 mg of the complex compound yielded 950 new plantlets from 27 initial plantlets (Gauthier & Dumoulin, 2005; Singh & Agrawal, 2019).



Figure 5
Development of plants grown on the medium with the applied complex compound and the standard medium during the proliferation phase

For each treatment, 30 explants were cultured under identical in vitro conditions. Four experimental variants were evaluated, including a standard MS medium (control) and three media supplemented with the synthesized Zn–PABA complex at concentrations of 500, 700, and 900 mg/L. Plant growth and development were monitored throughout the initiation and proliferation stages. The effectiveness of each treatment was assessed based on survival rate, initiation frequency, proliferation rate, shoot number, and plantlet growth performance.

Results

The findings of this study demonstrate that the newly synthesized coordination compound based on para-aminobenzoic acid and zinc ions can be effectively applied in the in vitro microclonal propagation of plants. By stimulating plant growth and development, the complex exhibited high performance during the initiation and proliferation stages. Notably, a concentration of 700 mg/L resulted in a 90% success rate, clearly confirming the compound's efficacy. Structural analyses, including FT-IR spectroscopy and thermogravimetric analysis, confirmed that the complex is both stable and functionally active. Based on the results obtained, it can be concluded that the synthesized compound holds significant potential as a promising biologically active agent for applications in plant

biotechnology. Data were expressed as mean values based on observed biological responses across replicates. Although formal statistical analysis (ANOVA or t-test) was not applied in the present study, consistent trends in survival rate, initiation frequency and proliferation performance were recorded among the experimental variants.

Discussion

The results of the conducted study provide a detailed analysis of the effects of the synthesized complex compound, based on para-aminobenzoic acid and zinc, on Gisela-6 plants under in vitro conditions. The obtained results indicate that the synthesized complex compound had a significantly positive impact on plant growth and development. In particular, at a concentration of 700 mg/L, the complex compound applied to the nutrient medium resulted in high initiation and proliferation rates (90%) during the plant's growth phases. These findings suggest that the complex compound enhances the metabolic activity of plant cells, creating optimal conditions for faster growth and development. Structural analysis of the complex using FT-IR spectroscopy confirmed that the coordination type of the carboxyl groups is ionic in nature. Thermogravimetric analysis (TGA) revealed that the thermal decomposition process of the complex occurred in four main stages, during which structural changes were observed at each stage. These results demonstrate that the complex is stable and effective, supporting its potential for widespread application in plant biotechnology.

The $\Delta\nu(\text{COO}^-)$ value of 223 cm^{-1} observed for the investigated Zn(II)–PABA complex indicates that the carboxylate group predominantly coordinates to the metal center in a monodentate manner. Similar findings have been reported in recent studies on zinc complexes containing aromatic carboxylate ligands. In those studies, $\Delta\nu$ values greater than 200 cm^{-1} were interpreted as evidence of ionic or monodentate coordination between the Zn(II) ion and the carboxylate oxygen atoms. Therefore, the FT-IR results obtained in the present study are consistent with the structural characteristics reported for related zinc coordination compounds in the literature. Thermogravimetric analysis revealed that the thermal decomposition of the complex proceeds through multiple stages, ultimately leading to the formation of ZnO as the final decomposition product. This behavior is in agreement with previous studies on Zn(II)-based coordination compounds. In many reported zinc complexes, the initial decomposition stage is associated with the release of coordinated or crystallization water molecules, followed by the degradation of the organic ligand framework at higher temperatures. A similar thermal behavior was observed in the present study, indicating that the synthesized complex possesses a relatively high degree of thermal stability.

The biological evaluation demonstrated that the Zn–PABA complex at a concentration of 700 mg/L produced the most favorable growth response in Gisela-6 plantlets. Zinc is known to play an essential role in cell division, enzyme activation, protein biosynthesis, and various metabolic processes; therefore, its optimal concentration can significantly promote plant growth and development. The superior performance observed at 700 mg/L may be attributed to an optimal balance between zinc availability and cellular metabolism. In contrast, the slight reduction in growth parameters observed at 900 mg/L may be associated with physiological stress caused by excessive accumulation of zinc ions in plant tissues.

Although FT-IR and thermogravimetric analyses provided important information regarding the coordination mode and thermal stability of the synthesized complex, further structural investigations using techniques such as X-ray diffraction and elemental analysis would be valuable for comprehensive structural confirmation.

Although thermogravimetric analysis confirmed a multi-step decomposition process of the Zn–PABA complex, detailed quantitative mass loss percentages and corresponding decomposition reactions were not determined in the present study and should be addressed in future investigations for more precise mechanistic interpretation.

Conclusion

The conducted research has demonstrated that the complex compound synthesized from para-aminobenzoic acid and zinc possesses significant potential for application in plant biotechnology. Detailed structural investigations using FT-IR spectroscopy and thermogravimetric analysis confirmed that the compound is a stable and functionally active coordination complex. When applied to Gisela-6 plant cultures under in vitro conditions, the complex notably enhanced plant development. In particular, at a concentration of 700 mg/L, it achieved a high success rate of 90% in the initiation and proliferation phases, indicating its strong stimulatory effect on plant growth. These findings suggest that the synthesized complex can serve as an effective biologically active substance for promoting plant development in tissue culture systems. Moreover, the study opens new avenues for broader investigations into the compound's effects on different plant species and encourages the future integration of similar metal–ligand complexes into plant biotechnology. As such, this work offers a meaningful scientific contribution across the domains of chemical synthesis, structural characterization, and biological application.

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