ARTEMISIA VULGARIS "HAIRY" ROOTS AS A SOURCE OF SAFE ANTIOXIDANT COMPOUNDS

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Abstract

The present study aimed to investigate flavonoid content and antioxidant activity of Artemisia vulgaris "hairy" roots of 3 lines. We also studied the safety of transgenic root extracts by determining their genotoxicity. The total flavonoid content in water extracts of the fresh "hairy" roots reached up to 1.976 ± 0.1 mg/g FW, which is 4.8 times higher than in control plant roots. Extracts of transgenic roots on average showed 1.5 times higher antioxidant activity compared to untransformed roots. The comet assay results indicated the absence of the genotoxic effect of all tested extracts. Therefore, these extracts can be the source of safe antioxidant compounds.

Keywords: *Artemisia vulgaris L., "hairy" roots, flavonoids, antioxidants, genotoxicity*

Introduction. *Artemisia vulgaris* L. plants are known as producers of compounds that exhibit various biological activities, including antioxidant, anti-inflammatory, anti-cancer, antimicrobial, anti-parasitic, neuroprotective, etc. [1]. Some of these compounds are flavonoids, which possess strong antioxidant properties and can reduce oxidative stress, which is a key factor in various pathological processes [2]. The biosynthesis of flavonoids can be intensified by genetic transformation even without the transfer of specific genes involved in the synthesis of these compounds. Thus, *Agrobacterium rhizogenes*-mediated transformation results in the formation of a "hairy" roots culture, which can produce a complex of valuable phytochemical components [3]. This study aimed to investigate the effect of *A. rhizogenes*-mediated transformation on the flavonoid content, antioxidant activity, and genotoxicity of *A. vulgaris* "hairy" root extracts.

Materials and methods. In the study, 3 "hairy" root lines of *A. vulgaris*, which were obtained earlier, were used [4]. Transgenic roots, as well as control plants of *A. vulgaris*, were cultivated on the Murashige and Skoog hormone-free medium at +25ºC for 30 days. Extracts were obtained by homogenization of 1 g of plant material and 1 mL of water with further centrifugation. The total flavonoid content in obtained extracts was determined by modified $AlCl_3$ method [5]. The antioxidant activity of root extracts was analyzed by the ability to scavenge DPPH radical [6]. The genotoxic effect of plant extracts was tested by comet assay [7]. For this purpose, 50 μL of human lymphocyte suspension and 100 μL of 1 g FW/mL plant extract were incubated with 650 μL RPMI medium, and the level of double- and single-stranded DNA breaks was estimated.

Results and discussion. Extracts of the transgenic roots had 2.1 – 4.8 times higher flavonoid content compared to the extract of control plant roots (0.415 ± 0.05 mg/g FW) (fig. 1). The "hairy" roots of line 3-7 were characterized by the highest flavonoid content (1.976 ± 0.1 mg/g FW). The concentration of these

bioactive compounds in extracts of transgenic lines 3-8 and 3-10 was relatively lower (0.877 ± 0.02 and 1.142 ± 0.05 mg/g FW respectively).

The results of the antioxidant activity analysis (fig. 2) indicated that the extract of the control plant roots had the lowest activity (EC_{50}=46.1 mg FW). "Hairy" roots of line 3-7 were characterized by the highest antioxidant activity (EC_{50} =22.9 mg FW). EC_{50} value for transgenic lines 3-8 and 3-10 was equal to 37.3 and 29.8 mg FW.

The comet assay showed that there was no significant effect of tested extracts on the level of DNA damage in lymphocytes (fig. 3). The percentage of DNA, that migrated to the anode during comet assay (which reflects the relative level of DNA breaks) in control experiments was 15.87 ± 4.5 %, while after treatment with extracts of "hairy" root lines 3-7, 3-8, 3-10, and control plant roots was equal to 13.22 ± 4.3, 2.59 ± 2.4, 4.12 ± 3.6, and 8.95 ± 3.4 % respectively.

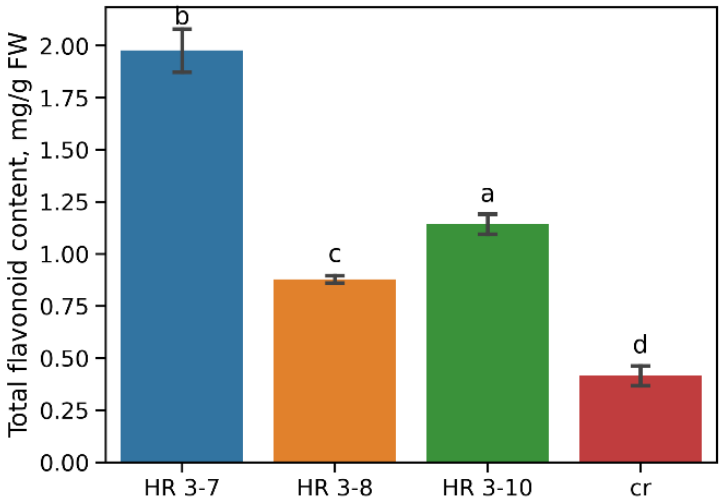

Fig. 1. Total flavonoid content of water extracts of *A. vulgaris* "hairy" root (HR) lines 3-7, 3-8, 3-10, and control roots (cr). Columns with different lowercase letters indicate significant differences in values between samples at p<0.05.

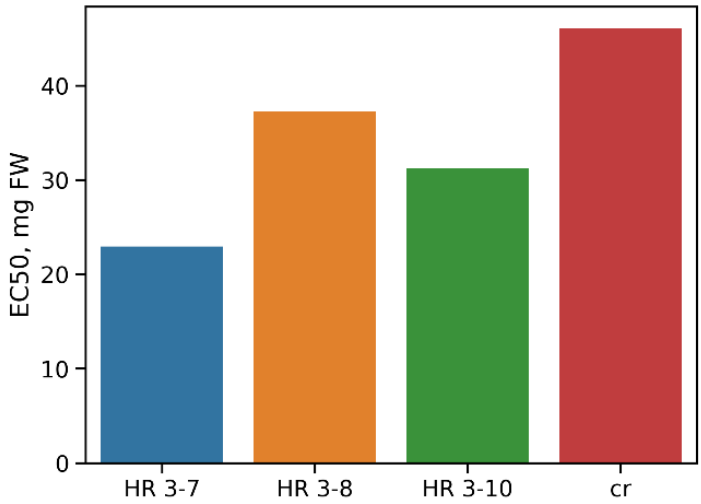

Fig. 2. Antioxidant activity (right) of water extracts of *A. vulgaris* "hairy" root (HR) lines 3-7, 3-8, 3-10, and control roots (cr).

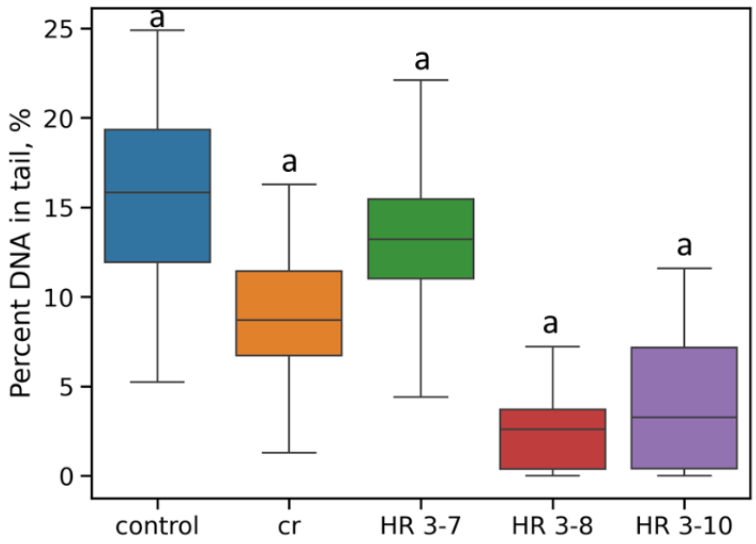

Fig. 3. Genotoxicity effect of *A. vulgaris* "hairy" root (HR) extracts of lines 3-7, 3-8, 3-10 and control roots (cr).

Conclusions. *A. rhizogenes*-mediated transformation led to increased flavonoid content in all "hairy" root lines of *A. vulgaris*. This also was correlated with increased antioxidant activity of transgenic lines. Extracts of "hairy" roots and roots of control plants had no genotoxic effect, thus they can be safely used in human disease therapy.

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