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PECULIARITIES OF THE GROWTH OF *Artemisia tilesii* Ledeb. "HAIRY" ROOTS WITH DIFFERENT FOREIGN GENES

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Aim. To compare *Artemisia tilesii* "hairy" root lines with different transferred genes in terms of the relationship between the total content of flavonoids, the levels of antioxidant activity (AOA) and reducing power (RP), as well as the activity of phenylalanine ammonia-lyase (*PAL*), chalcone synthase (*CHS*), *rol*B and *rol*C genes.

Methods. We compared the root lines Nos. 10 and 16, obtained by transformation with the wild *Agrobacterium rhizogenes* strain A4, lines Nos. 2 and 4, obtained using *A. rhizogenes* carrying pSV124 vector with *ifn-α2b* and *npt*II genes, as well as the roots of non-transformed plants that were cultivated *in vitro*. The presence and activity of *rol*B, *rol*C, *PAL*, and *CHS* genes were determined by PCR and real-time PCR, respectively. The content of flavonoids, AOA and RP were determined by standard tests with $AlCl_3$, DPPH (2,2-diphenyl-1-picrylhydrazyl) and $K_3[Fe(CN)_6]$ accordingly.

Results. The content of flavonoids in most of the lines was higher than in the control, and correlated with AOA and RP. Roots No. 10 were characterized by the fastest growth, which coincided with higher activity of *rol*B and *rol*C genes. The activities of *PAL* and *CHS* in "hairy" roots were lower than those in non-transformed ones.

Conclusions. Root lines carrying only *rol*B and *rol*C and lines with additional *ifn-α2b* and *npt*II genes had similar ranges of flavonoids concentration, AOA and RP levels that exceeded those in the control. The dependence of the root growth rate, and lack of the dependence of the flavonoids content with the activity of the *rol* genes were demonstrated. *PAL* activity inversely correlated with flavonoids content in all experimental lines, which may be the result of overproduction of compounds in transgenic roots.

Key words: *Artemisia tilesii* Ledeb., *Agrobacterium rhizogenes*, "hairy" roots, flavonoids, *rol* genes, *PAL* and *CHS* genes, real-time PCR, antioxidant activity.

Medicinal plants have long been used both in traditional medicine and in the pharmaceutical industry. This is due to the fact that they synthesize a number of biologically active compounds that can be used to treat human diseases. Among medicinal plants, the genus *Artemisia*, or wormwood, is quite famous. Many species belong to this genus. In particular, "Plants of the World Online", an online database published by the Royal Botanic Gardens, counts about 500 species of *Artemisia*, of which *A. annua* L. has recently become the most famous. It has been established that plants of this species are capable of synthesizing artemisinin, a compound with antimalarial and antitumor properties [1, 2]. The Nobel Prize was awarded to Chinese researcher Tu Youyou in 2015 for such studies of annual wormwood (https://www.nobelprize.org/prizes/medicine/2015/tu/lecture/). Recently, publications have appeared on the possibility of using wormwood to inhibit the replication of the dangerous SARS-CoV-2 virus [3].

Among the numerous representatives of the genus *Artemisia*, the little-studied plants *A. tilesii* Ledeb., which have a very limited natural area of growth [4], should be singled out. It is known that they are used by the population of Alaska, on the territory of which they grow, for the treatment of various diseases. Since this plant material is scarcely available, genetic transformation and establishing of "hairy" root cultures can be a way to obtain biologically active compounds from plants of this species. Such roots can be maintained *in vitro* for decades, they are able to grow in bioreactor conditions on a nutrient medium that does not contain high-value components [5]. In addition, "hairy" roots synthesize the same compounds that the mother plants contain. It should be noted that due to the transfer of the *rol* genes of *A. rhizogenes* in the process of transformation, the content of valuable compounds can be significantly increased [6–8]. Such features make "hairy" root cultures of medicinal plants a valuable source of biologically active compounds [9, 10]. This is especially attractive for those plants that are rare, endangered, or not readily available.

It was determined that the bacterial *rol*B gene is a powerful inducer of the secondary metabolism of plants, affecting the activity of the native genes of "hairy" roots [6, 11–13]. For example, increased expression of this gene positively correlated with increased isochorismate synthase gene and with anthraquinone production [14]. In this study, the effect of the *rol*B gene exceeded the similar effect of other *A. rhizogenes* genes, namely *rol*A and *rol*C. The transfer of *rol*B and *rol*C genes to *A. annua* plants led to an increase in the content of artemisinin, artesunate, and dihydroartemisinin in transgenic plants [15]. In addition, an increase in the activity of genes involved in the biosynthesis of artemisinin was observed in these roots.

Previously, we obtained the "hairy" roots of *A. tilesii* and analyzed the accumulation of flavonoids and the level of antioxidant activity [16]. Significant fluctuations of those parameters were found in different lines, which is probably due to the non-determined site of incorporation of transferred genes into the plant genome. At the same time, it is of great interest to find out the features of correlation between the activity of the transferred *rol* genes of *A. rhizogenes* and the synthesis of flavonoids in the lines transformed by the A4 wild strain of *A. rhizogenes* and the same strain that additionally carried a plasmid with *npt*II and *ifn*-α*2b* genes. The present work was aimed at comparing such "hairy" root lines regarding the presence of an interrelation between the total content of flavonoids and the level of antioxidant activity, as well as the activity of the plant's own genes — phenylalanine ammonia-lyase (*PAL*) and chalcone synthase (*CHS*), and the *rol*B and *rol*C genes of *A. rhizogenes*.

Materials and Methods

Plant material

Four *A. tilesii* "hairy" root lines from the collection of the Institute of Cell Biology and Genetic Engineering of the National Academy of Sciences of Ukraine were used as the plant material for the study [17]. We obtained two of them (Nos. 10 and 16) by the transformation using *A. rhizogenes* A4 wild strain. The other two (Nos. 2 and 4) — using *A. rhizogenes* carrying pCB124 vector with the human interferon-α2b gene *ifn*-α*2b* and the selective neomycin phosphotransferase *npt*II gene. The roots were grown at a temperature of +24 °C for two weeks on the solidified nutrient Murashige and Skoog medium (Duchefa Biochemie) with halved macrosalt content (1/2 MS) and the addition of sucrose at a concentration of 20 g/l. For research, the roots were separated from the nutrient medium and washed with deionized water.

PCR analysis

Total plant DNA for polymerase chain reaction (PCR) was isolated according to the protocol [18], using the CTAB method. The following pairs of primers were used: 5′-CCTGAATGAACTCCAGGACGAGGCA-3′ and 5′-GCTCTAGATCCAGAGTCCCGCTCAGAAG-3′ (*npt*II, 622 bp, 65 °C); 5′-TTGATGCTCCTGGCACAG-3′ and 5′-TTCTGCTCTGACAACCTC-3′ (*ifn*-α*2b*, 396 bp, 60 °C); 5′-ATGGATCCCAAATTGCTATTCCTTCCACGA-3′ and 5′-TTAGGCTTCTTTCTTCAGGTTTACTGCAGC-3′ (*rol*B, 592 bp, 56 °C); 5′-TGGAGGATGTGACAAGCAGC-3′ and 5′-ATGCCTCACCAACTCACCAGG-3′ (*rol*C, 473 bp, 56 °C). The reaction was carried out on a Mastercycler personal 5332 amplifier (Eppendorf). PCR products were analyzed by electrophoresis in 1.5% agarose gel in a Tris-borate buffer system.

Real-time qPCR analysis

Total RNA was isolated from "hairy" root samples (~200 mg) of transformed and control

Table 1. **Primer sequences of the genes used for qPCR amplification**

Gene name	Primer sequences, 5′–3′	Accession number in the Gene bank or reference
β-actin	Forward: ATCAGCAATACCAGGGAACATAGT Reverse: AGGTGCCCTGAGGTCTTGTTCC	EU531837
chalcone synthase (*CHS*)	Forward: AGGCTAACAGAGGAGGGTA Reverse: CCAATTTACCGGCTTTCT	GQ468548
phenylalanine ammonia-lyase (*PAL*)	Forward: ACACTCGGTTAGCTATTGCTGCAA Reverse: CCATGGCGATTTCTGCACCT	JF806362
*rol*B	Forward: CTCACTCCAGCATGGAGCCA Reverse: ATTGTGTGGTGCCGCAAGCTA	[16]
*rol*C	Forward: TGGAGGATGTGACAAGCAGC Reverse: ATGCCTCACCAACTCACCAGG	[16]

A. tilesii cultures according to the high-throughput phenol-based method described in the paper [19]. DNase treatment of each RNA sample was performed in a 20 µl reaction mixture containing 3 µg total RNA, 2 U RNase-free DNase I (Thermo Fisher Scientific), 20 U RiboLock™ RNase inhibitor (Thermo Fisher Scientific), 1× Reaction Buffer (Thermo Fisher Scientific), adjusted to the final volume with DEPC-treated Milli-Q water. The reaction was carried out at 37 °C for 30 min using an Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System (Thermo Fisher Scientific) and stopped by adding 2 µl of 25 µM EDTA, followed by incubation at 65 °C for 10 min. Reverse transcription (RT) was performed in a reaction mix containing 10 µl DNase-treated RNA sample, 40 U Maxima Reverse Transcriptase (Thermo Fisher Scientific), 20 U RiboLock™ RNase inhibitor (Thermo Fisher Scientific), 5 µM oligo(dT)$_{18}$ primer (Metabion, Germany), 1 µM dNTP (Thermo Fisher Scientific), 1× Reaction Buffer (Thermo Fisher Scientific), adjusted to a final volume of 20 µl with DEPC-treated Milli-Q water. The reaction was performed at 55 °C for 30 min in a thermocycler and stopped by incubation at 85 °C for 5 min.

Gene expression analysis was performed in 20 µl qPCR mix using HOT FIREPol → → EvaGreen → qPCR Mix Plus (no ROX; Solis BioDyne, Estonia) according to the manufacturer's protocol. The reaction mixture included gene-specific forward and reverse primers (0.5 mM each), and 2 µl RT mixture prepared above. The primer sets used for amplification of flavonoid biosynthetic genes and real-time qPCR conditions are given in Table 1. Three amplification reactions per sample were performed to obtain the average expression levels of the genes and internal standards. The relative expression levels of the genes of interest were calculated according to Pfaffl's equation [20].

Total flavonoids content

Determination of the content of flavonoids was carried out according to the Pekal and Pyrzynska method with modifications [21]. To prepare the extracts, the roots were separated from the medium, washed with deionized water, dried using filter paper, weighed 0.3 g each and homogenized in 3 ml of 70% ethanol. The homogenate was centrifuged in an Eppendorf Centrifuge 5415 C at 15 000 g for 10 min. The reaction mixture contained 0.25 ml of extract supernatant, 1 ml of deionized water, 0.075 ml of 5% $NaNO_2$ solution. After standing for 5 min, 0.075 ml of 10% $AlCl_3$ solution was added and held for another 5 min. Then 0.5 ml of 1M NaOH and 0.6 ml of deionized water were added. Absorption was determined at λ = 510 nm on a Fluorate-02-Panorama spectrofluorimeter. The calculation of the total content of flavonoids was carried out in the rutin equivalent (RE) according to the following formula, and converted to grams of fresh weight (FW):

$$C = (0.8842 \cdot OD) \cdot V / m, (R^2 = 0.9988)$$

where C — concentration of flavonoids in 1.0 g of fresh weight of plant material, mg RE/g FW; *OD* — optical density of the investigated solution, U; *V* — volume of ethanol used to prepare the extract; *m* — mass of plant material used for research.

Antioxidant activity (AOA)

The antioxidant activity of ethanol extracts of "hairy" roots was studied using the DPPH test according to the method described in [22]. The optical density of the solutions was

measured at a wavelength of λ = 515 nm on a Fluorate-02-Panorama spectrofluorimeter. The percentage of inhibition was calculated according to the following formula:

$$\%\ inhibition = (OD_1 - OD_2) / OD_1 \cdot 100,$$

where OD_1 — optical density of the DPPH solution, U; OD_2 — optical density of the of the reaction mixture after carrying out the reaction with DPPH, U.

The effective concentration (EC_{50}) was calculated as the fresh weight of the root (mg FW) required to scavenge 50% of DPPH in the reaction with the radical.

Reducing power (RP)

Determination of the ability of root extracts to reduce iron ions Fe^{3+} to Fe^{2+} was proceeded according to the method of Zhao *et al.* [23] with modifications [16]. The reaction mixture contained: 0.312 ml of 0.2 M phosphate buffer (pH 6.6); 0.312 ml of 1% potassium hexacyanoferrate(III) and ethanol root extract, the concentration of which was successively reduced. The cuvettes were incubated in a water bath at 50 °C for 30 min. After that, 0.312 ml of 10% trichloroacetic acid, 1.25 ml of deionized water and 0.25 ml of 0.1% iron(III) chloride were added to the reaction mixture. The optical density was measured at a wavelength of λ = 700 nm on a Fluorate-02-Panorama spectrofluorimeter. Reducing power was characterized by the effective concentration parameter ($EC_{0.5}$), which corresponded to the roots weight (mg FW) required to obtain OD = 0.5.

Weight gain

The terminal parts of the roots (growth points) with a length of about 10 mm were separated, weighed and cultivated on the surface of the agarized 1/2 MS medium at a temperature of +24 °C. After two weeks, the grown roots were separated from the agar, washed with distilled water, dried using filter paper and weighed on a Sartorius balance with a standard deviation of ± 0.005 g. Weight gain was determined as the difference between the final and initial weights in terms of one growth point.

Data analysis

All analyzes were performed in triplicate. Results were calculated in Microsoft Excel and presented as mean ± SD. The data were analyzed for statistical significance using ANOVA followed by Tukey HSD test using R software version 4.0.4. The difference between mean values were considered statistically significant at $P < 0.05$.

Results and Discussion

PCR analysis of four *A. tilesii* lines selected for research confirmed the presence of *rol*B and *rol*C genes in all the lines (Nos. 2, 4, 10, 16) and the absence of those genes in the control roots (C). This result affirms the successful transformation of all four lines (Fig. 1, *A* and *B*) and presence of the genes during 8 years of *in vitro* subcultivation.

Two lines (Nos. 10 and 16) were obtained by *A. rhizogenes*-mediated transformation with the A4 wild strain. The others two (Nos. 2 and 4) using *A. rhizogenes*, carrying the pCB124 vector with the human interferon-α2b gene and the selective neomycin phosphotransferase II gene, i.e., which additionally had *ifn-α2b* and *npt*II genes. PCR analysis confirmed the presence of these genes in lines Nos. 2 and 4 (Fig. 1, *C*). These genes were not detected in control roots.

It was established that the weight gain of the root lines varied significantly (Fig. 2, 3). Line No. 10 was characterized by the fastest growth (weight gain was 2.31 ± 0.22 g). Weight gain of the roots Nos. 2, 16, and 4 was 1.52 ± 0.09 g, 1.51 ± 0.19 g, and 1.77 ± 0.13 g, respectively.

The content of flavonoids in lines Nos. 2, 10 and 16 was higher than that in control roots (2.31 ± 0.42 mg RE/g FW) and varied from 3.86 ± 0.22 mg RE/g FW in line No. 16 to 9.47 ± 1.97 mg RE/g FW in line No. 2 (Fig. 4). It should be noted that the content of those compounds in line No.4 was 2.57 ± 0.28 mg RE / g FW and did not differ from the control.

Such an increase in the content of flavonoids in extracts from most of "hairy" root lines compared to non-transformed plants can be explained by an increase in the level of synthesis of secondary metabolites after the transfer of *rol* genes, as they are known to be activators of metabolism in plants. Significant variability in the parameters between the individual lines is probably caused by the non-determined incorporation of transferred genes into the plant genome. It is also important to indicate that no differences in flavonoids accumulation were observed between the two groups of lines. "Hairy" roots obtained as a result of transformation with a wild strain of agrobacteria (lines Nos. 10 and 16) and those containing *ifn-α2b* and *npt*II genes (lines Nos. 2 and 4) had approximately the same range of flavonoids concentration. Therefore, it can be concluded that these additional genes do not affect the biosynthesis of polyphenolic compounds.

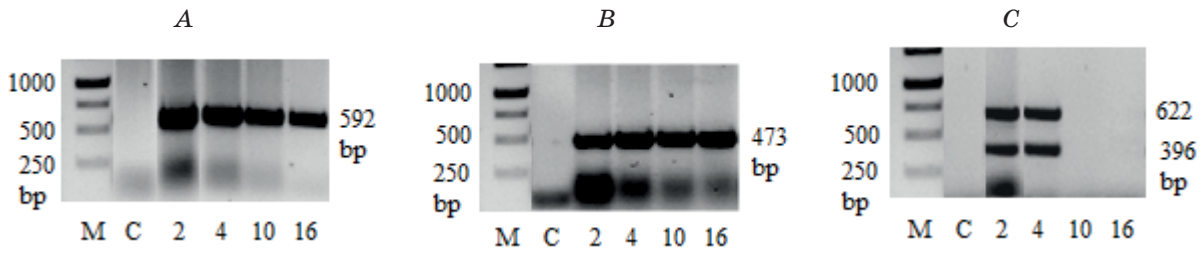

Fig. 1. **Electrophoregrams of DNA amplification products in samples of *A. tilesii* with gene-specific primers to *rol*B (*A*), *rol*C (*B*) and *npt*II i *ifn*-α*2b* (*C*)**
Tracks: M — marker GeneRuler 1 kb DNA Ladder (Thermo Scientific); c — DNA of control plant; 2, 4, 10, 16 — "hairy" root lines Nos. 2, 4, 10, 16.

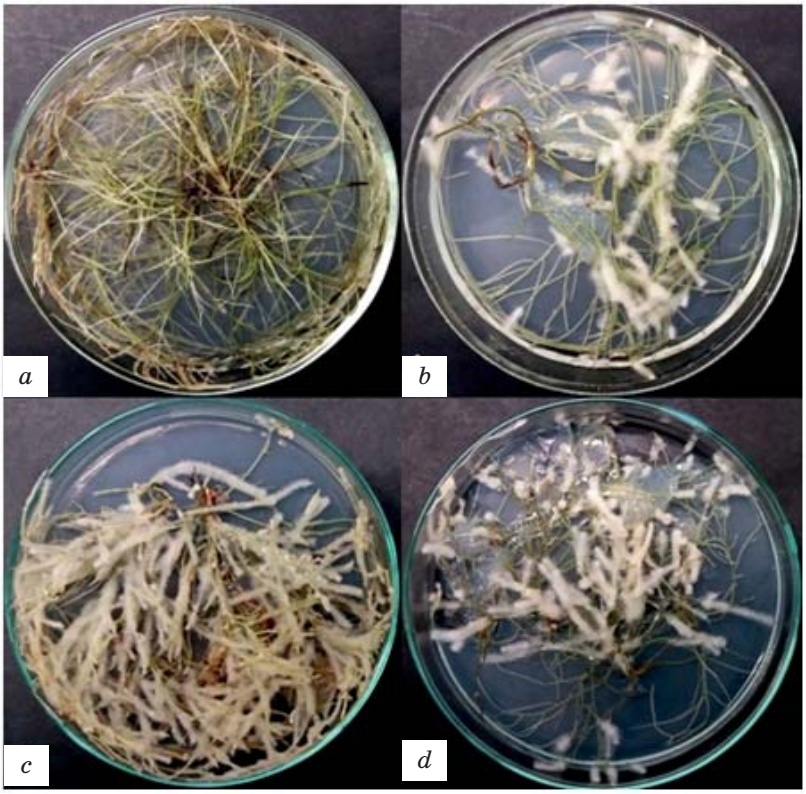

Fig. 2. **"Hairy" roots of *Artemisia tilesii:* lines No. 2 (*a*), No. 4 (*b*), No. 10 (*c*), No. 16 (*d*) after two weeks of *in vitro* cultivation:**
lines Nos. 2 and 4 were transformed with wild *A. rhizogenes* strain A4; lines Nos.10 and 16 were transformed with *A. rhizogenes* carrying *ifn*-α*2b* and *npt*II genes.

Similarly, the antioxidant activity and reducing power of the studied lines were higher than those of the control roots. For example, in the line No. 2 EC_{50} was 1.92 mg FW, and $EC_{0.5}$ was equal to 1.38 mg FW. It was 4.68 and 3.42 times higher, respectively, than the values in non-transformed roots. Both EC_{50} and $EC_{0.5}$ varied between different lines, but it was found out that in all lines, the higher was the flavonoid content, the lower was the EC_{50} and $EC_{0.5}$ values, and thus the higher was the antioxidant activity and reducing power. Such a correlation can be explained by the antioxidant nature of polyphenolic compounds. Polyphenols have the ideal chemical structure to activate the scavenging of free radicals, and they are more effective antioxidants than tocopherols and ascorbates. The antioxidant properties of polyphenols derive from their high reactivity as hydrogen or electron donors. The ability of flavonoids to change the kinetics of peroxidation by changing the order of lipid packing and reducing membrane fluidity is another mechanism of the effect as the radical scavengers [24, 25]. Similar to the total content of flavonoids in the studied

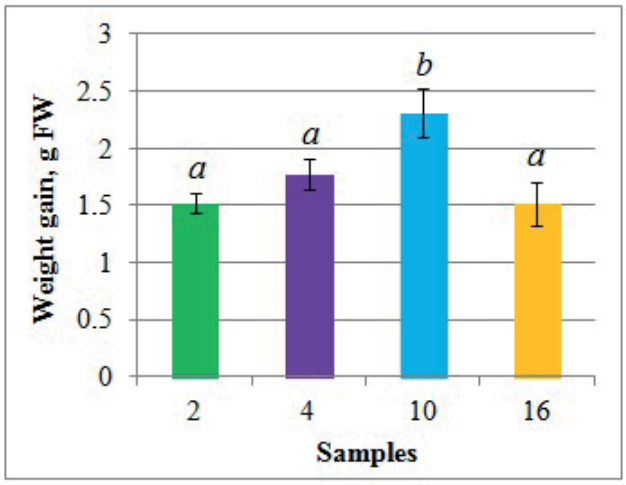

Fig. 3. **Weight gain of the *A. tilesii* "hairy" roots:**
columns 2 and 4 — lines Nos. 2 and 4 transformed with wild *A. rhizogenes* strain A4; 10 and 16 — lines Nos. 10 and 16 transformed with *A. rhizogenes* carrying *ifn*-α*2b* and *npt*II genes.
Error bars with different small letters denote significant differences in values among four samples at $P < 0.05$

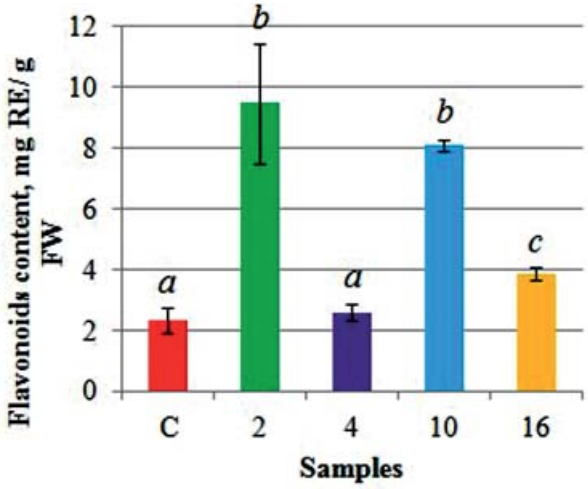

Fig. 4. **Total content of flavonoids in "hairy" and control (C) roots of *A. tilesii:***
columns 2 and 4 — lines Nos. 2 and 4 transformed with wild *A. rhizogenes* strain A4; 10 and 16 — lines Nos. 10 and 16 transformed with *A. rhizogenes* carrying *ifn*-α*2b* and *npt*II genes.
Error bars with different small letters denote significant differences in values among four samples at $P < 0.05$

plant extracts, no differences were found in the range of AOA and RP between the roots obtained as a result of transformation with *A. rhizogenes* wild strain and the bacterial strain carrying additional genes (*ifn*-α*2b* and *npt*II).

The results of real-time PCR showed that the activity of the transferred *rol*B (Fig., 5 *a*) and *rol*C (Fig. 5, *b*) genes was different in the studied lines, regardless of agrobacteria strain they were transformed with. It should be noted that the activity of both genes in line No. 10 was higher than in the other three lines. The relative activity of *rol*B was 3.05–9.34 times higher, and *rol*C was 2.46–5.38 times higher than in the other lines. The relationship between the *rol* genes activity and the weight gain of *A. tilesii* "hairy" roots was studied. Indeed, line No. 10 had the fastest growth rate compared to lines Nos. 2 and 16. The same result was obtained when analyzing the relative activity of the *rol*B gene. This can be explained by the mechanisms of *rol* genes action when they are transferred into the plant genome. Specific root formation in plants is the main function of *rol*B gene. It has a decisive influence on the formation of both lateral and adventive roots. Thus, the difference in growth rate among "hairy" root lines may be due to the difference in the *rol*B gene expression level [26].

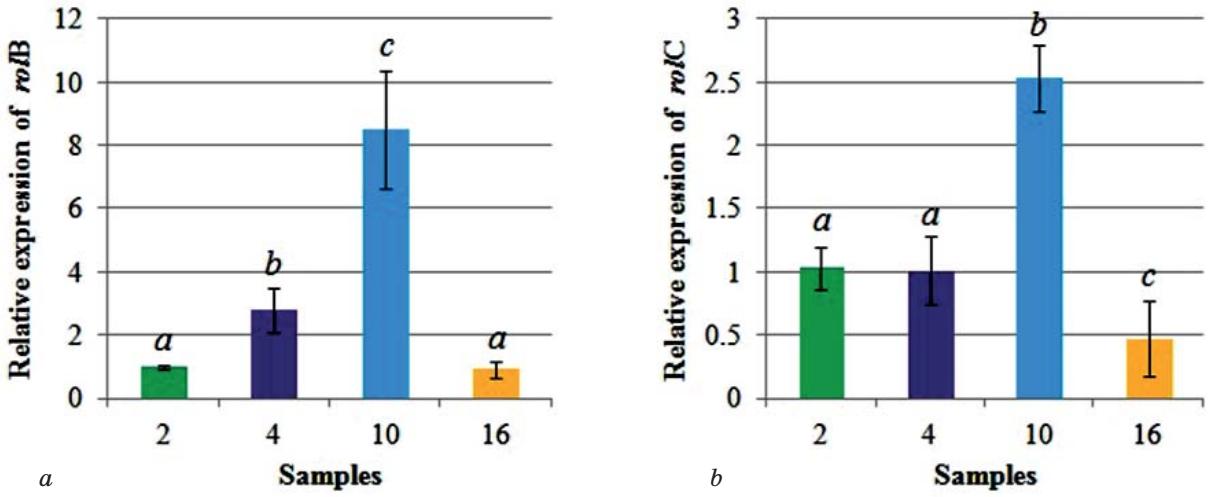

Fig. 5. **Relative activity of *rol*B (*a*) and *rol*C (*b*) genes in the "hairy" roots of *A. tilesii* after 2 weeks of cultivation:**
columns 2 and 4 — lines Nos. 2 and 4 transformed with wild *A. rhizogenes* strain A4; 10 and 16 — lines Nos. 10 and 16 transformed with *A. rhizogenes* carrying *ifn*-α*2b* and *npt*II genes.
Error bars with different small letters denote significant differences in values among four samples at $P < 0.05$

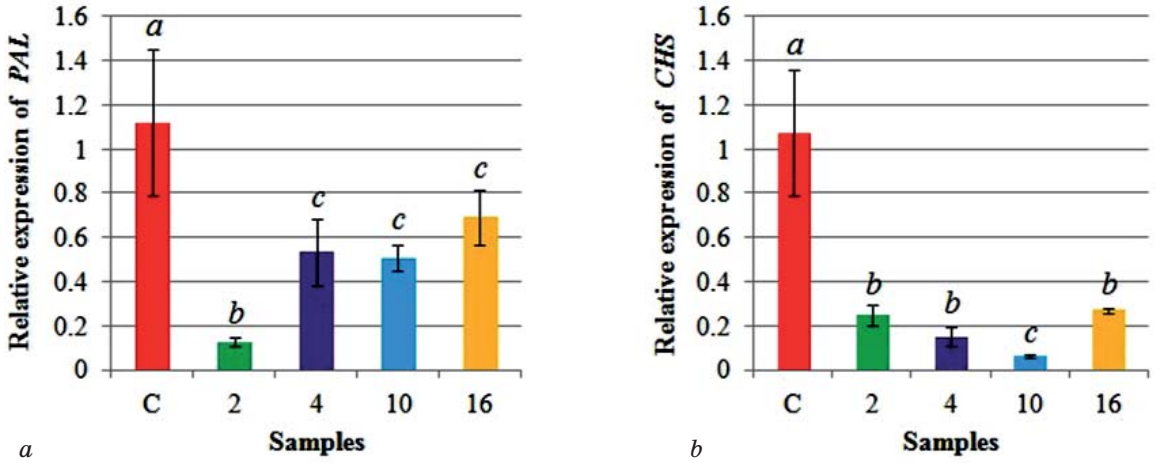

Fig. 6. **Relative activity of *PAL* (*a*) and *CHS* (*b*) genes in the "hairy" roots of *A. tilesii* after 2 weeks of cultivation:**
columns 2 and 4 — lines Nos. 2 and 4 transformed with wild *A. rhizogenes* strain A4; 10 and 16 — lines Nos. 10 and 16 transformed with *A. rhizogenes* carrying *ifn*-α*2b* and *npt*II genes
Error bars with different small letters denote significant differences in values among four samples at $P < 0.05$

Although the results of this study confirmed the influence of the activity of the transferred *rol* genes on the growth rate of the formed *A. tilesii* "hairy" roots, no interrelation between their activity and the synthesis of flavonoids was observed. This may be due to the fact that the function of *rol* genes as activators of secondary metabolism in plants is determined not by the activity of these transferred genes, but by the fact of their presence, and the site of their incorporation into the plant genome.

The study of the relative activity of phenylalanine ammonia-lyase (*PAL*) and chalcone synthase (*CHS*) showed that the level of activity of these genes in the root lines was different, but was lower than in the control. Since these genes are involved in the biosynthesis of polyphenolic compounds such as flavonoids and phenylpropanoids in plants, their activity may influence flavonoids content. As it turned out, in all experimental samples higher flavonoids content corresponded to lower *PAL* activity.

For example, the content of flavonoids in the line No. 2 was the highest among all samples and was 4.10 times higher than in control non-transformed plants (Fig. 6, *a*). Similarly, *PAL* activity in the line No. 2 was 8.62 times lower than in the control. Such an inverse correlation was observed in all experimental lines. This can be explained by the reverse inhibition of the *PAL* gene by the high content of the synthesized product.

The level of *CHS* gene activity in control plants was 4.01–16.72 times higher than in *A. tilesii* "hairy" roots (Fig. 6, *b*). This result could also be the outcome of reverse inhibition of the chalcone synthase gene involved in the synthesis of polyphenols. As in all previous studies, the dependence between the activity of the phenylalanine ammonia-lyase and chalcone synthase genes and the presence of either *rol* genes only or together with *ifn*-α*2b* and *npt*II genes among the two groups of "hairy" roots was not detected.

Conclutions

In this paper, for the first time, the potential dependence of the activities of bacterial *rol* genes and the plant's own *PAL* and *CHS* genes present in the "hairy" roots, as well as their growth rate, total flavonoid content, antioxidant activity and reducing power on the presence of additional genes in the transformed roots, namely *npt*II and *ifn-2b*, were studied. The study confirmed the effect of *rol* genes as activators of secondary metabolism, in particular, on increasing the content of flavonoids, AOA and RP in most of the experimental lines of *A. tilesii* wormwood compared to the control plants. However, no interdependence of the relative activity of *rol* genes and flavonoids synthesis was found. Nevertheless, according to the results of the study, it was established that the activity of the *rol*B gene affects the growth rate of "hairy" roots. In particular, in all root lines the greater weight gain was observed in samples with higher activity of *rol*B gene. In addition, the level of phenylalanine ammonia-lyase activity was inversely correlated with the content of flavonoids in all experimental lines: the higher was the content, the lower was the *PAL* activity. The relative activity of both *PAL* and *CHS* was highest in the control roots. Such a result may indicate the presence of reverse inhibition of these two genes by the products of biosynthesis in which they are involved, due to the high content of flavonoids in the transformed plants.

Of special interest was the confirmation or refutation of whether the additional presence of transferred *npt*II and *ifn-2b* genes in "hairy" roots affects all the above-mentioned indicators. As a result of the study, it was found that there were no fundamental differences in the ranges of flavonoids content, AOA and RP, as well as the relative activities of the *rol*B, *rol*C, *PAL* and *CHS* genes between the two groups of "hairy" roots.

Funding

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ОСОБЛИВОСТІ БІОСИНТЕЗУ ФЛАВОНОЇДІВ У «БОРОДАТИХ» КОРЕНЯХ *Artemisia tilesii* Ledeb. З РІЗНИМИ ПЕРЕНЕСЕНИМИ ГЕНАМИ

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Мета. Порівняти лінії «бородатих» коренів з різним набором перенесених генів стосовно наявності взаємозв'язку між загальним вмістом флавоноїдів, рівнем антиоксидантної (АОА) та відновлювальної (ВА) активності, а також активності власних генів рослин — фенілаланін-амоній-ліази (*PAL*) та халконсинтази (*CHS*) і генів *rol*B і *rol*C *A. rhizogenes*.

Методи. Лінії коренів №10 і 16 отримано з використанням *A. rhizogenes* A4, № 2 та 4 — з використанням *A. rhizogenes* з вектором pCB124 (*ifn*-α*2b* та *npt*II гени).

Результати. Вміст флавоноїдів у всіх лініях був вищим за контроль і корелював з АОА та ВА активністю. Корені №10 відзначались найшвидшим ростом, що збігалося з високою активністю *rol*B та *rol*C генів. Активність *PAL* та *CHS* у «бородатих» коренях була нижчою за активність у нетрансформованих коренях.

Висновки. Лінії коренів, до яких було перенесено лише *rol*B та *rol*C, і лінії з додатковими генами *ifn*-α*2b* та *npt*II мали близький діапазон концентрації флавоноїдів, рівнів АОА та ВА, які перевищували ці показники в контролі. Встановлено залежність швидкості росту коренів та відсутність залежності вмісту флавоноїдів від активності *rol* генів. Активність *PAL* обернено співвідносилась із вмістом флавоноїдів в усіх дослідних лініях, що може бути результатом надпродукції сполук у трансгенних коренях.

Ключові слова: *Artemisia tilesii* Ledeb., *Agrobacterium rhizogenes*–опосередкована трансформація, «бородаті» корені, флавоноїди, *rol* гени, антиоксидантна активність.