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Production Of Phenolic Acid And Antioxidant Activity In Transformed Hairy Root Cultures Of Common Buckwheat (*Fagopyrum Esculentum* M.)

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Abstract: Common buckwheat (*Fagopyrum esculentum* Moench) is recognized as a healthy food in many countries because it is nutrient-rich crop. This experiment was conducted to investigate the antioxidant activity and production of phenolic acids from hairy cultures of roots, leaves and stems of Common buckwheat. In these cultures, a large amount of chlorogenic, *p*-hydroxybenzoic, p-anisic and caffeic acids were produced together with small amounts of the related phenolic acids, vanilic acids, p-coumaric acid, hesperetic and cinamic acid. The DPPH and ABTS assays showed more high antioxidant activity in hairy root cultures(leaves, stems and roots). Especially DPPH assay showed increasing antioxidant activity in hairy root culture at 3 times compared to control. The highest hairy root growth reached up to 21.2 g/l dry weight with roots, 17.7 g/l with leaves and 14.6 g/l with stems at the third week after placement. Molecular analysis by PCR amplification confirmed that the *rol* B gene (652 bp) which is transferred into hairy roots from the Ri-plasmid in *Agrobacterium rhizogenes* is responsible for the induction of hairy root from plant species.

Key words: *Fagopyrum esculentum* Moench, hairy root transformationm, *Agrobacterium rhizogenes,* phenolic acids, antioxidant activity.

INTRODUCTION

Antioxidants help organisms to deal with oxidative stress, caused by free radical damage. Free radicals are chemical elements, which contains one or more unpaired electrons due to which they are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability (Ali *et al*. 2008). Some of these molecules can be physiologically useful, but they can also cause damage under certain circumstances. The most notorious among these damages being neurodegenerative conditions like Alzheimer's and Parkinson's disease. Other neurodegenerative diseases significantly associated with oxidative stress include multiple sclerosis, Creutzfeldt–Jacob disease, and Meningoencephalitis (Darley-Usmar and Halliwell 1996; Ali *et al*. 2008).

Common buckwheat (*Fagopyrum esculentum* Moench) is recognized as a healthy food in many countries because it is rich in phenolic compounds (Alvarez *et al*., 2010), flavonoids, vitamin E, (Sedej *et al*. 2010) and amino acids (Golisz *et al*. 2007). Gallardo, *et al*.(2006) and Gorinstein, *et al*. (2008) reported that buckwheat is the greatest sources of antioxidants amongst cereals and pseudo cereals. Nowadays is considerable interest in the consumption of alternative crops as potential recipes for gluten-free products production too. Therefore, the use of buckwheat for production of gluten-free pasta can have great interest for food industry. RP-HPLC-ESI-TOF-MS has been applied for the separation and characterization of free and bound phenolic compounds in buckwheat flour and buckwheat spaghetti (Verardo *et al*. 2011). In same time it was hypothesized that buckwheat, because it is rich in phenolic compounds, influences in tests *in vitro* foregut fermentation in ruminant animals which make great interest to find way to increase production of phenolic compounds regarding goals of agricluture and food industry too (Leiber *et al*. 2012).

The main phenolics are rutin, chlorogenic acid, and hyperoside, that possess high antioxidant activity (Azuma *et al*. 1999). Previous studies have confirmed that chlorogenic acid has the same biosynthesis pathway as quercetin-3-O-rutinoside and cyanidin-3-galactoside (Lancaster and Dougall 1992). Awad, *et al*.(2001) suggested that the accumulation of chlorogenic acid is apparently not affected by the synthesis of quercetin-3-O-rutinoside and cyanidin-3-galactoside. Rutin and quercetin levels in buckwheat depend greatly on growth

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location and cultivar (Oomah and Mazza 1996; Steadman *et al*. 2001). In common buckwheat groats, levels of rutin and quercetin are ≈0.20 mg/g and 0.001 mg/g, respectively. Buckwheat hulls contained higher levels of rutin (0.84–4.41 mg/g) and quercetin (0.009–0.029 mg/g) (Oomah and Mazza 1996). Tartary buckwheat is an excellent source of rutin because groats of Tartary buckwheat showed levels of rutin at 80.94 mg/g. Besides rutin and quercetin, Watanabe (1998) isolated four catechins with antioxidant activity from ethanol extracts from common buckwheat groats: epicatechin, catechin 7-O-β-D-glucopyranoside, epicatechin 3-O-p-hydroxybenzoate, and epicatechin 3-O-(3,4-di-O-methyl)- gallate. Catechins show a higher antioxidant activity than rutin. But both rutin and quercetin have another advantage in addition to their antioxidant activity: they can help with treatment of chronic venous insufficiency (Erlund *et al*. 2000).

It is well known that transformed root is highly differentiated and can cause stable and extensive production of secondary metabolites, whereas other plant cell cultures have a strong tendency to be genetically and biochemically unstable and often synthesize very low levels of useful secondary metabolites (Hu and Du 2006). Hairy root-infected *A. rhizogenes* is characterized by a high growth rate and genetic stability. Antioxidant activity and phenolic compound production by hairy root culture of *F. esculentum* has never been reported by using the roots as a source for transformation. Here we describe the productionof antioxidant activity and phenolic compounds by hairy root cultures of common buckwheat leaves, stems and roots transformed with *A. rhizogenes*. In this respect, hairy root transformation of common buckwheat has been reported (Trotin *et al*. 1993; Park and Park 2001; Lee *et al*. 2007; Kim *et al*. 2010). However, antioxidant activity by hairy root culture of *F. esculentum* to our knowledge has not been reported.

In this paper, we describe an efficient protocol for transformation of different common buckwheat explant sources, i.e., leaves, stems and roots, with *A. rhizogenes* A4 strain for production of Antioxidant activity and phenolic compounds.

MATERIALS AND METHODS

Seed Sterilization And Germination:

Common buckwheat Seeds (the kind Antariya (2009) year) were surface sterilized by washing in running water with soap for 20 min.Under aseptic condition in laminar air-flow cabinet seeds were rinsed with 70% (w/v) ethanol for 1 min and immersed for 20 min in 10% (w/v) chlorax (sodium hypochlorite solution), then rinsed three times in sterilized distilled water. Five seeds were placed on 25 ml of the basal MS (Murashige and Skoog 1962)agar-solidified culture medium in Petri dishes (100 x 15 mm). The MS medium was supplemented with sucrose (30 g/l) and agar (8 g/l), adjusted to pH 5.8 and autoclaved at 121°C for 25 min. The seeds were germinated in a growth chamber under standard cool white fluorescent tubes with a flux rate of 35 μmol $s^{-1}m^{-2}$ and a 16-h photoperiod and at 25 °C.

Preparation Of Agrobacterium Rhizogenes:

The culture of *A. rhizogenes* strain A4, obtained from Department of Methods in Food Biotechnology, Institute of Food Technology and Food Chemistry, Berlin University of Technology, was initiated from glycerol stock and maintained on MYA-solid medium (5.0 g/l Yeast extract, 0.5 g/l Casamino acids, 8.0 g/l Mannitol, 2.0 g/l Ammonium sulfate, 5.0 g/l NaCl and 15 g/l agar)(Petit and Tempé 1978) for 48 h at 28°C in the dark. The single clone was grown for 24 h in 20 ml MYA-liquid medium at 28°C on a rotary shaker at 100 rpm in the dark.

Establishment Of Hairy Root Cultures:

Excised stems, leaves and roots were collected from plants grown *in vitro* (21-day-old) and used as the explant materials for co-cultivation with *A. rhizogenes*. Stems (2 cm), leaves with petioles and the roots (2-3cm length and approximately 3 mm separated from the root tip). Then, each explant was immersed in bacterial suspension separately for 5 min. The explants were blotted dry on sterile filter-paper to remove excess bacteria and incubated in the dark at 28°C on 200mlErlenmeyerflaskwith50mlof liquid hormone-free MS medium with 30 g/l sucrose on a rotary shaker at 100 rpm. Uninfected explants (control) were cultured under the same conditions. After 24 h of co-cultivation, the explant tissues were transferred to new liquid hormone-free MS medium with 30 g/l and containing 500 mg/L cefotaxime to eliminate bacteria and then incubated in the dark.

Numerous hairy roots were observed emerging from the wound sites within 2 weeks. The hairy roots were separated from the explant tissues and sub-cultured in the dark at 25°C on liquid hormone-free MS medium. After repeated transfer to fresh medium, rapidly growing hairy root cultures were obtained. Isolated roots (300 mg) were transferred to 50 ml of MS liquid medium, containing 30 g/L sucrose, in 200-mL flasks. The root cultures were maintained at 25°C on a rotary shaker at 100 rpm in growth chamber under standard cool white fluorescent tubes with a flux rate of 35 μmol $s^{-1}m^{-2}$ and a 16-h photoperiod. After three weeks of culture, the hairy roots were harvested and their antioxidant capacity and phenolic compound contents were determined. Six flasks were used for each culture condition, and the experiments were repeated twice.

PCR Detection:

Plant genomic DNA of different buckwheat hairy root cultures (leaves, stems and roots) for polymerase chain reaction (PCR) analysis were extracted by using DNA-Kit (Wizard R genomic DNA purification kit, A1120, Promega, USA). Genomic DNA used as template on PCR was isolated from roots excised from infected and uninfected plants (control). The primer used for amplification of gene was 5'-ACTATAGCAAACCCCTCCTGC-3' and 5'-TTCAGGTTTACTGCAGCAGGC-3', with product size 652 bp (biomers. net GmbH, Ulm, Germany). The amplification cycle consisted of denaturation at 95 °C for 1 min, primer annealing at 55 °C for 1 min, and primer extension at 72 °C for 1 min. After 30 repeats of the thermal cycle and final extension 72 °C for 5 min, amplification products were analyzed on 1.5% agarose gel.

Sample Preparation

1.0 g of freeze dried collected samples dissolved in 10 ml of 70 % methanol were agitated for 10 seconds and then centrifuged at 6000 rpm for 10 min. The supernatant was collected and the same procedure was repeated for 2 more times.

Antioxidant Activity Of Extracts:

In light of the differences among the wide number of the systems available, the results of a single method can give only a reductive suggestion of the antioxidant properties of the extracts (Sacchetti et al. 2005). For that reason, the antioxidant capacity of each extracts was determined through two complementary assay procedures.

Determination Of DPPH· Radical Scavenging Capacity:

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay (Lee *et al*. 2003) was utilized with some modifications. The stock reagent solution (1×10^{-3}M) was prepared by dissolving 22 mg of DPPH in 50 ml methanol and stored at 20°C until use. The working solution (6×10^{-5} M) was obtained by mixing 6 ml of the stock solution with 100 ml methanol to obtain an absorbance value of 0.8 ± 0.02 at 515 nm, using a spectrophotometer (Jenway 6505 UV/Vis). The different extracts (0.1 ml of each) were allowed to react with 3.9 ml of the DPPH solution and vortex during 30 seconds and then the absorbance was measured at 515 nm, at reaction time 30 min. A control sample with no added extract was also analyzed and the scavenging percentage was calculated according to the following equation:

DPPH scavenging capacity (%) = $[(A_{control} - A_{sample})]/(A_{control})] \times 100$

A = absorbance at 515 nm

ABTS Radical Scavenging Assay:

For 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assay, the method of Re *et al*. (1999) was adopted. The stock solutions included 7 mM ABTS solution and 2.4 mM potassium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and allowing them to react for 12-16 h at room temperature in the dark. The solution was then diluted by mixing 1 ml $ABTS^+$ solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 units at 734 nm using the spectrophotometer (Jenway 6505 UV/Vis). $ABTS^+$ solution was freshly prepared for each assay. Sample extracts (1 ml of each) were allowed to react with 1 ml of the $ABTS^+$ solution and the absorbance was taken at 734 nm after 7 min using the spectrophotometer. The $ABTS^+$ scavenging capacity of the extract was calculated as percentage inhibition

ABTS radical scavenging activity (%) = $[(Abs_{control} - Abs_{sample})]/(Abs_{control})] \times 100$

Where $Abs_{control}$ is the absorbance of ABTS radical + methanol; Abs_{sample} is the absorbance of ABTS radical + sample extract.

Determination Of Phenolics Composition With HPLC Analysis:

Phenolic acidswere determined according to Sytar *et al*. (2011). A total of 20 mg freeze dried samples were extracted for 15 min using 750 µL 70% methanol (v/v, pH 4, phosphoric acid) in an ultrasonic water bath (Sonorex digital 10p , Bandelin) on ice. Samples were centrifuged for 5 min at 6000 rpm. The supernatants were collected and the pellets were re-extracted twice more with 500 µL 70% methanol. The combined supernatants from each sample were reduced to near dryness in a centrifugation evaporator (Speed Vac, SC 110) at 25°C. Samples were then made up to 1 ml with 40% acetonitrile. The samples were filtrated using 0.22µm filter, and then analyzed with HPLC (Dionex Summit P680A HPLC-System), equipped with P680 pump, ASI-100 automated sample injector, a Narrow-Bore Acclaim PA C16-column (3 µm, 2.1 × 150 mm, Dionex) and PSA-100 photodiode array detector (Dionex) and software Chromeleon 6.8 (Dionex, USA). The column was operated at a temperature of 35 °C.

The mobile phase consisted of 0.1% (v/v) phosphoric acid in ultrapure water (eluent A) and of 40% (v/v) acetonitrile in ultrapure water (eluent B). A multistep gradient was used for all separations with an initial

injection volume of 40 µL and a flow rate of 0.4 mL/min. The multistep gradient was as follows: 0-1 min: 0.5% (v/v) B; 1-10 min: 0.5-40% B; 10-12 min: 40% B; 12-18 min: 40-80% B; 18-20 min: 80% B; 20-24 min: 80-99% B; 24-30 min: 99- 100% B; 30-34 min: 100-0.5% B; 34-39 min: 0.5% B. Simultaneous monitoring was performed at 290, 330 and 254 nm at a flow rate of 0.4 mL/min. Phenolic acid quantity was calculated from HPLC peak areas at 290 nm against the internal standard and external standards. Identification and quantification of phenolic acids present was done by comparing retention time and area of the peaks in the extracts with that of the standard phenolic acids (chlorogenic acid, caffeic acid, cinnamic acid, coumaric acid, rosmarinic acid and sinapic acid).

RESULTS AND DISCUSSIONS

Establishment Of Hairy Root Cultures:

Hairy roots of common buckwheat were initiated with three different explant sources i.e., roots, leaves and stems, (Fig, 1) inoculated with *A. rhizogenes* A4 strain. After 2 days of co-cultivation, explant tissues were transferred to free hormone liquid-MS-medium containing 500 mg/l cefotaxime, to eliminated *A. rhizogenes*. Visible roots were formed after five to seven days at the site of bacterial inoculation of different explants. After 10–14 days, the roots began to grow more rapidly. About four weeks after co-cultivation with *A. rhizogenes*, hairy roots of the different explants were excised from the necrotic explant tissues and sub-cultured on fresh liquid-MS-medium. After isolation, hairy roots of each source were cultured in MS liquid medium for three weeks and their growth rate was determined by harvesting six flasks every week. During the culture period (Fig, 2) the dry weight of the roots increased from the original level of 0.3 g/L to 21.2 g/l after third week. The maximum growth 21.2, 17.7 and 14.6 g/l were recorded with roots, leaves and stems after the third week, respectively.

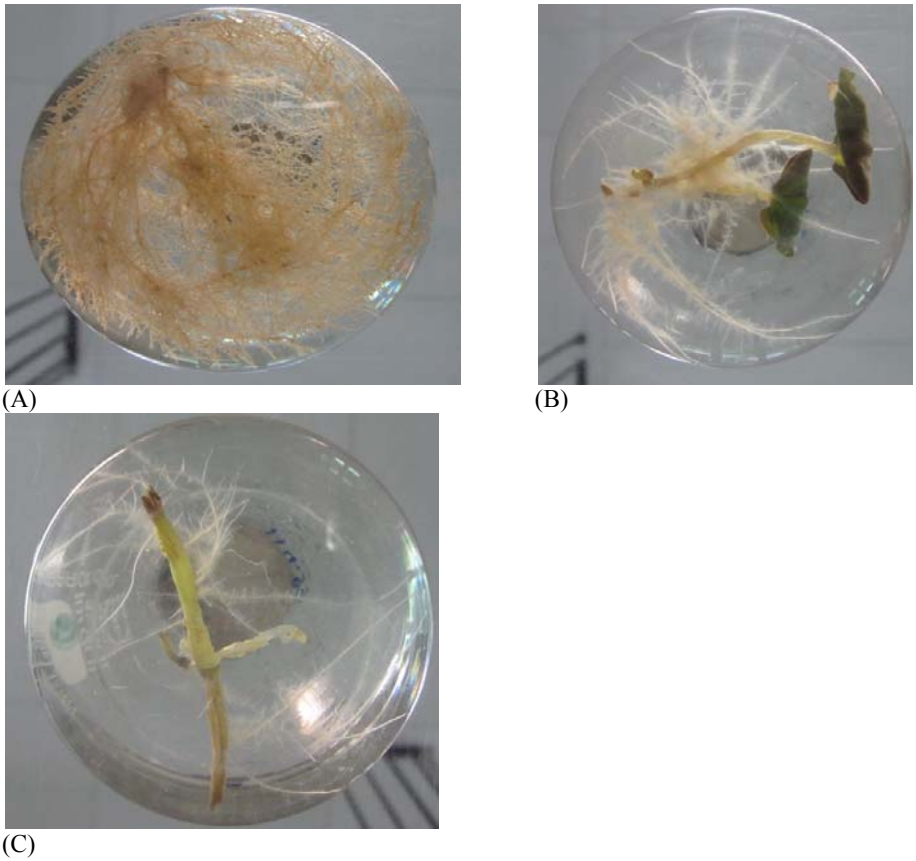

Fig 1: Development of hairy roots from different explant sources of *F. esculentum* (A) roots (B) leaves and (C) stems within ten days after inoculation with *A. rhizogenes* strain A4.

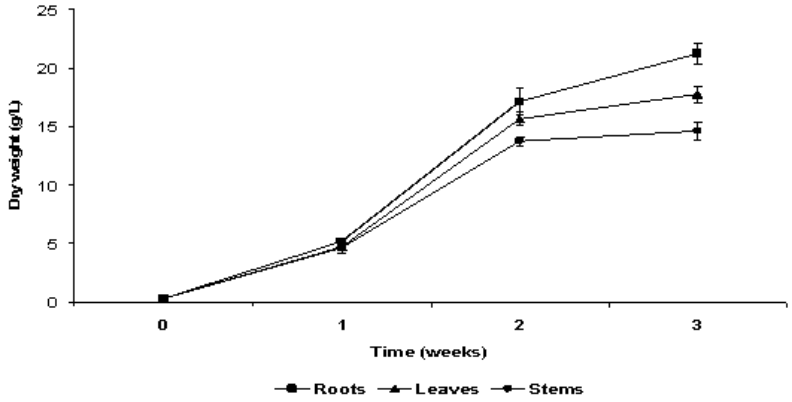

Fig. 2: Time course studies of growth in hairy root culture of common buckwheat grown in liquid-MS medium for three weeks. Values represent the mean ± SD of six independent measurements.

PCR Detection:

It is well known that each *rol* gene of the Ri-plasmid in *A. rhizogenes*is responsible for the induction of hairy root from plant species. To determine the insertion of *rol* genes, PCR was performed by using *rol* B primers to detect the transformation. PCR amplification (Fig, 3) confirmed that the gene was presented in the all transformed explants i.e., roots, leaves and stems.

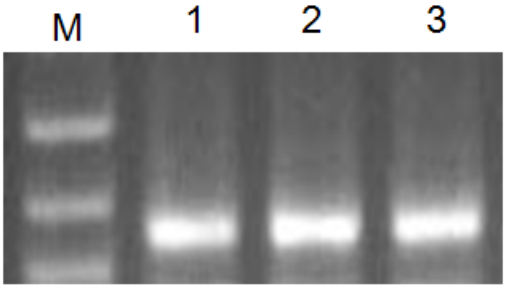

Fig. 3: PCR amplification of the rol/B gene from genomic DNA isolated from different hairy root explants of buckwheat *F. esculentum* (Moench).M: DNA ladder, 1: Stem, 2: Leaf and 3: root ; (652 bp).

Antioxidant Activity:

As stressed by Frankel and Meyer (2000) and Huang, *et al*. (2005) no single method is adequate for evaluating the antioxidant capacity, since different methods can yield widely diverging results. Several methods based on different mechanisms must be used. Here we applied assays of DPPH radical-scavenging activity and ABTS radical-scavenging activity.

DPPH Radical-Scavenging Activity:

Free radicals involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies such as cancer and cardiovascular diseases (Dorman *et al*. 2003).

DPPH is a stable radical, which could be easily used for the detection of antioxidant properties of different compounds in term of hydrogen donating ability. The advantages of the method are expressed mainly in its rapidity and selectivity. Because of these reasons DPPH· has been widely used in recent years for the assessment of different antioxidants (Grayer *et al*. 2003; Georgiev *et al*. 2006).

The scavenging activity of hairy roots cultures against DPPH• are represented in (Fig. 4). Significant ($P < 0.05$) differences between samples were observed, but the results clearly indicate that all explant sources (roots, leaves, and stems) exhibited antioxidant activity. The highest scavenging capacity was obtained from methanolic extracts of hairy roots culture with stems, which represent 78.3% inhibition of DPPH radicals more than its control. Furthermore,significant higher antioxidant activity (DPPH assay) has been found in hairy roots cultures with explant sources of leaf and roots also. This rsults are semilar with those of Adeolu, *et al*. (2009)Who mentioned that DPPH radical scavenging activity of the methanol extract of the stem had higher activity than that of the leaves.

ABTS Radical-Scavenging Activity:

Although the DPPH• free radical is ubiquitously used to estimate the potential free radical-scavenging activity of natural products, the ABTS•+ free radical is commonly used when issues of solubility or interference arise and the use of DPPH•-based assays becomes inappropriate (Arnao 2000; Dorman *et al*. 2004). Having considered the solubility of the test samples and the advantages and disadvantages of the use of the DPPH• free radical, it was considered necessary to further asses of extracts against the ABTS+ free radical. Proton radical scavenging is an important attribute of antioxidants. ABTS•+, a protonated radical, has a characteristic absorbance maximum at 734 nm that decreases with the scavenging of proton radicals(Mathew and Abraham 2006). The extracts demonstrated a range of ABTS•+-scavenging activities from 83.7 to 98% (fig 5). In our study, hairy roots cultures with all explant sources (roots, leaves, and stems) exhibited significant antioxidant activity.

Awika, *et al*. (2003)observed high correlation between ABTS, DPPH assays and antioxidant products. In this research work, the scavenging of the ABTS+ radical by the hairy roots cultures was found to be much higher than that of DPPH radical. Factors like stereoselectivity of the radicals or the solubility of the extract in different testing systems have been reported to affect the capacity of extracts to react and quench different radicals (Yu *et al*. 2002). Wang, *et al*. (1998) found that some compounds which have ABTS+ scavenging activity did not show DPPH scavenging activity. This is not the case in this study. This further showed the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage.

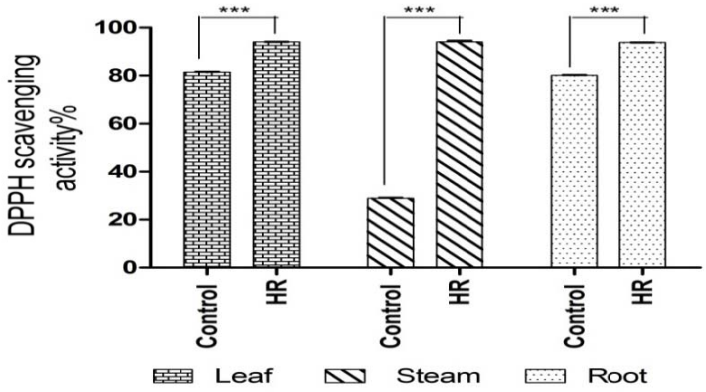

Fig. 4: DPPH▪ radical scavenging activity of methanolic extracts of transgenic root (HR) and wild type root cultures. Values are expressed as mean ± SD; n=3; significance of differences between treatments is indicated (*** p≤0.001) significance was checked using an unpaired t-test.

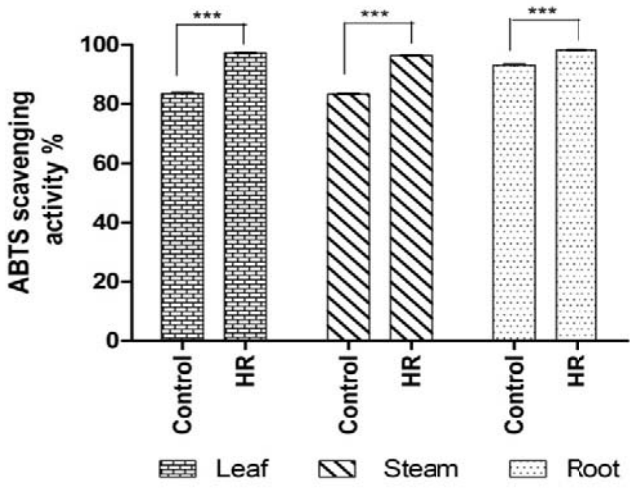

Fig. 5: ABTS▪ radical scavenging activity of methanolic extracts of transgenic root (HR) and wild type root cultures. Values are expressed as mean ± SD; n=3; significance of differences between treatments is indicated (*** p≤0.001) significance was checked using an unpaired t-test.

Phenolic Acids Composition:

A. rhizogenes causes hairy root disease in plants. The neoplastic (cancerous) roots produced by *A. rhizogenes* infection are characterized by high growth rate, genetic stability and growth in hormone free media. These genetically transformed root cultures can produce levels of secondary metabolites comparable to that of intact plants (Srivastava and Srivastava 2007). Production of differ phenolic compounds in hairy root cultures of common buckwheat has been reported too (Lee *et al*. 2007; Trotin *et al*. 1993). The hairy root culture of *F. tataricum* is produced in high content (-)-epigallocatechin, caffeic acid, chlorogenic acid, and rutin after 21 days of culture (Park *et al*. 2011).

Among phenolic compounds special place have phenolic acids. Phenolic acids occur in plants in free form as glycosides and can be integrated into larger molecules in an ester form. They are common as depsides — the intermolecular ester of two or more units composed of the same or different phenolic acids such as: caffeic, coumaric, ferulic, gallic, and syringic. Depsides are, for example, a ubiquitous chlorogenic as well as isochlorogenic, ellagic, lithospermic and rosmarinic acids. Due to the presence of a high number of hydroxyl groups and a carboxyl moiety, their antioxidant properties are very pronounced (Sroka 2005).

We suppose that in hairy root cultures with different explant sources (roots, leaves and stems) are important to study phenolic acids composition. In buckwheat hairy root cultures with different explant sources i.e., roots, leaves and stems, were detected next phenolic compounds: *p*-hydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, hesperetic acid, p-anisic and cinamic acids (Table 1). The high content of *p*-hydroxybenzoic acid, hesperetic and p-anisic acids in hairy roots culture of explant sources (roots, leaves and stems) has been presented. Results obtained revealed that, the content of detected phenolic acids has been different in variants with differ explants sources of hairy roots culture. It was found that, hairy root culture with root has been characterized higher production of identified phenolic acids compared with hairy root culture with leaves or stems. Generally, the all hairy roots cultures of the different sources has been showed high production of phenolic acids compared to control.

Table 1: Phenolic acids composition in wild type and hairy roots cultures extracts of buckwheat (mg g^{-1} DW).

Phenolic acids	Roots	Roots	Leaves		Stems	
	Control	HR	Control	HR	Control	HR
P-hydoxibenzoic acid	0.399±0.016	0.455±0.023	0.402±0.010	0.441±0.018	0.398±0.009	0.432±0.019
Caffeic acid	0.013± 0.002	0.025+0.003	0.013±0.001	0.025+0.005	0.013± 0.002	0.023+0.009
Chlorogenic acid	0.035±0.007	0.115±0.008	0.036±0.004	0.109±0.010	0.035±0.005	0.103±0.006
Vanilic acid	0.024±0.002	0.029±0.003	0.022±0.003	0.025±0.002	0.023±0.002	0.025±0.002
P-coumaric acid	0.013±0.002	0.020±0.007	0.014±0.001	0.018±0.004	0.012±0.002	0.016±0.005
Hesperetic acid	0.110±0.012	0.116±0.010	0.108±0.009	0.110±0.014	0.110±0.023	0.109±0.011
P-Anisic acid	3.430±0.180	3.891±0.250	3.429±0.226	3.752±0.198	3.428±0.156	3.560±0.101
Cynamic acid	0.035±0.006	0.042±0.014	0.036±0.003	0.038±0.008	0.035±0.002	0.038±0.007

*HR: Hairy root culture

In literature the data shown that *p*-hydroxybenzoic acid increases the impermeability of the cell wall, leading to increase resistance against pathogen infection (Horváth *et al*. 2007). Transgenic hairy root cultures from *Datura stramonium* expressing a bacterial enzyme of 4-hydroxycinnamoyl-CoA hydratase/lyase (HCHL) were found to accumulate a very-high amount of *p*-hydroxybenzoic acid in soluble form (Mitra et al. 2002). A detectable amount of soluble *p*-hydroxybenzoic acid accumulation (390 μg/g DW) was observed in hairy roots culture of *D. carota* after 7 days of incubation. In hairy root culture of *F. esculentum* with root the content of soluble *p*-hydroxybenzoic acid has been increased on 12% compared to control and it was 0.455 μg/g DW. Chlorogenic acid is an extremely widespread plant metabolite that appears to provide protection against certain forms of stress too (Grace and Logan 2000). HPLC analyses of methanolic extracts from hairy roots culture of *Echinacea purpurea* revealed the presence of high content (0.93 mg g^{-1} DW) of chlorogenic acid (Liu *et al*. 2006). In the extracts of hairy root culture of *Lactuca virosa* was identified also high content of chlorogenic acid (Stojakowska *et al*. 2012). The hairy root cultures of *F.esculentum* showed 3 times higher content of chlorogenic acid compared to control in all explants sources of hairy roots culture (roots, leaves, stems). The most higher content of chlorogenic acid was found in hairy roots culture with roots (0.115 mg g^{-1} DW). In the same time content of p-anisic acid has been increased on 12% and caffeic acid twise in hairy root cultures with roots compared to control. The less but significant increasing of p-anisic acid and caffeic acid has been observed in variants with hairy roots culture with leaves and stems. The similar increasing of chlorogenic and caffeic acids has been observed for hairy roots culture which were obtained after inoculating sterile young stems of *F. tataricum* with *A. rhizogenes* R1000 (Park *et al*. 2011). Caffeic acid was also identified in ethanol extract of the hairy roots of *Salvia miltiorrhiza* by bioassay-guided fractionation as compound with good antimicrobial and antifungal activities (Zhao *et al*. 2011). Contents of vanilic acid, p-coumaric acid, hesperetic and cinamic acids were without significant changes as in the hairy root cultures with explant sources (roots, leaves and stems) and in the control variant.

Our results indicate that hairy root culture is a valuable alternative approach for obtaining *p*-hydroxybenzoic acid, chlorogenic acid, caffeic acid and p-anisic acids from *F. esculentum*. Selection of the optimal explant sources of hairy roots is an important factor for improved secondary metabolite production *in vitro*.Further experiments will be focused on the application of different strategies for the exploitation of *F. esculentum* secondary metabolism.

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Conflict of Interest:

The authors declare no conflict of interest.

Abbreviations: MS:

Murashige and Skoog medium, DPPH: 1,1-diphenyl-2-picrylhydrazyl, ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid).

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