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Gallic Acid as a Non-Specific Regulator of Phenol Synthesis and Growth of Regenerate Plants of *Corylus avellana* (L.) H. Karst. and *Salix alba* L. *in vitro*

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Abstract. Gallic acid is found in plant tissues in free form, as well as in the composition of complex esters and hydrolysed tannins. These phenolic compounds have significant antioxidant activity and protect plant cells from damage by free radicals. In the conditions of stress that occurs during the introduction of plants into *in vitro* culture, the vast majority of explants are characterised by an intensive synthesis of phenols, which quickly oxidise, polymerise, block the explants' nutrition pathways, and cause tissue necrosis. The addition of gallic acid in millimolar concentrations to the nutrient medium reduces the risk of autointoxication of tissues by secondary metabolic products. The purpose of this study was to investigate the effect of exogenous gallic acid on organogenesis and phenolic synthesis of *Salix alba* and *Corylus avellana* plants *in vitro*. For this purpose, the study used methods of tissue and organ culture *in vitro*, spectrophotometric determination of total phenols and flavonoids in leaves, methods of dispersion and nonparametric analysis. It was established that gallic acid at a concentration of 1 mmol·l^{-1} in the composition of the DKW nutrient medium caused the awakening of dormant buds, stimulated the growth of shoots, and also promoted the branching of stems, the development and growth of lateral roots of S*alix alba in vitro*. It also inhibited the synthesis of phenols in *Corylus avellana* plants of the varieties 'Tonda Romana', 'Tonda Gentile Dele Lange', 'Barcelona', while contributing to an increase in the content of phenolic compounds in the leaves of the varieties 'Tonda Di Giffoni', 'Mortarella', and 'Epsilon'. It was established that the varieties recommended for fruiting have a higher content of phenolic compounds ('Tonda Gentile Dele Lange' and 'Tonda Di Giffoni') compared to pollinator varieties ('Mortarella'). Therefore, exogenous gallic acid at a concentration of 1 mmol·l^{-1} has the properties of a non-specific regulator of phenol synthesis in regenerating plants of hazel (*Corylus avellana*), which is relevant for plants with a high content of phenols, especially at the stage of their introduction into *in vitro* culture

Keywords: development, hydroxybenzoic acid, organogenesis, phenolic compounds, flavonoids, nutrient medium

Introduction

A unique property of plants is the ability to synthesise a wide range of organic compounds that belong to the class of secondary metabolites (SM) [1]. The vast majority of SMs exhibit specific bioactivity that supports the physiological stability and viability of the plant organism [2-4]. These organic compounds, which are quite diverse in structure and chemical properties, protect plant cells and tissues from stress factors in conditions of excess or lack of moisture, light, organ injury, heavy metals and xenobiotics [5; 6],

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as well as pathogens – phytoviruses, mycoplasmas, pathogenic bacteria, fungi, and phytophages [7-9]. At the same time, SMs serve as attractants for pollinating insects [10]. Tannins and flavonoids protect tissues and organs from the harmful effects of ultraviolet light [11], increase drought resistance [12], and provide root protection, especially in acidic soils [13].

SM are responsible for the interaction of plants with the natural environment at the individual, population, and ecosystem levels [10; 14]. At the same time, the current vision of secondary plant metabolism is not limited only to the functionality of protective mechanisms and adaptive responses. It is believed that individual products of SM are components of regulatory molecular programmes that positively affect plant growth and development [15; 16]. Transcription factors are associated with secondary synthesis due to complex regulatory networks that control the processes of metabolite generation [16]. Some products of secondary metabolism actively affect the course of plant growth and development [17]. In particular, polyphenols and flavonoids regulate the polar transport of phytohormones through tissues [18-20].

Phenol carboxylic acids: vanillic, syringic, ferulic, salicylic, and gallic acids, even in low concentrations, affect the development of plant tissues and organs in *in vitro* conditions [21; 22]. Phenol carboxylic acids containing 4-oxy and 3-methoxy groups stimulate the growth of shoots of essential oil rose [23]. It is shown that hydroxybenzoic (vanillic, syringic, gallic) and hydroxycinnamic (p-coumaric, caffeic, ferulic) acids in the nutrient medium (concentration of 1mm·L^{-1}) in the area of contact with living tissues initiate callusogenesis [23]. Vanillic acid in the nutrient medium increases the activity of phenylpropanoid synthesis. At the same time, an increase in the number of hydroxyl groups in the molecular structure of hydroxybenzoic acids (gallic acid), on the contrary, slows down the synthesis of total phenols and catechins.

Gallic acid (GA) is found in plant tissues in free form (Figure 1), but mainly in the composition of esters and hydrolysed tannins (gallotannins).

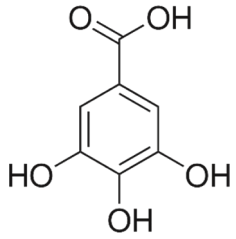


Figure 1. Structural formula of gallic (3,4,5-trihydroxybenzoic) acid

Tannins have high antioxidant activity and protect cell membranes and other intracellular structures from free radical damage, especially under stressful conditions [12]. Although in some plant species (*Populus tremula* L., *Salix alba* L.) the synthesis of phenolic compounds in response to injury slightly increases [24; 25]. This creates prerequisites for the successful introduction of primary explants into the *in vitro culture*. For other species (*Quercus robur* L., *Corylus avellana* (L.) H.Karst.), on the contrary, it is characterised by intensive synthesis of hydrolysed tannins and other polyphenols, which are able to chelate metals and participate in redox reactions [26; 27]. GA complexes, as the main component of gallotannins, with iron ions have antibacterial activity [28]. Due to the polymerisation of condensed tannins, the nutrition of explants is difficult, which over time can cause tissue necrosis. Slowing down the accumulation and release of phenols at the sites of tissue damage to donor plants is critical. Therefore, at the first stages of the introduction into *in vitro* culture of plants with active polyphenol synthesis, the addition of GA to the nutrient medium would be appropriate to reduce the risk of tissue autointoxication due to active oxidation under traumatic stress conditions.

The purpose of this study was to investigate the effect of GA on organogenesis and phenolic synthesis of *Salix alba* and *Corylus avellana* in plant tissue culture in the aspect of reducing autointoxication of explant tissues by polyphenol oxidation products.

Materials and Methods

Studies were conducted on *Salix alba* regenerant plants and 6 varieties of hazel (varieties of Italian selection – 'Tonda Romana' (Lazio), 'Tonda Di Giffoni', 'Mortarella' (Campania) [29], 'Tonda Gentile Dele Lange' and varieties of North American selection – 'Barcelona', 'Epsilon'. A wild form *Corylus avellana* was used as a reference object.

Apical buds from 1-2 annual hazel plants (*Corylus avellana* L.) (container culture) and willows (*Salix alba* L.) were used as primary explants.

70% ethanol (30-60 seconds) and 0.1% mercury chloride ($HgCl_2$) solution with an exposure of 7-8 minutes were used to obtain an aseptic culture, which provided 80% of sterile and viable material.

Explant were cultivated on a modified Driver-Kuniyuki walnut medium (DKW) [30] with the addition of 2.5-5.0 mg·l^{-1} BAP (6-benzylaminopurine), 100-200 mg·l^{-1} Fe-EDDHA, 30 g·l^{-1} sucrose, and 6 g·l^{-1} agar at 5.8-6.0 pH (control). To investigate the effect of trihydroxybenzoic acid (GA) on explant

morphogenesis, it was added to a nutrient medium at a concentration of 1 mmol·l^{-1}. All experiments on the morphogenesis of hazel and willow tissues and organs *in vitro* were conducted in a controlled climate room with 16-hour lighting (2.5-3.0 klx), at a temperature of +26.0°C. The cultivation period – 30-45 days. Determination of the total content of phenolic compounds and flavonoids in the leaves of regenerant plants was performed 14 days after cultivation.

Methods for quantitative determination of total phenols and flavonoids. Extraction of polyphenols from macerated plant tissues was performed with cold methyl alcohol (80%). The volume ratio of solvent to raw mass of leaves was 1/10 (v/v). The total content of phenols in the leaves was determined using spectrophotometry with Folin-Ciocalteu reagent [31]. The calibration graph was plotted using gallic acid. The flavonoid content was determined spectrophotometrically at λ=419 nm. 250 µl of methanol (80%), 200 µl of 0.1 mol aluminium chloride ($AlCl_3$), and 300 µl of 1 mol sodium acetate (CH_3COONa) were added to 50 µl of the obtained ethanol extract of the leaves. The calibration graph was constructed using quercetin (Sigma, Germany). Repeatability of measurements n=4.

Biochemical profiling of solutions of iron and GA chelate complex. The separation of substances was performed by high-performance thin-layer chromatography (HPTLC) on cellulose plates (Merck) in solvent systems: ethyl acetate–formic acid–acetic acid–water (v/v/v/v – 5:1:1:2) and n-butanol–acetic acid–water (v/v/v – 3:1.2:0.8). The resulting chromatograms were treated with 0.5% NP reagent in ethyl acetate, followed by heating at 90°C for 1 min. GA was detected on the chromatogram in ultraviolet light (λ_{max}= 365 nm). The Rf (retention factor) values of individual compounds were determined photodensitometrically using the Sorbfil TLC Videodensitometer computer programme.

Method of statistical data processing. The measurement results are presented as the average value ± standard error (x±SE). The significance of the difference ($p<0.05$) between the obtained data was determined by variance analysis (one-way ANOVA) using Tukeys post-hoc test in the XLSTAT software suite [Addinsoft Inc., USA, 2010]. The principle component analysis (PCA) was performed in the XLSTAT.

Results and Discussion

According to the findings, GA as part of the DKW nutrient medium (NM) actively awakened dormant buds and stimulated the growth of shoots of *Salix alba* L. *in vitro*. At a concentration of 1 mmol·l-1, hydroxybenzoic acid stimulated the awakening of lateral buds, caused branching of stems, development and growth of lateral roots (Fig. 2).

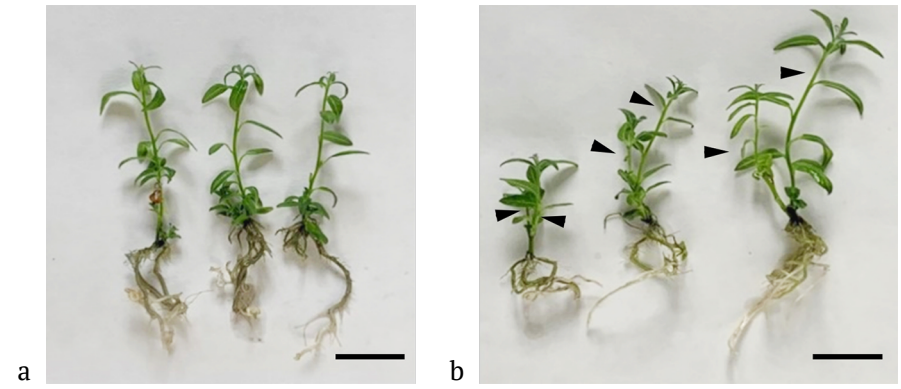


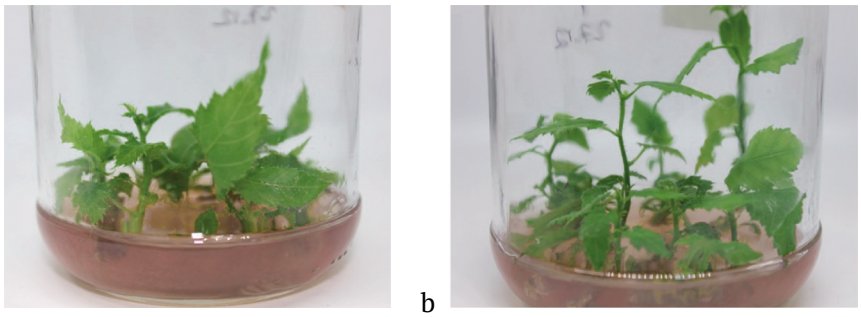
Figure 2. *Salix alba* regenerant plants, which are grown in the following conditions: *in vitro* on the basic (a) and modified gallic acid (b) DKW nutrient medium; scale bar – 10 mm

However, during the experiment, a difference was found in the individual sensitivity of regenerant plants to free GA. Thus, some plants showed signs of suppressed growth, while the morphometric parameters of others (the length of shoots and lateral roots, the length and width of leaf blades) exceeded the control by 1.3-1.5 times. This fact may indicate the ability of free GA, or in combination with metal ions, to influence plant morphogenesis. The variability of growth processes indicates a non-specific nature of *Salix alba* regenerant plant reactions to non-hormonal stimuli of 3,4,5-trihydroxybenzoic acid, which is of scientific and practical interest from the standpoint of identifying genotypes that are particularly sensitive to GA.

The role of GA and other hydroxybenzoic and hydroxycinnamic acids in the regulation of physiological

processes is quite complex and multi-vector. GA is a part of hydrolysed tannins, has a high antioxidant potential, and is able to chelate metal ions [32; 33]. In the experiment, it contributed to a decrease in the effect of apical plant dominance conditioned by the vertical gradient of auxin concentration. The development of lateral roots and lateral shoots is usually associated with changes in the transport and distribution of phytohormone in tissues. Differences in organogenesis and features in stem alkalinisation indicate that this hydroxybenzoic acid acts on explants not locally, at the point of contact with the medium, but systemically. This suggests that it not only enters the tissues with NM, but is also transported through the tissues.

A similar process was observed in an experiment with different varieties of hazel (*Corylus avellana* L.). At the initial stages of cultivation of GA as part of DKW NM contributed to the awakening of lateral buds and the growth of regenerant plants compared to the control (Fig. 3).



a b

Figure 3. *Corylus avellana* regenerant plants of 'Tonda Romana' varieties on basic (a) and gallic acid-modified (b) DKW nutrient medium

However, over time, the difference between the growth rate indicators in the control and experimental groups of plants was levelled. The index of the ratio of leaf width to its length, and the total surface area of leaf blades, decreased on the modified medium with GC. This may indicate a slowdown in the proliferation of marginal meristem cells. Regarding the synthesis of polyphenols, it was found that according to the reaction to GA, the hazel varieties under study were divided into two groups. In the first (varieties 'Tonda Romana', 'Tonda Gentile Dele Lange', 'Barcelona') – GK, in comparison with the control, slowed down the synthesis of phenols; varieties of the second group ('Tonda Di Giffoni', 'Mortarella', 'Epsilon') – with the addition of exogenous hydroxybenzoic acid, increased the synthesis of polyphenols (Table 1). Interestingly, the distribution in these groups coincided with the complementarity of varieties for pollination. Thus, the Italian early-maturing variety 'Tonda Gentile Dele Lange' is pollinated by a larger calibre variety 'Tonda Romana', and the early, rather frost-resistant Italian variety 'Tonda Di Giffoni' is the main one for fruiting, and another Italian variety 'Mortarella' is recommended to be used as a pollinator. The 'Barcelona' variety, selected by the University of Oregon (USA), is an early and frost-resistant variety. Another variety of the same selection – 'Epsilon' is recommended as a pollinator.

Table 1. Effect of exogenous gallic acid on the content and ratio of polyphenols in leaves of *Corylus avellana* in vitro (x±SE; n=4)

Type/variety	NM	PH	FL	PH/FL
Wild form	Control	36.5±2.19	8.6±0.69	4.3
	GA	32.1±1.93	4.5±0.36*	7.1
'Tonda Romana'	Control	40.8±3.45	6.9±0.55	5.9
	GA	37.2±2.23	4.9±0.39*	7.6
'Tonda Gentile Dele Lange'	Control	62.8±3.77	6.4±0.51	9.8
	GA	45.8±4.15*	4.5±1.36*	10.2
'Mortarella'	Control	26.2±1.57	5.1±0.40	5.2
	GA	43.4±2.61*	5.6±0.45	7.8

Table 1, Continued

Type/variety	NM	PH	FL	PH/FL
'Tonda Di Giffoni'	Control	40.0±2.80	6.0±0.78	6.6
	GA	44.8±3.19	8.0±1.64*	5.6
'Barcelona'	Control	36.1±2.17	6.5±0.52	5.5
	GA	26.4±1.59*	4.1±0.33*	6.4
'Epsilon'	Control	26.6±1.60	5.4±0.43	5.0
	GA	47.0±2.82*	5.2±0.42	9.0

Note: PH – phenolic compounds, FL – flavonoids, PH/FL – ratio, GA – gallic acid, * – the difference with the control is significant ($p<0.05$)

Notably, the varieties recommended for fruiting have a higher content of phenolic compounds ('Tonda Gentile Dele Lange' and 'Tonda Di Giffoni'), compared to pollinating varieties ('Mortarella'). In general, Italian varieties, in comparison with the varieties of the selection centre of the University of Oregon (USA), are characterised by a higher content of phenolic compounds in the leaves. As for flavonoids, GA significantly increased their content in the leaves of only Italian varieties 'Tonda Di Giffoni' and 'Mortarella', which are usually recommended as a complementary pair. In *Corylus avellana* plants (classical species form) the flavonoid content decreases by 1.9, and in varieties 'Tonda Romana', 'Tonda Gentile Dele Lange' and 'Barcelona' – by almost 1.5 times. Thus, GA at a concentration of 1 mmol·L^{-1} can influence plant morphogenesis, stimulate cell proliferation, and accelerate or inhibit phenylpropanoid synthesis. At the same time, its regulatory effect is not specific and depends on the characteristics of the plant genotype.

Based on the results of PCA analysis, which are represented in the coordinates F1 and F2 of the main components of the *Corylus avellana* regenerant plants in terms of the content of phenols in the leaves, it was shown that the central position with the lowest contribution to the total variance is occupied by the varieties 'Tonda Romana', 'Tonda Di Giffoni' and 'Barcelona' (Fig. 4a).

These varieties are close to each other in terms of the intensity of accumulation of phenolic compounds and occupy an intermediate position between the high-phenolic variety 'Tonda Gentile Dele Lange' and the low-phenolic 'Epsilon' and 'Mortarella'. Accordingly, these varieties had the greatest contribution to the overall variance (F1 axis), which mainly determines the content of total phenols in the leaves (Table 2).

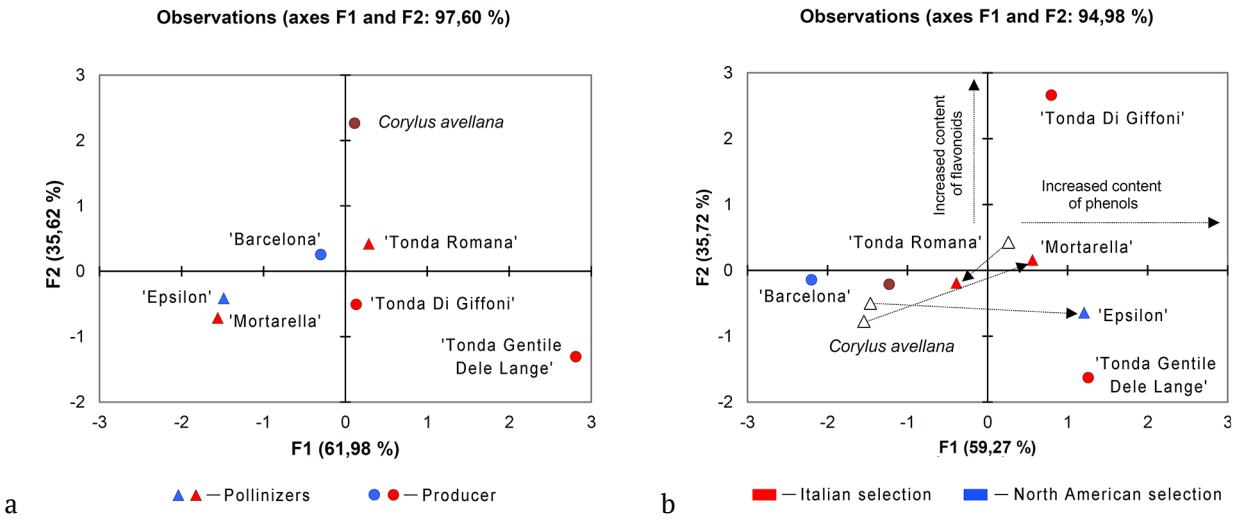


Figure 4. The results of PCA analysis of Italian and North American varieties of *Corylus avellana* under conditions of cultivation on basic (a) and gallic acid-modified (b) nutrient media

Table 2. Introduction of *Corylus avellana* species and varieties to the total variance (%) according to the results of PCA analysis of plant phenolic compounds i*n vitro*

Variety	F1	F2	F3
Basic DKW			
Corylus avellana	0.1	**68.4**	**37.8**
'Tonda Romana'	0.6	2.4	0.0
'Tonda Gentile Dele Lange'	**60.8**	**22.8**	**9.6**
'Barcelona'	0.7	0.9	0.0
'Tonda Di Giffoni'	0.1	3.4	1.3
'Mortarella'	**18.6**	6.7	1.5
'Epsilon'	**17.0**	2.3	0.6
A modified DKW with GA (1mmol·l^{-1})			
Corylus avellana	**12.1**	0.6	3.4
'Tonda Romana'	1.2	0.5	0.3
'Tonda Gentil Dele Lange'	**12.7**	**35.4**	0.2
'Barcelona'	**38.9**	0.3	**9.1**
'Tonda Di Giffoni'	5.1	**94.3**	2.9
'Mortarella'	2.5	0.3	3.2
'Epsilon'	**11.6**	5.5	**5.3**

The relatively high content of flavonoids in the leaves was typical for *Corylus avellana* plants (wild form) in plant tissue culture. The wild form of regenerant plants had the greatest contribution to the overall dispersion (F2 axis), which explains their distant position from the group of varieties along the second main component (Fig. 4a). At the same time, the flavonoid content decreased by 1.9 times in natural regenerant plants on GA-modified NM. A similar effect was observed in varieties 'Tonda Romana', 'Tonda Gentile Dele Lange' and 'Barcelona'. However, GA almost did not affect the amount of flavonoids in the leaves of the 'Epsilon' and 'Tonda Di Giffoni' varieties, which also indicates a non-specific nature of the action of 3,4,5-trihydroxybenzoic acid. In the coordinates of the main components, after the addition of GA, the wild form among the group of varieties moved down along the F2 axis (a decrease in the content of flavonoids in the leaves) and slightly less along the F1 axis (due to a decrease in the content of polyphenols) (Fig. 4b), the movement of some varieties in the coordinates of the main components under the influence of GC is shown by arrows). A more noticeable decrease in the content of phenols compared to the control group of plants was observed only in the 'Tonda Gentile Dele Lange' variety, which was also noted for the largest contribution to the overall variance (Table 2).

The revealed feature of the action of hydroxybenzoic acid may be related to the variety-specific sensitivity of plants to it by type: dose – effect. In the case of isothermal conditions and a regular photoperiod (16 hours), its effect on phenylpropanoid and flavonoid synthesis depended on the NM composition, GA concentration, and pH of the medium. It is known that GA can form coordination complexes with metal ions, which depend on the acidity of the medium [33]. Accordingly, under the conditions of adding GA to the NM composition, the mobility and availability of trace elements for plants may vary. In a slightly alkaline medium, a stable Fe^{3+} ion complex is combined by three coordination bonds with gallic acid, and in a slightly acidic medium, it gradually dissociates with the transition to bis-coordination [34]. In the pH range from 3.52 to 5.50, GA interacts with Fe^{2+} ions in the ratio of 1:1 [28].

In this experiment, a chelated form of iron (Fe-EDDHA) was added to NM, which is more stable and accessible to hazel plants compared to Fe-EDTA [35]. Iron in the form of Fe-EDTA at pH 5.7 is photooxidised to form iron oxide [36], which causes acute deficiency of an extremely important element for hazel explants [35; 37]. The system of phenolic synthesis enzymes is very sensitive to iron ion deficiency [38]. Their lack, for example, activates the synthesis of coumarin, which is released by the roots of some plants with the participation of a transporter protein. As a result, an organometallic complex with a physiologically accessible form of iron is formed.

It is known that the equilibrium constant of the complex Fe^{2+} with GA (log(K)~7) [39] is significantly less than the equilibrium constant of its binding to Fe^{3+} ions (log(K)~34) [40]. This indicates that under the conditions of adding GA to the nutrient medium,

the stability of the chelate complex Fe-EDDHA (log(K)~35.09) should be maintained. However, for different volume ratios of aqueous solutions Fe-EDDHA and GA, certain interactions occur between the components, which is determined by the adsorption spectra of light energy (Fig. 5a).

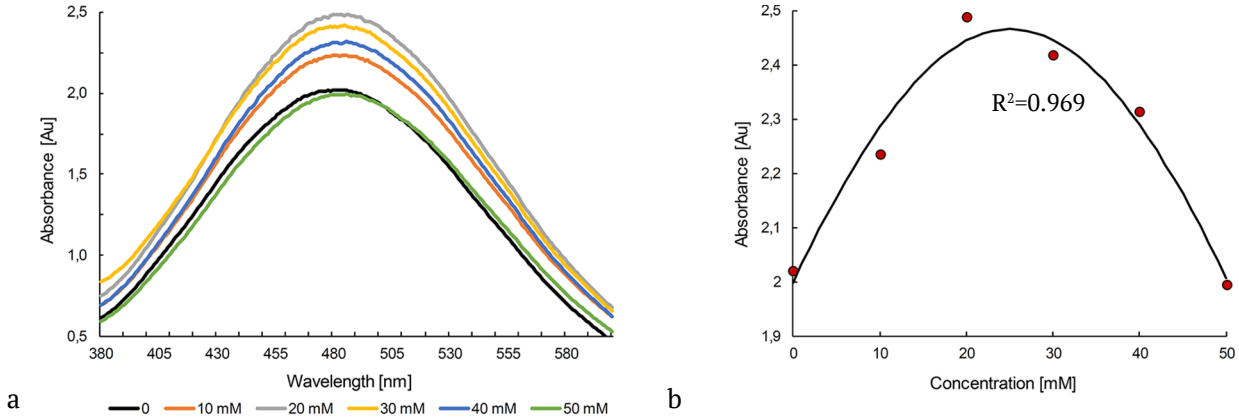


Figure 5. Light energy absorption spectra of the chelated form of iron (10 mmol Fe-EDDHA): a – at different wavelengths after adding a gallic acid solution; b – at the maximum absorption (λ_{max}=480 nm) with the addition of gallic acid

When adding 10 mmol GA solution to chelated iron, the light absorption index (λ_{max}= 480 nm) the resulting solution reached its maximum volume ratio (v/v) – 1:2. With a further increase in the volume fraction of GA, the absorption of the solution gradually decreased (Figure 5b). A possible explanation for the detected effect is the partial protonation of EDDHA in solution, which creates prerequisites for the competition of GA for coordination bonds with iron ions. After reaching a certain equilibrium, the interaction of hydroxybenzoic acid with the chelate complex slows down.

The stability of Fe-EDDHA in an aqueous solution was confirmed by chromatography (Fig. 6). At the same time, in the case of chromatographic separation of a 10 mmol aqueous solution of iron chelate, two products were detected (Fig. 6a). The EDDHA ligand is known to exist in three forms. Of these, the ortho- and ortho-isomer EDDHA has the best complexing ability, which has six binding sites and forms stable chelates [32]. Two peaks on the chromatogram probably correspond to Fe(III)-rac-o,o-EDHHA (Rf~0.50) and Fe(III)-meso-o,o-EDHHA (Rf~0.55), which are the main components in the chelate complex [41]. The stability coefficients of these isomers are 35.86 and 34.15, respectively [42]. When the GA solution was added, the peak area of one of the metal chelate isomers with a retention factor (Rf~0.55) on chromatography initially increased, and when the volume fraction of GA increased, it decreased until it almost completely disappeared (Figs. 6c-6f), which is consistent with spectrophotometry data (Fig. 5b). Considering that equilibrium constant of meso Fe-(o,o)EDDHA is very close to the Fe^{3+} complex with GA, it interacted with the latter when added to the iron chelate, as observed in the densitograms. The total area of the main peaks on the densitogram for the introduction of GA into the Fe-EDDHA solution in a volume ratio of 1:2 first increased, and then gradually decreased with an increase in the volume fraction of hydroxybenzoic acid. Under other chromatography conditions, the chelate complex and GA were separated differently (Fig. 6b). This revealed a direct relationship between the increase in the Fe-EDDHA retention factor (Rf~0.90 to 0.93) and the volume fraction of hydroxybenzoic acid. This also confirms that under such conditions, GA affects the coordination complexes of at least one of the iron chelate isomers.

At low concentrations in plant tissues, GA accelerates the desoxydation of Fe^{3+} to Fe^{2+}. Divalent iron is more accessible to plants, but under stress, cells actively synthesise H_2O_2, and in the presence of Fe^{2+}, the Fenton reaction (catalytic decomposition of hydrogen peroxide by iron ions to form a hydroxyl radical) is triggered [26]. Under such conditions, GA is able to reduce energy barriers and accelerate the oxidation of organic compounds [43]. In addition, as a result of desoxydation of Fe^{3+} to Fe^{2+}, GA is converted to a semiquinone radical, which can then be reduced by Fe^{3+} ions to biologically active quinones [44; 45]. In the presence of oxygen, semiquinone is oxidised to form a superoxide anion radical that has mutagenic

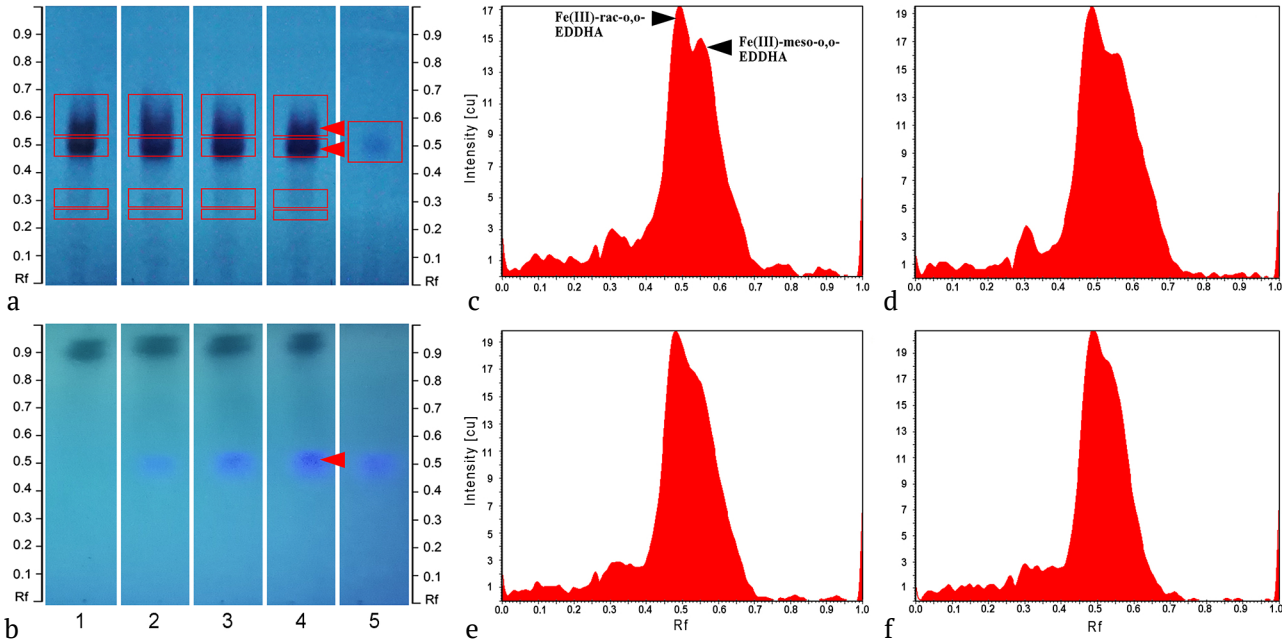


Figure 5. Chromatograms (a-b) and densitograms (c-f) of solutions: 1, (c) – 10 mmol Fe-EDDHA, for adding gallic acid solution to iron chelate: 2, (d) – 10 mmol, 3, (e) – 20 mmol, 4, (f) – 30 mmol, 5, (d) – 10 mmol gallic acid; arrows on chromatograms show GA (a) and Fe-EDDHA forms (b)

activity [46]. In living cells, a general increase in the concentration of free radicals causes oxidative stress due to the establishment of oxidised proteins, lipids, and biopolymers [47]. Thus, at the GA concentration of 10^{-5}-10^{-3} mol, an order of magnitude higher than that used in this experiment (10^{-6} mol), the processes of *Cucumis sativus* L. seed germination and seedling growth are suppressed [48]. In addition, it is known that hydrolysed tannins, which include GA, form chelates with metal ions depending on their valence, ionic radius, and polarity coefficient, which directly affects the physiological availability of elements for plants. In polycationic solutions, which also include nutrient media, metals with a higher valence and a larger ionic radius are primarily chelated [32]. Thus, by the presence of Fe^{3+} and Fe^{2+} ions in the medium under conditions of competitive complexation, chelation occurs mainly with a trivalent iron ion, which is conditioned by a higher stability constant of the complex. Chelates with Cu^{2+} can also form quite quickly. With macro- and microelements Zn^{2+}, Mg^{2+}, Sa^{2+}, and K^{+}, this process may be somewhat slower. With the addition of Fe-EDDHA to the NM, not only iron ions but also the ligand itself, which is capable of chelating other trace elements inside the tissues, enter the tissues of *Corylus avellana* plants [49].

The formation of gallic acid chelate complexes with metal ions can significantly affect the mobility of trace elements in the nutrient medium and their bioavailability, which determines the physiological state of plants. In addition, the concentration of trihydroxybenzoic acid used in the experiment is not sufficient for the complete chelation of existing metal ions. Therefore, the vast majority of macro- and microelement salts in the nutrient medium remained relatively unchanged.

The study found that exogenous GA increased the morphometric parameters of the leaf plate, shoots, and roots. Similar results were obtained on *Oryza sativa* L. plants, where an increase in shoots and roots was observed by 1.28 and 2.05 times, respectively, under the action of GA (60 µg/ml) in relation to the control [50]. According to cold stress, GA had a positive effect on plant growth indicators of *Glycine max* (L.) Merr. [51]. Based on the results obtained by A. Singh et al. [50] during the treatment of *Oryza sativa* with GA, the total level of phenolic compounds increased by 1.31 times compared to the control. A similar effect was observed in *Lepidium sativum* L. plants. Exogenous gallic acid not only increased the total content of phenolic compounds (by 19%) and antioxidant activity (46%), but also increased plant resistance to salt stress [52]. GA and its derivatives have been shown to increase plant resistance to low positive temperatures and drought [53], and reduce the toxic effect of copper

on *Zea mays* L. [54]. In callus tissues of *Solanum lycopersicum* L. after the introduction of GA into NM, the concentration of phenols and flavonoids increased by 1.6 and 1.8 times, respectively [55], which indicates a significant effect of GA on the phenolic synthesis of the plant. However, in this study, the response of regenerant plants to exogenous GA was significantly different. Thus, in half of the 6 *Corylus avellana* varieties under study, the phenol content in the leaves also increased. At the same time, in the wild form and other varieties, on the contrary, it decreased. This may indicate the species- and variety-specific sensitivity of these plants to GA and its concentration. Thus, when treated with 10 mmol ferulic, coumaric, and vanillic acids, seed germination and seedling growth of *Chenopodium album* L., *Solanum nigrum* L., *Amaranthus retroflexus* L. were almost completely suppressed The effect of GA on these plant species was insignificant and depended on the concentration of hydroxybenzoic acid. Thus, for *Chenopodium album* and *Solanum nigrum*, the optimal concentration for growth was 0.01 mmol, for *Amaranthus retroflexus* – 0.1 mmol. Other oxybenzoic acids showed both growth-stimulating and inhibiting effects on different species [56].

Based on the results of assessing the general condition of regenerant plants of *Salix alba* and *Corylus avellana* in the *in vitro* conditions, there were no signs of suppressed growth, abnormal stem development, or leaf pigmentation disorders. According to physiological indicators, the presence of 1 mmol·L^{-1} GA in NM is mainly regulatory in nature. Variety-specific and individual sensitivity of plants to exogenous GA are primarily conditioned by the redox status of regenerant plants, which determines the direction of metabolism, in particular, associated with morphogenesis, transport of active forms of phytohormones, and other processes in which 3,4,5-trihydroxybenzoic acid and its chelated complexes with metals directly or indirectly participate.

Conclusions

The presence in the nutrient medium of individual phenolcarboxylic acid, which is part of hydrolysed tannins as one of the main components, even in micromolar concentrations can significantly affect the functionality of plant tissues. Exogenous gallic acid at a concentration of 10^{-6} mol as part of the DKW nutrient medium (pH 6.0) contributed to the branching of stems, the development and growth of lateral roots of *Salix alba* L. regenerant plants. In *Corylus avellana* L. regenerant plants, gallic acid caused an increase in the total number and elongation of internodes. Availability in nutrient medium composition of gallic acid and chelate complex Fe-EDDHA did not cause pigmentation disorders in willow and hazel leaves. Given that equilibrium constant of meso Fe-(o,o)-EDDHA as one of the main isomers of the chelate complex is very close to the equilibrium constant of the ion complex Fe^{3+} with gallic acid, the latter is able to interact with the iron chelate isomer and affect its physiological activity and accessibility for plants. This hydroxybenzoic acid caused a decrease in the total content of phenolic compounds, in particular flavonoids, in the leaves of *Corylus avellana* regenerant plants, which is especially important for plants at the stage of their introduction into plant tissue culture. Special attention should be paid to the fact that the phenolic synthesis of the studied hazel varieties of Italian and North American selection for gallic acid had varietal differences, which confirms its non-specific regulatory properties, which may be related to its ability to interact with the corresponding isoenzymes. Given the ability of gallic acid to form chelated complexes with other trace elements, it remains an open question in what form it enters explants and is subsequently transported in tissues. Further studies of the specificity of the effect of gallic acid on plant phenolic synthesis would provide a better understanding of its role in secondary metabolism, and determine the range of functional possibilities for its practical application in plant biotechnology.

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Галова кислота як неспецифічний регулятор фенольного синтезу і росту рослин-регенерантів *Corylus avellana* (L.) H.Karst. та *Salix alba* L. *in vitro*

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Анотація. Галова кислота міститься у рослинних тканинах у вільній формі, а також у складі складних ефірів та гідролізованих танінів. Ці фенольні сполуки мають значну антиоксидантну активність і захищають клітини рослин від пошкодження вільними радикалами. В умовах стресу, який виникає під час введення рослин в культуру in vitro, для переважної більшості експлантатів характерним є інтенсивний синтез фенолів, які швидко окислюються, полімеризуються, блокують шляхи живлення експлантатів і викликають некротизацію тканин. Додавання галової кислоти у мілімолярних концентраціях до складу живильного середовища зменшує ризики автоінтоксикації тканин продуктами вторинного метаболізму. Метою даної роботи було дослідити вплив екзогенної галової кислоти на органогенез і фенольний синтез рослин *Salix alba* і *Corylus avellana* в умовах *in vitro*. Для цього використовували методи культури тканин і органів *in vitro*, спектрофотометричного визначення у листках загальних фенолів і флавоноїдів, методи дисперсійного і непараметричного аналізу. Встановлено, що галова кислота в концентрації 1 мМ·л$^{-1}$ у складі живильного середовища Драйвера-Куньюки (DKW) викликала пробудження сплячих бруньок, стимулювала ріст пагонів, сприяла галуженню стебел, а також розвитку і росту бічних коренів *Salix alba* в культурі *in vitro*. Фенолкарбонова кислота гальмувала синтез фенолів у рослин *Corylus avellana* сортів 'Тонда Романа', 'Тонда Гентіль Делє Ланге', 'Барселона', водночас сприяла підвищенню вмісту фенольних сполук у листках сортів ‹Тонда Ді Джифоні›, 'Мортарелла', 'Епсилон'. Встановлено, що рекомендований у якості запилювача сорт 'Мортарелла' містить у листках менше фенольних сполук ніж сорти для плодоношення ('Тонда Гентіль Делє Ланге' і 'Тонда Ді Джиффоні'). Отже екзогенна галова кислота в концентрації 1 мМ·л$^{-1}$ має властивості неспецифічного регулятора фенольного синтезу у рослин-регенерантів ліщини (*Corylus avellana*). Вона знижує негативні наслідки автоінтоксикації тканин продуктами окислення поліфенолів, що актуально для рослин з високим вмістом фенольних сполук особливо на етапі їх введення в культуру *in vitro*. Чутливість рослин-регенерантів до 3,4,5-тригідроксибензойної кислоти має практичне значення в аспекті виявлення генотипів з потенційно активним ростом

Ключові слова: розвиток, гідроксибензойна кислота, органогенез, феноли, флавоноїди, живильне середовище