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INFLUENCE OF COLD STRESS ON GROWTH AND FLAVONOIDS ACCUMULATION IN *ARTEMISIA TILESII* «HAIRY» ROOT CULTURE

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The aim of the work was to investigate the effect of short-term cold stress on the growth, antioxidant activity and flavonoids synthesis in the *Artemisia tilesii* "hairy" root cultures. The effect of low temperature was studied by roots cultivating during the first 1, 2 and 5 days at +10 °C, then the plants were grown at +24 °C The total flavonoids content in Rutin equivalent was determined spectrophotometrically using alumunium chloride assay. Antioxidant activity of ethanolic extracts was studied using the DPPH method. The root lines differed in sensitivity to short-term temperature decrease up to +10 °C. Stress factor action resulted in a reduction of mass increment in 1.7–3 times. At the same time, two lines were found to be less sensitive to the cold stress since the cultivation for a short period at the low temperatures did not suppress their growth. Transgenic root lines also differed in the flavonoids content which was up 3–15 mg RE/g. Cultivation of "hairy" roots under short-term cold stress has led to decrease of the flavonoids content in all "hairy" root lines except line No. 5. Cold stress did not lead to the significant changes of antioxidant activity in any of the studied root lines except root line No. 5 where the greater antioxidant activity was observed in one- and two-day cold stress. Thus, short-term cold stress have suppressed the "hairy" roots growth, inhibited flavonoids accumulation, and had no effect on the antioxidant activity (except one roots line).

Keywords: "hairy" roots culture; *Artemisia tilesii*; cold stress; mass increment; flavonoids; antioxidant activity

Introduction

The study of abiotic stress influence on the secondary metabolites accumulation in plants has both a fundamental and practical importance since these compounds are used as pharmaceuticals, fragrances, food additives etc. The stress factors such as ultraviolet radiation, low and high temperature, drought, soil overwetting, technogenic pollution, other factors (Ramakrishna et al., 2011) lead to the changes of secondary metabolites accumulation in plant tissues. Low temperature as one of abiotic stress factors causes deviations in the optimal development of plants. Phenotypical changes after low temperatures (cold stress) effect include the shift-down, chlorosis, wilting and tissue necrosis (Yadav, 2010). The effect of short-term cold stress does not lead to death and significant damage of plants, but can cause the changes in the synthesis of inherent compounds, including secondary metabolites. It was demonstrated in *in vitro* studies that stress factors including low temperatures

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can lead to the increase of flavonoids and antioxidants synthesis in plants (Chalker-Scott, 1999; Winkel-Shirley, 2002; Fini et. al., 2011; Schulz et. al., 2016).

It was found that cold stress led to the changes in plant gene expression and resulted in the increased accumulation of anthocyanins and flavonols in *Arabidopsis thaliana* (L.) Heynh. plants (Schulz et. al., 2016). The influence of cold stress on the secondary metabolites accumulation in plant shoots was determined in numerous studies (Chalker-Scott, 1999; Fini et. al., 2011; Schulz et. al., 2016), but the effect of abiotic stresses on plant roots is still underexplored.

The study of abiotic stress influence on the synthesis of biologically active compounds in medicinal plants is of a particular interest. Plants of the *Artemisia* L. genus are the medicinal ones and traditionally are used in America, Europe and Asia. Antioxidants, flavonoids and other biologically active compounds (BAC) were found to synthesize in different *Artemisia* spp.

Artemisia rhizogenes-mediated genetic transformation is one of the methods for increasing of the content of biologically active compounds. It was shown that transgenic plants and "hairy" roots can accumulate BAC in a larger quantity than initial non-transformed plants. This is due to the transfer of alien genes into the plant genome after agrobacterial transformation and their influence on the activity of other plant genes.

However, the influence of stress factors including cold stress on the secondary metabolites accumulation in transgenic roots is still remained underexplored. Therefore, the aim of this work was to determine the effect of short-term cold stress on the growth, antioxidant activity and flavonoids accumulation in *Artemisia tilesii* Ledeb. "hairy" roots culture.

Materials and methodology

We have used 5 *Artemisia tilesii* "hairy" root cultures obtained earlier by *Artemisia rhizogenes*-mediated transformation as the starting material for our study (Matvieieva et. al., 2016). The control "hairy" root cultures were grown in the sterile conditions on the Petri dishes with Murashige and Skoog (½ MS) (Murashige and Skoog, 1962) basal medium at the temperature +24 °C during 4 weeks. The influence of the cold stress was investigated by roots cultivating during the one, two and five days at +10 °C and subsequent growing (up to 4 weeks) at +24 °C. The increment of roots mass was determined by direct weighing. The amount of total flavonoids in rutin equivalent (RE) in 70% ethanolic extracts was determined by modified aluminium chloride colorimetric essay (Pękal and Pyrzynska, 2014). Antioxidant activity of "hairy' root ethanolic extracts was studied by DPPH-method (Semenov, 1985). Statistical analysis of the results was performed using Microsoft Excel (p <0.05) application software packages.

Results and discussion

The lines of transgenic roots differed in the growth rate. For example, mass increase of root line No. 3 was twice then mass increase of root line No. 2 in case of cultivation in the control conditions (+24 °C). Transgenic root lines also differed in growth rate in case of short-term cold stress (+10 °C). Particularly, the growth rate of root lines No. 2 and No. 3 did not fall during one-day cultivation at the reduced temperature. At the same time, the growth rate of the root lines No. 1, No. 4 and No. 5 significantly decreased even under one-day low temperature stress. Inhibition of roots growth for the all lines after 5 days cultivation at the temperature +10 °C was quite expected (Figure 1).

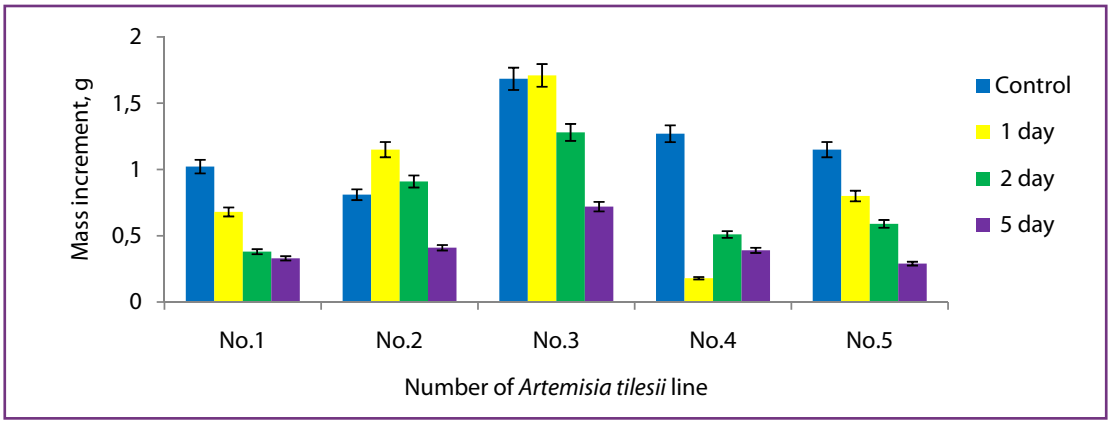

Figure 1 The influence of cold stress on mass increment of *Artemisia tilesii* "hairy" root cultures during one, two and five days cultivation at +10 °C

It was shown that transgenic root lines differed in flavonoid content during cultivation under control conditions. The highest flavonoids content was found in the root line No. 4–15 mg RE/g. The lowest content of flavonoids was found in transgenic root line No. 5.

Response of root lines No.1, No. 2, No. 3 to short-term cold stress did not differ significantly and resulted in a decrease of flavonoid content in 1.7–2 times as compared to the control. The flavonoids content in root line No. 4 under these cultivation conditions was in three times less than in the control.

Root line No.5 differed from all other lines and was characterized by the absence of flavonoids content decrease for cultivating at lowed temperature. Root line No. 5 differed from all studied ones because of absence of the flavonoids content decrease after the cultivation at low temperature (Figure 2).

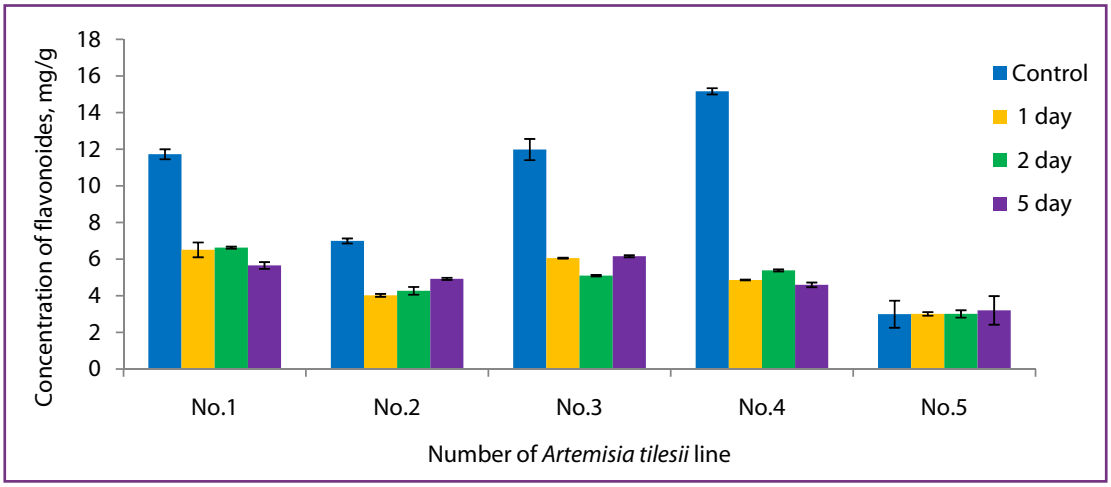

Figure 2 The influence of cold stress (one, two and five days cultivation at +10 °C) on flavonoids accumulation of *Artemisia tilesii* "hairy" roots cultures

In our experiments it was found out that there were any significant differences in antioxidant activity of root lines No. 1–4 extracts after cold stress. However, the antioxidant activity of the root line No. 5

extract has increased during cultivation at the low temperature cultivation during one and two days (Figure 3). Thus, the transgenic root line No.5 differed from other ones by the response to the cold stress effect both for the flavonoids content and for level of antioxidant activity.

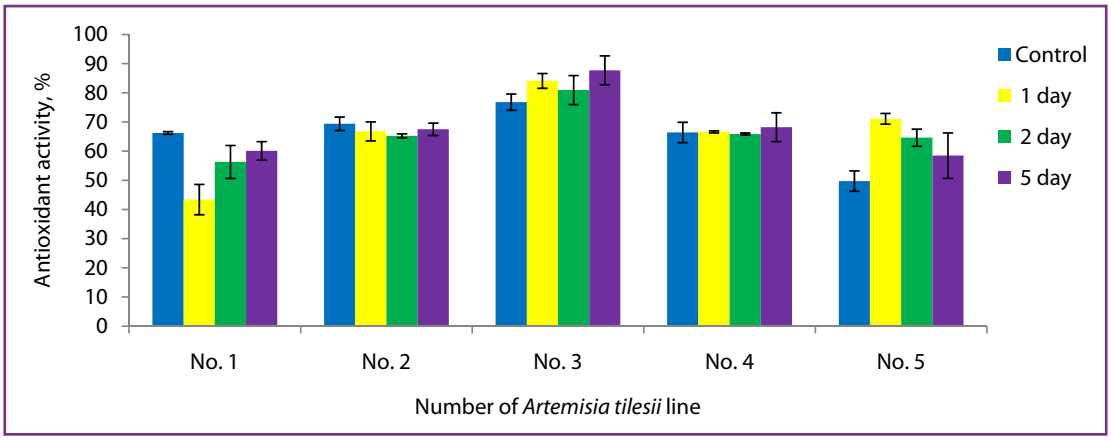

Figure 3 The influence of cold stress (one, two and five days cultivation at +10 °C) on antioxidant activity of *Artemisia tilesii* "hairy" roots cultures

Conclusions

The root lines differed in sensitivity to short-term temperature decrease up to +10 °C. Stress factor action resulted in a reduction of mass increment in 1.7–3 times. At the same time, two lines were found to be less sensitive to the cold stress since the cultivation for a short period at the low temperatures did not suppress their growth. Transgenic root lines also differed in the flavonoids content which was up 3–15 mg RE/g. Cultivation of "hairy" roots under short-term cold stress has led to decrease of the flavonoids content in all "hairy" root lines except line No. 5. Cold stress did not lead to the significant changes of antioxidant activity in any of the studied root lines except root line No. 5 where the greater antioxidant activity was observed in one- and two-day cold stress. Thus, short-term cold stress have suppressed the "hairy" roots growth, inhibited flavonoids accumulation, and had no effect on the antioxidant activity (except one roots line).

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