J Plant Mol Breed (2019) 7(1): 10 - 21
DOI: 10.22058/jpmb.2019.112410.1188

RESEARCH ARTICLE

Agrobacterium rhizogenes-mediated hairy root induction and plant regeneration from transgenic roots in *Ficus carica* L.

Shahla Amani[1], Mehdi Mohebodini[1*], Shahram Khademvatan[2], Morad Jafari[3]

[1] Department of Horticulture Sciences, University of Mohaghegh Ardabili, Ardabil, Iran
[2] Cellular and Molecular Research Center &, Department of Medical Parasitology and Mycology, Urmia University of Medical Sciences, Urmia, Iran
[3] Department of Plant Production and Genetics, Urmia University, Urmia, Iran

ABSTRACT: One of the most effective biotechnological techniques for producing plant metabolites is the hairy roots (HRs) culture system. HRs are genetically and biologically stable and able to produce secondary metabolites in a short time. *Ficus carica* L. is one of the most important plant sources of valuable medicinal compounds, especially polyphenolic compounds. The aim of this study was to investigate the growth and morphological features of HRs, methyl jasmonate (MeJA) elicitation and plant regeneration potential of HRs induced by different strains of *Agrobacterium rhizogenes* on *Ficus carica* cv. Siah. Four bacterial strains (A4, A7, A13 and ATCC 15834) were used for HR induction in leaf and shoot samples. The MS medium containing 2 mg/l of 2,4-D in combination with 1 mg/l of TDZ or BAP was used to induce callus from HRs, and then the callus regeneration ability was evaluated in MS medium containing TDZ and NAA. Depending on explant type and bacterial strain, the roots were induced directly or indirectly (via callus formation) from the wound sites, and exhibited different morphology. The HRs showed high levels of phenolic compounds. A 4-day elicitation with MeJA, in dependence on the concentration, enhanced the phenolic capacity and antioxidant capacity of HRs. The calli obtained from HRs showed root (70-80%) and bud (23.33%) regeneration potential. The current study described that the HR culture systems, in addition to providing the possibility of plant regeneration from transgenic roots, could be a promising in vitro technique for high production of secondary metabolites through elicitation.

KEYWORDS: Biotransformation, Callus, Hairy root, Phenolic compounds.

INTRODUCTION

Ficus carica L. (Moraceae) known as common fig is one of the most valuable medicinal fruit trees and healthy food products (1). All parts of plant (fruit, leaf, latex, and root) have been considered medicinally important. The isolation of many different compounds from *F. carica* such as flavonoids from fruit and leaf, coumarins from leaf and root, sterols from leaf and triterpenoids from latex and root have been reported in previous investigations (6, 16, 40). The most important medicinal properties of the fig including the antibacterial, antiviral, antifungal, anti-cancer and antioxidant effects have been attributed to the poly-phenolic compounds (6, 31, 39). However, little information is available about root metabolic activity.

The HR syndrome induced by *Agrobacterium rhizogenes* infection due to its unique ability to produce a wide variety of secondary metabolites in large scale has recently received much attention (41, 51), is the focus of many studies. The HR cultures are genetically stable, so their phytochemical biosynthetic potential can be

*Corresponding author (✉): mohebodini@uma.ac.ir
Received: 3 August 2019/ Last revised: 23 November 2019
Accepted: 21 December 2019

preserve for a long time. The T-DNA transfer from *A. rhizogenes* root inducing plasmid (pRi) and integration of its rol genes (rolA, rolB, rolC, rolD) into the host genome is responsible for inducing the hairy roots (10, 21). Each transformed cell at the infection site develops an independent HR line. The HR lines have distinct features, including high growth power, no need for growth regulators, high branching and plagiotropic growth (19). Although morphological characteristics and biosynthetic traits of the HRs are genetically controlled, they are also affected by various environmental and nutritional conditions (48). Therefore, the phenomenon of HR will be a reflection of the interaction between the type of plant species, bacterial strain, explants, infection location, culture medium and growth conditions (22, 45). In this regard, one of the main advantages of the HR culture system is the possibility of using different types of abiotic and biotic elicitors to increase the production of secondary metabolites (41).

The plant regeneration capability from the HRs has been reported in a number of plant species (13, 23, 46, 55). The regeneration might be occurred spontaneous or induced by growth regulators. The regenerated plants showed different phenotypes in comparison with normal plants and were able to transmit their traits to progeny (46, 52).

Since most herbaceous species are susceptible to infection by *A. rhizogenes*, there are many reports of successful cultivation of HRs, which is still increasing. The efficient genetic transformation of *F. carica* with A. tumefaciens strain EHA 105 have been reported by Yancheva et al. (58), but so far no study has been done on the use of *A. rhizogenes* in the fig culture. Regarding to importance of HR culture, the overall objective of the present study was to investigate morphogenesis, phenolic compounds biosynthesis and regeneration of *F. carica* hairy roots. The results of this study can provide an optimal tool (HR culture and plant regeneration from transgenic roots) for molecular plant breeding programs and thus improve the pharmacological and agronomic properties of *F. carica.*

MATERIALS AND METHODS

Plant materials and hairy root cultures

F. carica cv. Siah (Smyrne fig) seeds were collected from Estahban (29° 08'N, 54° 03'E, and 1767 m average elevation from sea level) by Zarringiah Company (Zarringiah Medicine Plant Co. Ltd. West Azerbaijan Province, Iran). Seed sterilization was performed by soaking in ethanol (70%, 2 min) and hypochlorite sodium (2.5%, 20 min), followed by three times rinsing with sterilized deionized-water. The seeds were cultured on WP medium (33) supplemented with 20 g/l sucrose and 6g/l agar and incubated at 25±2°C under a 16 h light/8 h dark photoperiod. The leaves and shoots of three weeks-old in vitro seedlings were used as explant for *A. rhizogenes*-mediated transformation for HR induction.

The single colonies of bacterial cultures (A4, A7, A13 and ATCC 15834 *A. rhizogenes* strains, provided from the National Institute of Genetic Engineering and Biotechnology, Iran) were grown in LB medium (pH 7.0) containing 50 mg/l rifampicin, in a shaking incubator at 180 rpm in dark condition at 28°C to an optical density of 0.6 at 600 nm. After centrifugation of the bacterial cultures (3500 rpm, 10 min) and re-suspension of the pellets in ½ MS liquid medium, bacterial cultures were used to inoculate the explants. Induction of HR was performed by immersion of explants in suspensions of A4, A7, A13 and ATCC 15834 *A. rhizogenes* strains followed by three-day co-cultivation on ½ MS solid medium (20 g/l sucrose, 6 g/l agar, 100 µM acetosyringone, pH 5.8) at room temperature in darkness.

After emergence of HRs tip at wounded sites (1-2 weeks after co-cultivation), 1-2 cm of each HR line were picked up and cultured on solid ½ MS medium (pH 5.8) supplemented with 200 mg/l cefotaxime (decreased regularly by successive subcultures). The HRs which were free from any bacterial contamination were transferred into liquid ½ MS and incubated on shaker incubator (120 rpm) at 25±2ºC in darkness. After PCR confirmation of the transformed nature of HRs, the morphology of HR lines was studied in solid and liquid cultures. The superior HR line was screened based on morphology and growth pattern (fast growth and high branching) from different lines.

Methyl jasmonate treatment of HRs

Established HR cultures of superior line in late exponential phase growth (30 days, determined by growth pattern) were elicited by MeJA as an abiotic elicitor. The MeJA solution was prepared by dissolving in absolute methanol. Elicitation of HR cultures was conducted by adding different

MeJA concentrations of 0, 100, 150 and 200 μM to the HR culture medium followed by HR sampling at exposure time of 96 h. At the end, the HRs was harvested and the effects of elicitation on growth index and biochemical properties of elicited HRs were evaluated. Growth index (GI) was measured by the formula: where FDW is final dry weight (g) and IDW is initial dry weight (g).

Extraction and biochemical analysis of elicited hairy roots

The dried HRs (elicited and non-elicited HRs) and NRs (non-transformed roots) were powdered and macerated in pure methanol at room temperature for 48-72 h. The solution was filtered (paper filter), the methanol solvent was removed by evaporation under vacuum, and the extract was re-solved in methanol. Total phenolic content (TPC) of HRs was assessed using Folin-Ciocalteu colorimetric method (49) by a spectrophotometry (HALO DB-20 UV-VIS double beam spectrophotometer, Dynamica, UK) and expressed as mg Gallic Acid Equivalent per g of dry weight (mg GAE/g DW). Total flavonoid content (TFC) based on Aluminum chloride spectrophotometry method (4) was measured in samples as mg Quercetin Equivalent per g of dry weight (mg QE/g DW). Antioxidant capacity of samples was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging colorimetric assay (3) and Ferric Reducing Antioxidant Power (FRAP) colorimetric assay (7).

Plant regeneration from hairy roots

In order to callus induction, the fragments of superior HR line were inoculated on solid MS medium supplemented with 30 g/l sucrose, 2 mg/l 2,4-D in combination with 1 mg/l TDZ or 1 mg/l BAP and incubated at room temperature in 16/8 h (light/dark) photoperiod. After 3 weeks, the induced calli were transferred to regeneration mediums including solid MS medium containing 2mg/l TDZ alone or combined with 1 mg/l NAA (α-naphthalene acetic acid) and 30 g/l sucrose. Stem cuttings (at least 2 buds and 1 cm in length) from regenerated plantlets were grown on solid ½ MS without growth regulators.

PCR analysis

The genomic DNA of putative HR lines was extracted by CTAB-based extraction method (43). Molecular conformation of regenerated HRs was carried out by PCR analysis using *rol*A-B (1724A-F: 5′gtgctttcgcatcttgacag3′, 1724B-R: 5′tctcgcgagaagatgcagaa3′) and *rol*B (*rol*B-F: 5′atggatcccaaattgctattccccacga3′, *rol*B-R: 5′ttaggcttctttcattcggtttactgcagc3′) genes-specific primers. PCR amplification was performed in a 12.5 μL final reaction volume with 1 μl of template DNA (~ 25ng/μl), 0.75 μL of each primer (20 pmol), 6.25 μl of master mix (YTA, Yekta Tajhiz Azma, Iran) and 3.75 μl of distilled H2O, under thermal conditions as follow: 94 °C (5 min), 30 cycles of three stages of 94 °C (1 min), 58°C (rolA-B)/60°C (rolB) (60 s) and 72 °C (70 s) and a final extension at 72 °C for 10 min. The PCR products were loaded onto 1.2% agarose gel stained with ethidium bromide.

Statistical analysis

The experiments were conducted in a factorial based on completely randomized design (CRD). All experiments of HR induction, elicitation and regeneration of HRs were performed in three replications. Morphological observations were recorded regularly after induction of HRs. The results were expressed as means ± standard error (SE). Statistical data analysis was done using IBM SPSS Statistics 22 software. The significant differences between the mean values were determined through analysis of variance (ANOVA) with Student-Newman-Keuls's multiple range tests at $P<0.05$.

RESULTS AND DISCUSSION

Effects of *A. rhizogenes* strain on morphology of hairy roots

Non-infected explants were unable to grow and induce any hairy roots when cultured on hormone-free medium but infected explants showed a different response to strains of *Agrobacterium*. In general, the higher transformation efficiency (~ 100% HR induction rate with 1-7 HRs at infection sites) was obtained using shoot explants and A7 bacterial strain (data not shown). The HR induction was occurred directly or indirectly through callus formation on infected sites of explants. In leaf

explants, HR was induced at wounded sites of major veins and petiole. In shoot explants, HRs was induced directly (without callus formation) at wounded sites of internodes and indirectly (after callus formation) at the bottom end of explants (cutting site) (Fig 1). Interestingly, depending on bacterial strain, the HRs exhibited distinct morphology features that were classified into three different types.

It has been reported that the HR morphology was related to the presence of *Agrobacterium* genes in the plant genome (38). *A. rhizogenes* strains 15834, A4 and A7 have been classified as agropine-type strains by having agropine catabolism genes on the plasmid Ri (27) and *Agrobacterium* strain of A13 has been classified as mikimopine-type strain (42). Morphology of induced HRs using two strains of A4 and ATCC 15834 was similar (classified as type I), as observed in the cases of using A7 and A13 strains (classified as type II and type III).

The type I HRs had slow growth rate, non-callus morphology and low branching phenotypes with thin lateral roots. The color of the HRs of type I was white-yellowish, but after cutting and fragmenting, with the exception of the newly developing parts, the HR fragments turned brownish color (Fig 2, A and B). The type II HRs showed non-callus morphology (Fig 2, G and H) with higher branching potential, fast growth and thicker roots compared to type 1 HRs. The type III HRs showed typical HR phenotypes with a high degree of growth rate and branching. The type III HRs were distinguished from type II by having the callus-like friable, white, soft and watery cells at the main branch that were surrounded by a pod-like brown structure which was gradually dried and opened after branching (Fig 2, C-E). As the branching progressed, the callus-like tissue gradually decomposed and eventually the main branch of HR became apparent (Fig 2, E and F). The fresh weight of HRs lines with type III morphology was much higher than HR lines with type I and II morphology because of their thick roots and high branching (Table 1; Fig 2, I). According to the previous experiments, the superior high-branching line of HRs was determined with an average fresh weight (6 weeks after transferred of 100 mg into liquid medium) of 14.12 g/flask (1.17 g/flask DW) from induced HRs of the type III in leaf explants inoculated by *A. rhizogenes* strain A7.

Variety in morphological phenotypes and growth rate of HRs induced by different strains of *A. rhizogenes* has been studied in numerous plants. The induction of three phenotypes of typical transformed root, transformed callus, and rooty callus from HR lines by *A. rhizogenes*

Figure 1. Induction of hairy roots at different infected sites from *Ficus carica* explants inoculated with A7 bacterial strain, A: indirect HRs induction on major veins of Leaf explant, B: HR induction directly in the middle of the petiole and indirectly at the end of petiole of leaf explant, C: direct HR induction on the internodes of shoot explant, D: indirect HR induction at the bottom end of shoot explant.

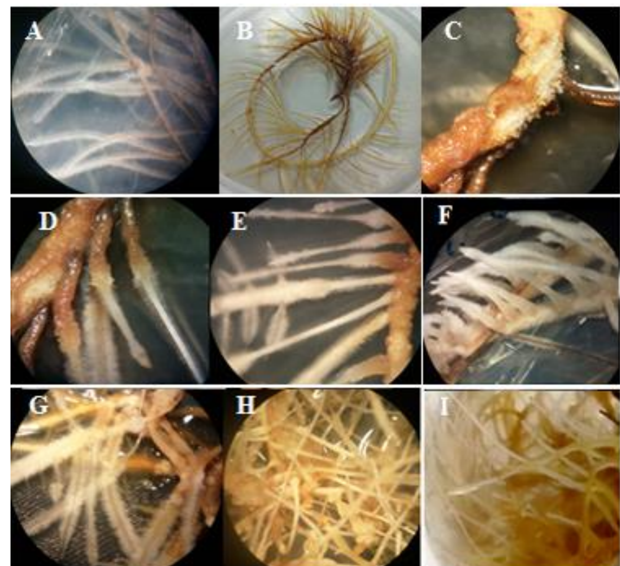

Figure 2. Morphology of *Ficus carica* hairy roots, A: HR type I cultured on solid ½ MS medium and B on liquid ½ MS medium (horizontal growth, non-callus type and limited growth of branches), C-I: HRs type III, C: morphology of HR type III (opening the pod-like structure of callus-like cells protector tissue), D: gradual branching of the HRs type III, E: appearance of white branches and gradual detachment of protector tissue in HR type III, F: Complete removal of protective tissue and appearance of main branch from HR type III, G: HRs mikimopine type II, H: type II and I HRs type III at exponential growth phase on liquid media.

Table 1. Fresh weight (g/flask) of *F. carica* HRs with three different morphology types within 1-6 weeks of growth

Phenotypes	week0	week1	week2	week3	week4	week5	week6
Type I	0.1	0.89±0.078[b]	1.85±0.31[b]	3.17±0.50[c]	4.24±0.34[c]	5.11±0.37[c]	5.74±0.42[c]
Type II	0.1	1.46±0.13[a]	3.31±0.25[a]	5.44±0.43[b]	7.23±0.39[b]	8.69±0.51[b]	9.69±0.46[b]
Type III	0.1	1.16±0.13[ab]	2.48±0.24[ab]	7.35±0.63[a]	11.19±0.24[a]	13.07±0.20[a]	14.06±0.13[a]

Values represent the means ± SE. Mean values followed by different lowercase letters are significantly different ($P < 0.05$).

Table 2. Effect of methyl jasmonate elicitation on biomass of *F. carica* hairy roots

MeJA (μM)	Dry weight (g)			4 days Growth Index
	DW_1 (before MeJA)	DW_2 (4 days after MeJA)	DW difference	
0	0.98±0.03[a]	1.00±0.02[a]	0.02±0.00[a]	0.02±0.00[a]
100	1.04±0.02[a]	0.97±0.01[a]	-0.07±0.01[a]	-0.06±0.01[ab]
150	1.00±0.02[a]	0.88±0.01[b]	-0.12±0.01[a]	-0.12±0.01[b]
200	1.10±0.09[a]	0.62±0.01[c]	-0.49±0.1[b]	-0.44±0.05[c]

Values represent the means ± SE. Mean values followed by different lowercase letters are significantly different ($P < 0.05$).

strains LBA 9402 in *Withania somnifera* (5), three phenotypes of typical morphology, callus-like morphology and thin morphology from HR lines by *A. rhizogenes* A4 in *Panax ginseng* (36), two phenotypes of typical and callus-like morphology from HR by LBA 9402 *Agrobacterium* strain in *Dracocephalum kotschyi* Boiss have been reported (17). It seems that depending on integration of T-DNA fragments (TR-DNA and TL-DNA) and their copy number into the host plant genome of HRs show different phenotypes (9, 11).

Effects of MeJA elicitation on growth index and biochemical capacity of hairy roots

In our study, the effect of MeJA on growth index, total phenolic and flavonoid production and antioxidant capacity in *F. carica* HRs were investigated. MeJA elicitation for 4 days significantly decreased the growth index, so that in the presence of the highest concentration of MeJA (200 μM), the lowest growth rate of HRs was observed (Table 2). On the other hand, MeJA affected the secondary metabolism of HRs. Adding 100 and 150 μM MeJA to HRs medium significantly increased the content of total flavonoid and phenolic compounds, respectively (Fig 3, A and B).

Antioxidant capacity of samples was measured using DPPH and FRAP assays. The DPPH method is a decolorization test based on the electron transfer ability of extract' secondary metabolites as free radical scavengers. Some of antioxidant compounds of sample may be inert to DPPH or react with it slowly (44), so the FRAP method was also used in the present study to determine the antioxidant capacity. FRAP method is a colorization assay based on the reduction power of sample antioxidants to reduce ferric iron ($Fe3+$) reduction to ferrous iron ($Fe2+$) with no involvement of free radicals (7, 44).

A four-day treatment with MeJA resulted in a significant reduction in DPPH radical scavenging capacity at concentration of 100 and 150 μM compared to HR control. At 200 μM concentration of MeJA no significant difference was observed in DPPH-based antioxidant activity compared to HR control (Fig 3, C). However, FRAP antioxidant capacity of HRs increased gradually with the addition of MeJA and peaked at 200 μM treatments (Fig 3, D). It should be noted that in all treatments, non-hairy roots had the lowest value in secondary metabolism and antioxidant capacity compared to HRs.

According to the results, it can be said that at the identical exposure time of MeJA depending on concentration, MeJA elicitation can affect biosynthesis of phenolic and flavonoids compounds involved in antioxidant activity.

MeJA as one of the derivatives of jasmonic acid is an important plant signal for defense responses to a variety of membrane destructive stresses (56). One of the clear effects of methyl jasmonate, which has been mentioned in many studies, is the growth-inhibitory effects from the treated samples. The inhibitory effect of MeJA on growth rate can be due to important role of MeJA in negative regulation of cell division cycle, reduction of cell number

and triggering plant senescence (32). However, MeJA can play a decisive role in the secondary metabolite biosynthesis pathway by activating cell defense responses due to altering the pattern of expression of genes (12, 24, 29, 59). It has been reported that MeJA elicitation of HRs enhanced synthesis of phenolic compounds in *Polygonum multiflorum* (26), flavonoids in *Hypericum perforatum* (54), alkaloids and flavonoids in *Isatis tinctoria* L. (20), isoflavonoids in *Astragalus membranaceus* (18), phenolic compounds in *Scutellaria lateriflora* (37), phenolics secretion from *Coleus blumei*, tanshinones in *Salvia miltiorrhiza* (60).

In the current study, although total phenolic content of HRs was lower than the TPC reported of leaves from different cultivars of *F. carica* by Mahmoudi et al. (35), but TPC value of both non-elicited and elicited HRs (100 and 150 µM) of *F. carica* (29.63 - 35.89 mg GAE/g DW) was considerably higher than TPC values of fruits reported from studies as follows: TPC value of 19.1 – 21.8 mg/100 g FW from dry and fresh fig fruits reported by Vallejo et al. (53); TPC value of 21.901 mg CE/g DW from dry extract of fig fruit reported by Amessis Ouchemoukh et al. (2); TPC value of 2 - 3.5 mg GAE/g FW from dry fruit fig (*F. carica* L. var. 'Sabz') under rain-fed condition reported by Sedaghat and Rahemi (47); TPC value of 19.4 – 220 mg GAE/100g FW from fig fruits of green, yellow, purple, black and brown cultivars reported by Caliskan and Polat (8) and TPC value of 10.1 – 14.8 mg chlorogenic acid equivalents /g DW from three cultivars fig fruits reported by Loizzo et al. (34). Based on results, HR culture system of *F. carica* could be introduced as an appropriate tool for high production of secondary metabolites.

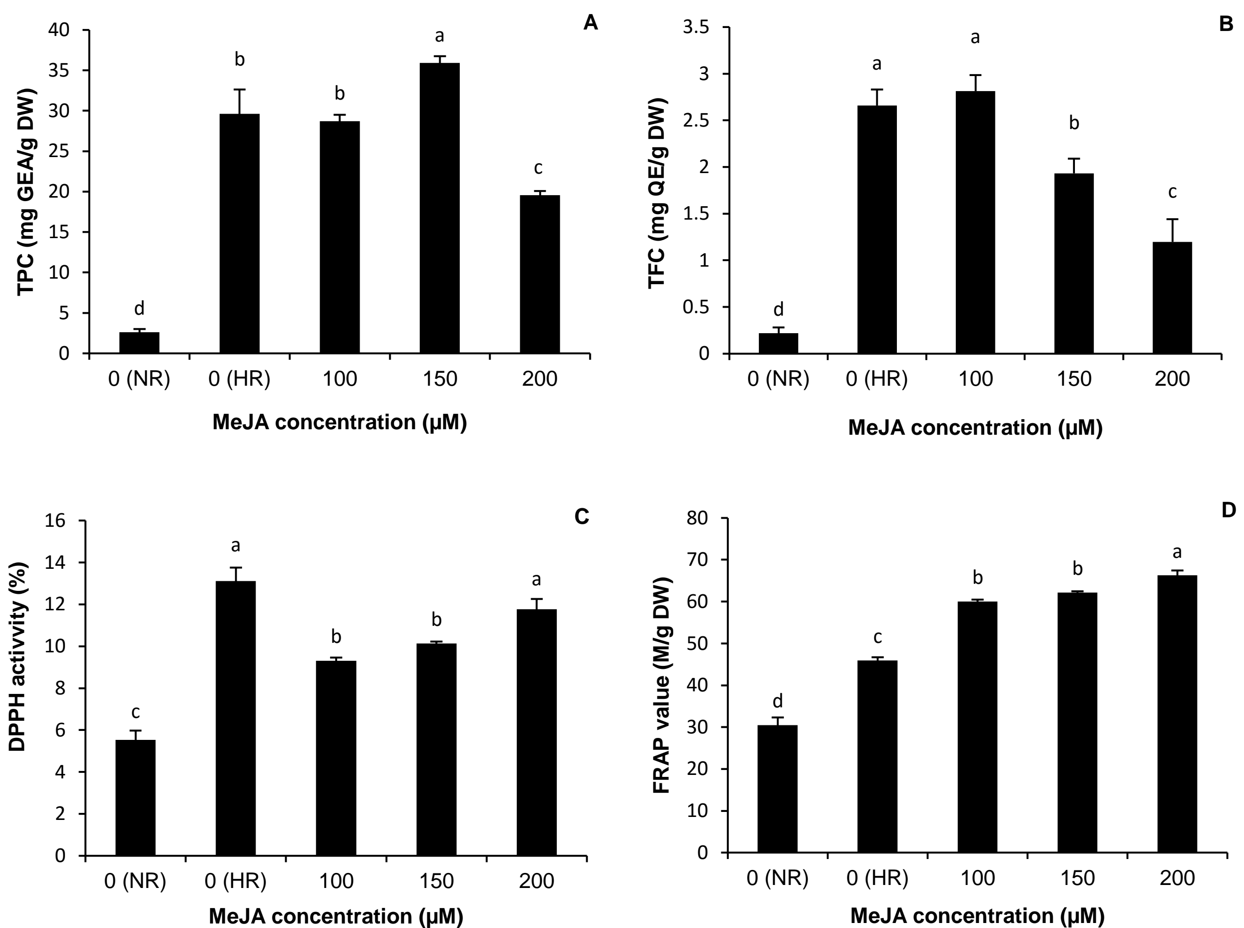

Figure 3. Effect of 4 days methyl jasmonate elicitation on biochemical properties, A: total phenolic content (mg GAE/g DW), B: total flavonoid content (mg QE/g DW), C: DPPH radical scavenging capacity (%) and D: FRAP antioxidant capacity (M/g DW). Values represent the means ± SE. Mean values followed by different lowercase letters are significantly different ($P < 0.05$).

Plant regeneration ability of hairy root cultures

Axenic superior HR line was cut off and subcultured into callus induction medium (MS medium containing 2 mg/l 2,4-D and 1 mg/l TDZ/BAP). The formation of the callus tissue (90-95 %) started from the beginning of cultivation (Table 3). After 20 days of culture, the callus covered the entire surface of explants, so that only the callus tissue was visible. The early-formed callus from the root originally was white, fragile, but rapidly growing, which later turned into yellowish-brown or pale green, compact and slowly growing (Fig 4, A-C). The obtained calli continued to grow after transferring to regeneration medium. Regeneration process started with an intense coloring and resulted in the induction of small roots and shoots within 15 days (D-I). Although root regeneration occurred from about 70-80% of growing calli, regeneration of shoot was achieved only in 23% of calli and in the presence of 2 mg/l TDZ alone. Since regeneration of shoot and root occurred separately (Fig 4), regenerated shoots were transferred to a growth regulator-free medium to establish and develop the root system. These plants were successfully rooted and their root system exhibited morphological characteristics of HRs, such as high growth rate, high-branching, and plagiotropic growth (Fig 5).

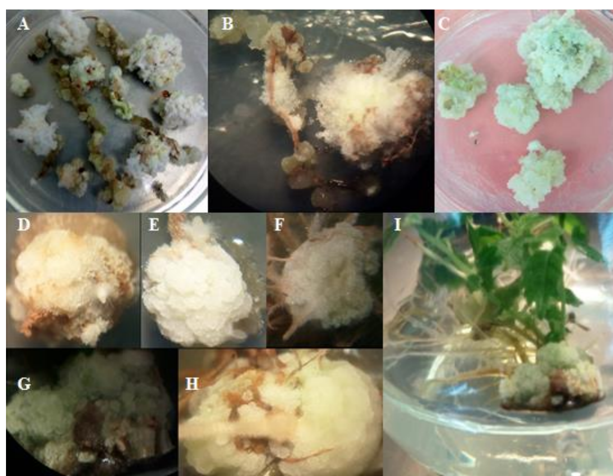

Figure 4. Induction and plant regeneration of *Ficus carica* hairy roots, A-C: callus induced using MS medium containing 2 mg/l 2,4-D and 1 mg/l TDZ, A: different color types of callus from white and yellowish-brown to pale green, B: white, friable and watery callus, C: Gradual color change and compression of callus after transferring to the regeneration medium, D: root regeneration of HR callus on MS medium containing 1 mg/l NAA in combination with 2 mg/l TDZ, E: 1 mg/l TDZ and F: 2 mg/l TDZ, G-I: from pale green and compact callus to root and shoot regeneration, separately (MS medium containing 2 mg/l TDZ).

The confirmation of transformed regenerated plants was performed by PCR analysis. As shown in Fig 6, PCR product of regenerated plants, HRs and *A. rhizogenes* (positive control), DNA bands of 1794 bp and 780 bp, were responsible for rolA-B and rolB, respectively.

In similar to reported studies (13, 14, 30) the regenerated plants showed bushy phenotype with shortened internodes and it was seemed that the apical dominance in these plants had been lost. On the other hand, contrary to the previous reports (11, 28, 57) the regenerated plant leaves were low wrinkling. The leaf color of the regenerated plants was initially light green and changed over time to a deeper green (Fig 5).

Within the genus *Ficus*, no study of HR induction has been done and the present study is the first report that focused on morphology, secondary metabolites production and regeneration potential of *Ficus carica* HR culture.

Figure 5. Regenerated plant from *Ficus carica* hairy roots after subcultured on growth regulator-free ½ MS medium, A: dwarf regenerated plants with bright green leaves (shortened internodes and without apical dominance), B: color change of regenerated plant leaves to deep green.

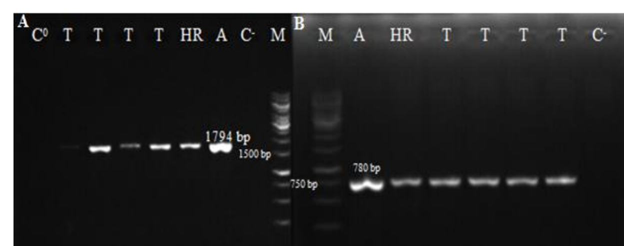

Figure 6. PCR analysis of *rol* genes from bacterial plasmid DNA, hairy roots and regenerated plants, A: PCR amplification of *rol*A-B gene (1794 bp), B: PCR amplification of *rol*B gene (780 bp). M: Marker (1 Kb), A: *Agrobacterium rhizogenes* DNA plasmid as positive control, HR: *F. carica* hairy root, T: transformed regenerated plants, C^-: normal plants as negative control, C^0: blank reaction PCR (without DNA).

Table 3. Effect of different concentrations of growth regulator on callus induction and plant regeneration

Media	% callus induction			
Control	0.00[b]			
MS + 2mg/l 2,4-D + 1 mg/l TDZ	95.00±5.00[a]			
MS + 2 mg/l 2,4-D + 1 mg/l BAP	90.00[a]			
	% root formation	Mean root number	% shoot formation	Mean shoot number
Control	0.00[c]	0.00[b]	0.00[b]	0.00[b]
MS + 2 mg/l TDZ	70.00±5.00[b]	2.67±0.58[a]	23.33±5.77[a]	4.00±1.00[a]
MS + 1mg/l TDZ	76.67±2.88[ab]	3.33±0.58[a]	0.00[b]	0.00[b]
MS + 1 mg/l NAA + 2 mg/l TDZ	80.00[a]	4.00±1.00[a]	0.00[b]	0.00[b]

Values represent the means ± SE. Mean values followed by different lowercase letters are significantly different ($P < 0.05$).

The HR has been introduced as an optimum tool for large scale production of biochemical compounds (41). It has been demonstrated that HRs will be able to transfer their distinctive features to its regenerated plants (46). Induction of callus and regeneration of plant from HRs was reported in many plant species under different cultivation conditions such as direct regeneration of HRs from *Hypericum tomentosum* using 2 µM thidiazuron (TDZ) and 40 or 60 µM p-chlorophenoxyisobutyric acid (PCIB) (25), indirect transgenic plant regeneration of *Catharanthus roseus* using MS medium containing 13.32 or 31.08 mM BAP and 5.37 or 10.74 mM NAA (13), direct regeneration from *Rhaponticum carthamoides* HRs using MS medium supplemented with 0.5 mg/L BA and 0.1 mg/L IAA (50), callus induction from *Alhagi pseudoalhagi* HRs using MS medium containing 2.0 mg/L 2,4-D and 0.5 mg/L 6-BA and plant regeneration on MS medium with 3 mg/L BAP. (55) and induction of callus from *Medicago truncatula* HRs using MS medium containing 5mg/l 2,4-D, 0.5 mg/l BAP, 5.0 mg/1 PPT (phosphinothricin) and 3% (w/v) sucrose and regeneration on MS medium supplemented with 2.5 mg/l PPT, 2% (w/v) sucrose (15). Although the morphology of transgenic plants may be different or similar to normal plants, it is undeniable that the ability to produce the HR system, and thus the high secondary metabolites biosynthetic capacity can be maintained in transgenic plants. It is also possible to create germplasm resistant to various stresses through the transfer of resistance genes during the process of transferring the T-DNA from the bacterial plasmid to the plant genome, inducing the hairy root and regenerating transgenic plant. Therefore, the results of this research will be useful in the molecular breeding and metabolic engineering programs.

CONCLUSION

This study provides an initial step to investigate hairy roots and plant regeneration from hairy roots in *F. carica.* The type of explant and *A. rhizogenes* strain could affect the HR induction and morphology features of HRs in *F. carica.* MeJA elicitation could increase the production of secondary compounds in HR cultures. Depending on used growth regulators, the HR cultures could be regenerated and maintained the HRs ability in transgenic plants. In conclusion, HR system culture can be considered as a promising in vitro method for plant breeding and pharmaceutical purposes.

ACKNOWLEDGEMENT

The authors acknowledge the support of the Department of Horticultural Sciences from University of Mohaghegh Ardabili, the Department of Plant Production and Genetics from Urmia University and the Cellular and Molecular Research Center from Urmia University of Medical Sciences.

REFERENCES

[1] Aljane, F., Essid, A., and Nahdi, S. 2018. Improvement of Fig (*Ficus carica* L.) by Conventional Breeding and Biotechnology, in Advances in Plant Breeding Strategies: Fruits. Springer. p. 343-375.

[2] Amessis Ouchemoukh, N., Ouchemoukh, S., Meziant, N., Idiri, Y., Hernanz, D., Stinco, C.M., Rodriguez Pulido, F.J., Heredia, F.J., Madani, K., and Luis, J. 2017. Bioactive metabolites involved in the antioxidant, anticancer and anticalpain activities of *Ficus carica* L., *Ceratonia siliqua* L. and *Quercus ilex* L. extracts. Ind Crops Prod, 95: 6-17. DOI: https://doi.org/10.1016/j.indcrop.2016.10.007.

[3]Argolo, A., Sant'Ana, A., Pletsch, M., and Coelho, L. 2004. Antioxidant activity of leaf extracts from *Bauhinia monandra*. Bioresour Technol, 95(2): 229-233. DOI: https://doi.org/10.1016/j.biortech.2003.12.014.

[4]Bakar, M.F.A., Mohamed, M., Rahmat, A., and Fry, J. 2009. Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang*) and tarap (*Artocarpus odoratissimus*). Food Chem, 113(2): 479-483. DOI: https://doi.org/10.1016/j.foodchem.2008.07.081.

[5]Bandyopadhyay, M., Jha, S., and Tepfer, D. 2007. Changes in morphological phenotypes and withanolide composition of Ri-transformed roots of *Withania somnifera*. Plant Cell Rep, 26(5): 599-609. DOI: https://doi.org/10.1007/s00299-006-0260-0.

[6]Barolo, M.I., Mostacero, N.R., and López, S.N. 2014. *Ficus carica* L.(Moraceae): an ancient source of food and health. Food Chem, 164: 119-127. DOI: https://doi.org/10.1016/j.foodchem.2014.04.112.

[7]Benzie, I.F. and Strain, J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay. Anal Biochem, 239(1): 70-76. DOI: https://doi.org/10.1006/abio.1996.0292.

[8]Caliskan, O. and Polat, A.A. 2011. Phytochemical and antioxidant properties of selected fig (*Ficus carica* L.) accessions from the eastern Mediterranean region of Turkey. Sci Hortic, 128(4): 473-478. DOI: https://doi.org/10.1016/j.scienta.2011.02.023.

[9]Cardarelli, M., Mariotti, D., Pomponi, M., Spano, L., Capone, I., and Costantino, P. 1987. *Agrobacterium rhizogenes* T-DNA genes capable of inducing hairy root phenotype. Mol Gen Genet, 209(3): 475-480. DOI: https://doi.org/10.1007/BF00331152.

[10] Chandra, S. 2012. Natural plant genetic engineer *Agrobacterium rhizogenes*: role of T-DNA in plant secondary metabolism. Biotechnol Lett, 34(3): 407-415. DOI: https://doi.org/10.1007/s10529-011-0785-3.

[11] Chaudhuri, K.N., Ghosh, B., Tepfer, D., and Jha, S. 2006. Spontaneous plant regeneration in transformed roots and calli from *Tylophora indica*: changes in morphological phenotype and tylophorine accumulation associated with transformation by *Agrobacterium rhizogenes*. Plant Cell Rep, 25(10): 1059-1066. DOI: https://doi.org/10.1007/s00299-006-0164-z.

[12] Choi, D.-W., Jung, J., Im Ha, Y., Park, H.-W., In, D.S., Chung, H.-J., and Liu, J.R. 2005. Analysis of transcripts in methyl jasmonate-treated ginseng hairy roots to identify genes involved in the biosynthesis of ginsenosides and other secondary metabolites. Plant Cell Rep, 23(8): 557-566. DOI: https://doi.org/10.1007/s00299-004-0845-4.

[13] Choi, P., Kim, Y., Choi, K., Chung, H., Choi, D., and Liu, J.R. 2004. Plant regeneration from hairy-root cultures transformed by infection with *Agrobacterium rhizogenes* in *Catharanthus roseus*. Plant Cell Rep, 22(11): 828-831. DOI: https://doi.org/10.1007/s00299-004-0765-3.

[14] Christey, M.C. and Braun, R.H. 2005. Production of hairy root cultures and transgenic plants by *Agrobacterium rhizogenes*-mediated transformation, in Transgenic plants: methods and protocols. Springer. p. 47-60.

[15] Crane, C., Wright, E., Dixon, R.A., and Wang, Z.-Y. 2006. Transgenic *Medicago truncatula* plants obtained from *Agrobacterium tumefaciens*-transformed roots and *Agrobacterium rhizogenes*-transformed hairy roots. Planta, 223(6): 1344-1354. DOI: https://doi.org/10.1007/s00425-006-0268-2.

[16] Duenas, M., Perez-Alonso, J.J., Santos-Buelga, C., and Escribano-Bailon, T. 2008. Anthocyanin composition in fig (*Ficus carica* L.). J Food Compos Anal, 21(2): 107-115. DOI: https://doi.org/10.1016/j.jfca.2007.09.002.

[17] Fattahi, M., Nazeri, V., Torras-Claveria, L., Sefidkon, F., Cusido, R.M., Zamani, Z., and Palazon, J. 2013. A new biotechnological source of rosmarinic acid and surface flavonoids: Hairy root cultures of *Dracocephalum kotschyi* Boiss. Ind Crops Prod, 50: 256-263. DOI: https://doi.org/10.1016/j.indcrop.2013.07.029.

[18] Gai, Q.-Y., Jiao, J., Luo, M., Wang, W., Gu, C.-B., Fu, Y.-J., and Ma, W. 2016. Tremendous enhancements of isoflavonoid biosynthesis, associated gene expression and antioxidant capacity in *Astragalus membranaceus* hairy root cultures elicited by methyl jasmonate. Process Biochem, 51(5): 642-649. DOI: https://doi.org/10.1016/j.procbio.2016.01.012.

[19] Gai, Q.-Y., Jiao, J., Luo, M., Wei, Z.-F., Zu, Y.-G., Ma, W., and Fu, Y.-J. 2015. Establishment of hairy root cultures by *Agrobacterium rhizogenes* mediated transformation of *Isatis tinctoria* L. for the efficient production of flavonoids and evaluation of antioxidant activities. PLoS One, 10(3): e0119022. DOI: https://doi.org/10.1371/journal.pone.0119022.

[20] Gai, Q.-Y., Jiao, J., Wang, X., Zang, Y.-P., Niu, L.-L., and Fu, Y.-J. 2019. Elicitation of *Isatis tinctoria* L. hairy root cultures by salicylic acid and methyl jasmonate for the enhanced production of pharmacologically active alkaloids and flavonoids. Plant Cell Tissue Organ Cult, 137(1): 77-86. DOI: https://doi.org/10.1007/s11240-018-01553-8.

[21] Georgiev, M.I., Agostini, E., Ludwig-Müller, J., and Xu, J. 2012. Genetically transformed roots: from plant disease to biotechnological resource. Trends Biotechnol, 30(10): 528-537. DOI: https://doi.org/10.1016/j.tibtech.2012.07.001.

[22] Giri, A. and Narasu, M.L. 2000. Transgenic hairy roots recent trends and applications. Biotechnol Adv, 18(1): 1-

22. DOI: https://doi.org/10.1016/S0734-9750(99)00016-6.

[23] Habibi, P., de Sa, M.F.G., da Silva, A.L.L., Makhzoum, A., da Luz Costa, J., Borghetti, I.A., and Soccol, C.R. 2016. Efficient genetic transformation and regeneration system from hairy root of *Origanum vulgare*. Physiol Mol Biol Plants, 22(2): 271-277. DOI: https://doi.org/10.1007/s12298-016-0354-2.

[24] Hao, X., Shi, M., Cui, L., Xu, C., Zhang, Y., and Kai, G. 2015. Effects of methyl jasmonate and salicylic acid on tanshinone production and biosynthetic gene expression in transgenic *Salvia miltiorrhiza* hairy roots. Biothechnol Appl Biochem, 62(1): 24-31. DOI: https://doi.org/10.1002/bab.1236.

[25] Henzelyova, J. and Cellarova, E. 2018. Modulation of naphthodianthrone biosynthesis in hairy root-derived *Hypericum tomentosum* regenerants. Acta Physiol Plant, 40(5): 82. DOI: https://doi.org/10.1007/s11738-018-2664-1.

[26] Ho, T.-T., Lee, J.-D., Ahn, M.-S., Kim, S.-W., and Park, S.-Y. 2018. Enhanced production of phenolic compounds in hairy root cultures of *Polygonum multiflorum* and its metabolite discrimination using HPLC and FT-IR methods. Appl Microbiol Biotechnol, 102(22): 9563-9575. DOI: https://doi.org/10.1007/s00253-018-9359-9.

[27] Jouanin, L., Tourneur, J., Tourneur, C., and Casse-Delbart, F. 1986. Restriction maps and homologies of the three plasmids of *Agrobacterium rhizogenes* strain A4. Plasmid, 16(2): 124-134. DOI: https://doi.org/10.1016/0147-619X(86)90071-5.

[28] Kang, H., Anbazhagan, V., You, X., Moon, H., Yi, J., and Choi, Y. 2006. Production of transgenic Aralia elata regenerated from *Agrobacterium rhizogenes*-mediated transformed roots. Plant Cell Tissue Organ Cult, 85(2): 187-196. DOI: https://doi.org/10.1007/s11240-005-9070-2.

[29] Kim, O.T., Bang, K.H., Kim, Y.C., Hyun, D.Y., Kim, M.Y., and Cha, S.W. 2009. Upregulation of ginsenoside and gene expression related to triterpene biosynthesis in ginseng hairy root cultures elicited by methyl jasmonate. Plant Cell Tissue Organ Cult, 98(1): 25-33. DOI: https://doi.org/10.1007/s11240-009-9535-9.

[30] Koike, Y., Hoshino, Y., Mii, M., and Nakano, M. 2003. Horticultural characterization of Angelonia salicariifolia plants transformed with wild-type strains of *Agrobacterium rhizogenes*. Plant Cell Rep, 21(10): 981-987. DOI: https://doi.org/10.1007/s00299-003-0608-7.

[31] Lansky, E.P., Paavilainen, H.M., Pawlus, A.D., and Newman, R.A. 2008. *Ficus* spp.(fig): Ethnobotany and potential as anticancer and anti-inflammatory agents. J Ethnopharmacol, 119(2): 195-213. DOI: https://doi.org/10.1016/j.jep.2008.06.025.

[32] Li, C., Wang, P., Menzies, N.W., Lombi, E., and Kopittke, P.M. 2018. Effects of methyl jasmonate on plant growth and leaf properties. J Plant Nutr Soil Sc, 181(3): 409-418. DOI: https://doi.org/10.1002/jpln.201700373.

[33] Lloyd, G. and McCown, B. 1980. In: proceedings of the international plant propagation society. 30: 421-427.

[34] Loizzo, M.R., Bonesi, M., Pugliese, A., Menichini, F., and Tundis, R. 2014. Chemical composition and bioactivity of dried fruits and honey of *Ficus carica* cultivars Dottato, San Francesco and Citrullara. J Sci Food Agr, 94(11): 2179-2186. DOI: https://doi.org/10.1002/jsfa.6533.

[35] Mahmoudi, S., Khali, M., Benkhaled, A., Benamirouche, K., and Baiti, I. 2016. Phenolic and flavonoid contents, antioxidant and antimicrobial activities of leaf extracts from ten Algerian *Ficus carica* L. varieties. Asian Pac J Trop Biomed, 6(3): 239-245. DOI: https://doi.org/10.1016/j.apjtb.2015.12.010.

[36] Mallol, A., Cusido, R.M., Palazon, J., Bonfill, M., Morales, C., and Pinol, M.T. 2001. Ginsenoside production in different phenotypes of *Panax ginseng* transformed roots. Phytochemistry, 57(3): 365-371. DOI: https://doi.org/10.1016/S0031-9422(01)00062-0.

[37] Marsh, Z., Yang, T., Nopo-Olazabal, L., Wu, S., Ingle, T., Joshee, N., and Medina-Bolivar, F. 2014. Effect of light, methyl jasmonate and cyclodextrin on production of phenolic compounds in hairy root cultures of *Scutellaria lateriflora*. Phytochemistry, 107: 50-60. DOI: https://doi.org/10.1016/j.phytochem.2014.08.020.

[38] Mirjalili, H.M., Fakhr- Tabatabaei, S.M., Bonfill, M., Alizadeh, H., Cusido, R.M., Ghassempour, A., and Palazon, J. 2009. Morphology and withanolide production of *Withania coagulans* hairy root cultures. Engineering in Life Sciences, 9(3): 197-204. DOI: https://doi.org/10.1002/elsc.200800081.

[39] Nadeem, M. and Zeb, A. 2018. Impact of maturity on phenolic composition and antioxidant activity of medicinally important leaves of *Ficus carica* L. Physiol Mol Biol Plants, 24(5): 881-887. DOI: https://doi.org/10.1007/s12298-018-0550-3.

[40] Oliveira, A.P., Baptista, P., Andrade, P.B., Martins, F., Pereira, J.A., Silva, B.M., and Valentao, P. 2012. Characterization of *Ficus carica* L. cultivars by DNA and secondary metabolite analysis: Is genetic diversity reflected in the chemical composition? Food Res Int, 49(2): 710-719. DOI: https://doi.org/10.1016/j.foodres.2012.09.019.

[41] Ono, N.N. and Tian, L. 2011. The multiplicity of hairy root cultures: prolific possibilities. Plant Sci, 180(3): 439-446. DOI: https://doi.org/10.1016/j.plantsci.2010.11.012.

[42] Otani, M., Shimada, T., Kamada, H., Teruya, H., and Mii, M. 1996. Fertile transgenic plants of *Ipomoea trichocarpa* Ell. induced by different strains of *Agrobacterium rhizogenes*. Plant Sci, 116(2): 169-175. DOI: https://doi.org/10.1016/0168-9452(96)04394-4.

[43] Pirttila, A.M., Hirsikorpi, M., Kamarainen, T., Jaakola, L., and Hohtola, A. 2001. DNA isolation methods for medicinal and aromatic plants. Plant Mol Biol Rep, 19(3): 273-273. DOI: https://doi.org/10.1007/BF02772901.

[44] Prior, R.L., Wu, X., and Schaich, K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem, 53(10): 4290-4302. DOI: https://doi.org/10.1021/jf0502698.

[45] Roychowdhury, D., Majumder, A., and Jha, S. 2013. *Agrobacterium rhizogenes*-mediated transformation in medicinal plants: prospects and challenges, in Biotechnology for Medicinal Plants. Springer. p. 29-68.

[46] Sarkar, S., Ghosh, I., Roychowdhury, D., and Jha, S. 2018. The Effects of rol genes of *Agrobacterium rhizogenes* on morphogenesis and secondary metabolite accumulation in medicinal plants, in Biotechnological approaches for medicinal and aromatic plants. Springer. p. 27-51.

[47] Sedaghat, S. and Rahemi, M. 2018. Effects of physio-chemical changes during fruit development on nutritional quality of fig (*Ficus carica* L. var.'Sabz') under rain-fed condition. Sci Hortic, 237: 44-50. DOI: https://doi.org/10.1016/j.scienta.2018.04.003.

[48] Sharafi, A., Sohi, H.H., Mousavi, A., Azadi, P., Razavi, K., and Ntui, V.O. 2013. A reliable and efficient protocol for inducing hairy roots in *Papaver bracteatum*. Plant Cell Tissue Organ Cult, 113(1): 1-9. DOI: https://doi.org/10.1007/s11240-012-0246-2.

[49] Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enol Viticult, 16(3): 144-158.

[50] Skała, E., Picot, L., Bijak, M., Saluk-Bijak, J., Szemraj, J., Kicel, A., Olszewska, M.A., and Sitarek, P. 2019. An efficient plant regeneration from *Rhaponticum carthamoides* transformed roots, enhanced caffeoylquinic acid derivatives production in pRi-transformed plants and their biological activity. Ind Crops Prod, 129: 327-338. DOI: https://doi.org/10.1016/j.indcrop.2018.12.020.

[51] Sujatha, G., Zdravkovic-Korac, S., Calic, D., Flamini, G., and Kumari, B.R. 2013. High-efficiency *Agrobacterium rhizogenes*-mediated genetic transformation in *Artemisia vulgaris*: hairy root production and essential oil analysis. Ind Crops Prod, 44: 643-652. DOI: https://doi.org/10.1016/j.indcrop.2012.09.007.

[52] Tsuro, M. and Ikedo, H. 2011. Changes in morphological phenotypes and essential oil components in lavandin (*Lavandula× intermedia* Emeric ex Loisel.) transformed with wild-type strains of *Agrobacterium rhizogenes*. Sci Hortic, 130(3): 647-652. DOI: https://doi.org/10.1016/j.scienta.2011.08.011.

[53] Vallejo, F., Marin, J., and Tomas-Barberan, F.A. 2012. Phenolic compound content of fresh and dried figs (*Ficus carica* L.). Food Chem, 130(3): 485-492. DOI: https://doi.org/10.1016/j.foodchem.2011.07.032.

[54] Wang, J., Qian, J., Yao, L., and Lu, Y. 2015. Enhanced production of flavonoids by methyl jasmonate elicitation in cell suspension culture of *Hypericum perforatum*. Bioresour Bioprocess, 2(1): 5. DOI: https://doi.org/10.1186/s40643-014-0033-5.

[55] Wang, Y.M., Wang, J.B., Da, L., and Jia, J.F. 2001. Regeneration of plants from callus cultures of roots induced by *Agrobacterium rhizogenes* on *Alhagi pseudoalhagi*. Cell Res, 11(4): 279. DOI: https://doi.org/10.1038/sj.cr.7290097.

[56] Wasternack, C. and Parthier, B. 1997. Jasmonate-signalled plant gene expression. Trends Plant Sci, 2(8): 302-307. DOI: https://doi.org/10.1016/S1360-1385(97)89952-9.

[57] Wu, J., Wang, Y., Zhang, L.-X., Zhang, X.-Z., Kong, J., Lu, J., and Han, Z.-H. 2012. High-efficiency regeneration of *Agrobacterium rhizogenes*-induced hairy root in apple rootstock Malus baccata (L.) Borkh. Plant Cell Tissue Organ Cult, 111(2): 183-189. DOI: https://doi.org/10.1007/s11240-012-0182-1.

[58] Yancheva, S.D., Golubowicz, S., Yablowicz, Z., Perl, A., and Flaishman, M.A. 2005. Efficient *Agrobacterium*-mediated transformation and recovery of transgenic fig (*Ficus carica* L.) plants. Plant Sci, 168(6): 1433-1441. DOI: https://doi.org/10.1016/j.plantsci.2004.12.007.

[59] Zhang, L., Yang, B., Lu, B., Kai, G., Wang, Z., Xia, Y., Ding, R., Zhang, H., Sun, X., and Chen, W. 2007. Tropane alkaloids production in transgenic *Hyoscyamus niger* hairy root cultures over-expressing putrescine N-methyltransferase is methyl jasmonate-dependent. Planta, 225(4): 887-896. DOI: https://doi.org/10.1007/s00425-006-0402-1.

[60] Zhou, J., Ran, Z.-F., Liu, Q., Xu, Z.-X., Xiong, Y.-H., Fang, L., and Guo, L.-P. 2019. Jasmonic acid serves as a signal role in smoke-isolated butenolide-induced tanshinones biosynthesis in *Salvia miltiorrhiza* hairy root. S Afr J Bot, 121: 355-359. DOI: https://doi.org/10.1016/j.sajb.2018.10.029.

القای ریشه‌های مویین با استفاده از اگروباکتریوم رایزوژنز و باززایی گیاهچه از ریشه‌های تراریخته در ***Ficus carica*** **L.**

شهلا امانی[۱]، مهدی محب‌الدینی[۱*]، شهرام خادم‌وطن[۲]، مراد جعفری[۳]

[۱] گروه باغبانی، دانشگاه محقق اردبیلی، اردبیل، ایران

[۲] گروه انگل شناسی و قارچ‌شناسی و مرکز تحقیقات سلولی و مولکولی، دانشکده پزشکی، دانشگاه علوم پزشکی ارومیه، ارومیه، ایران

[۳] گروه تولید و ژنتیک گیاهی ، دانشگاه ارومیه، ارومیه، ایران

*نویسنده مسئول: mohebodini@uma.ac.ir

چکیده

سیستم کشت ریشه مویین از جمله تکنیک‌های پربازده بیوتکنولوژی جهت سنتز متابولیت‌های ثانویه در مقیاس وسیع و بدون کاربرد تنظیم کننده‌های رشد محسوب می‌شود. ریشه‌های مویین از لحاظ ژنتیکی و زیستی پایدار بوده و قادر به تولید متابولیت‌های ثانویه در مدت زمان اندک می باشند. انجیر یکی از منابع ارزشمند ترکیبات دارویی بویژه ترکیبات پلی فنولیک است. در همین راستا، بررسی خصوصیات رشدی و مورفولوژی ریشه‌های مویین، تحریک با متیل جاسمونات و همچنین پتانسیل باززایی ریشه مویین در رقم سیاه انجیر (*Ficus carica* L.) از اهداف تحقیق حاضر بود. به منظور القای ریشه مویین، ریزنمونه‌های برگ و شاخه‌ی انجیر توسط سویه‌های A4، A7، A13 و ATCC15834 آگروباکتریوم رایزوژنز تلقیح شدند. از محیط کشت جامد ½ MS محتوی دو میلی‌گرم در لیتر از 2,4-D همراه با یک میلی‌گرم در لیتر از TDZ و یا BAP جهت القای کالوس استفاده گردید و قدرت باززایی کالوس‌ها در محیط کشت ½ MS محتویTDZ و NAA بررسی شد. بسته به نوع ریزنمونه و سویه‌ی باکتری، ریشه‌های مویین به طور مستقیم بدون تشکیل کالوس) و یا غیر مستقیم (بواسطه‌ی تشکیل کالوس) از محل زخم در ریزنمونه‌ها القا شد. لاین‌های ریشه مویین از قدرت بیوسنتزی بالای ترکیبات فنولی برخوردار بودند و خصوصیات مورفولوژیک متفاوتی را بروز نمودند. تیمار چهار روزه با الیسیتور متیل جاسمونات، پتانسیل تولید ترکیبات فنولی و آنتی اکسیدانی ریشه‌های مویین را بسته به غلظت مورداستفاده افزایش داد. کالوس‌های بدست آمده از ریشه‌های مویین از پتانسیل باززایی ریشه (٪۷۰-۸۰) و جوانه (٪ ۲۳/۳۳) برخوردار بودند. بنا بر مطالعه‌ی حاضر، سیستم کشت ریشه‌های مویین علاوه بر فراهم نمودن زمینه‌ای جهت باززایی گیاه از ریشه‌های تراریخته، می‌تواند به عنوان یک تکنیک بهینه جهت افزایش سنتز متابولیت‌های ثانویه از طریق اعمال محرک معرفی گردد.

کلمات کلیدی: ترانسفورماسیون، کالوس، ریشه مویین، ترکیبات فنولیک