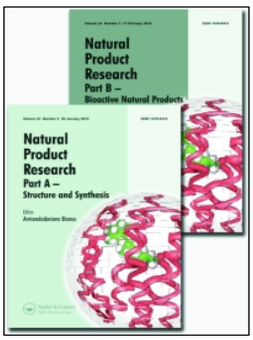


Taylor & Francis
Taylor & Francis Group

Natural Product Research

Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: http://www.tandfonline.com/loi/gnpl20

Determination of lignans, phenolic acids and antioxidant capacity in transformed hairy root culture of *Linum usitatissimum*

Ahmed M. M. Gabr, Hoda B. Mabrok, Emam A. Abdel-Rahim, Mohamed K. El-Bahr & Iryna Smetanska

To cite this article: Ahmed M. M. Gabr, Hoda B. Mabrok, Emam A. Abdel-Rahim, Mohamed K. El-Bahr & Iryna Smetanska (2018) Determination of lignans, phenolic acids and antioxidant capacity in transformed hairy root culture of *Linum usitatissimum*, Natural Product Research, 32:15, 1867-1871, DOI: 10.1080/14786419.2017.1405405

To link to this article: https://doi.org/10.1080/14786419.2017.1405405

View supplementary material

Published online: 20 Nov 2017.

Submit your article to this journal

Article views: 137

View Crossmark data

Citing articles: 2 View citing articles

Full Terms & Conditions of access and use can be found at
http://www.tandfonline.com/action/journalInformation?journalCode=gnpl20

NATURAL PRODUCT RESEARCH, 2018
VOL. 32, NO. 15, 1867–1871
https://doi.org/10.1080/14786419.2017.1405405

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Check for updates

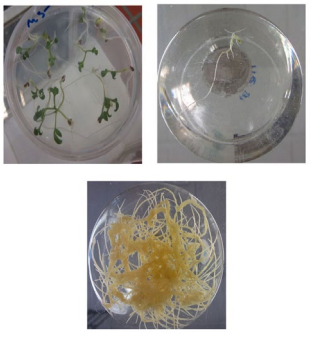
Determination of lignans, phenolic acids and antioxidant capacity in transformed hairy root culture of *Linum usitatissimum*

Ahmed M. M. Gabr[a,b], Hoda B. Mabrok[c], Emam A. Abdel-Rahim[d], Mohamed K. El-Bahr[a] and Iryna Smetanska[b]

[a]Department of Plant Biotechnology, Genetic Engineering & Biotechnology Division, National Research Centre (NRC), Cairo, Egypt; [b]Agricultural Faculty, Department of Plant Food Processing, University of Applied Science Weihenstephan-Triesdorf, Weidenbach, Germany; [c]Department of Nutrition and Food Science, Food industry and Nutrition Division, National Research Centre (NRC), Cairo, Egypt; [d]Faculty of Agriculture, Department of Biochemistry, Cairo University, Cairo, Egypt

ABSTRACT

Hairy root culture is a promising alternative method for the production of secondary metabolites. In this study, transformed root of *Linum usitatissimum* was established using *Agrobacterium rhizogenes* A4 strain from root cultures for lignans, phenolic acids and antioxidant capacity determination. Total lignin content (secoisolariciresinol diglucoside, secoisolariciresinol and matairesinol) was 55.5% higher in transformed root cultures than in the non-transformed root culture. Secoisolariciresinol was detected in higher concentration (2.107 µmol/g DM) in the transformed root culture than non-transformed culture (1.099 µmol/g DM). Secoisolariciresinol diglucoside and matairesinol were exclusively detected in the transformed root culture, but were not found in the non-transformed root culture. The overall production of phenolic acids in transformed roots was approximately 3.5 times higher than that of the corresponding non-transformed culture. Free radical scavenging $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ assays showed 2.9-fold and 1.76-fold higher anti-oxidant activity in transformed root culture as compared to non-transformed.



ARTICLE HISTORY
Received 4 April 2017
Accepted 7 November 2017

KEY WORDS
Linum usitatissimum; hairy root culture; *Agrobacterium rhizogenes*; lignans; phenolic acids; antioxidant capacity

CONTACT Ahmed M. M. Gabr a_m_gabr2@yahoo.com

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2017.1405405.

© 2017 Informa UK Limited, trading as Taylor & Francis Group

1. Introduction

Flax belongs to the genus *Linum* and family *Linaceae*. Linnaeus (1857) gives the botanical name, *Linum usitatissimum* in his book 'Species Plantarum'. Flaxseed is the richest source of lignans, mainly represented by secoisolariciresinol diglucoside (SDG) and secoisolariciresinol (SECO), but also matairesinol (MAT) is presented in small amount (Mazur and Adlercreutz 1998). Lignans have anticancer effects in breast, prostate and colon cancers (Mabrok et al. 2012; Papandreou et al. 2015).

Hairy root cultures are established by transformation of plants with *Agrobacterium rhizogenes*. This kind of *in vitro* system is characterised with fast growth and low-cost nutrient supply (Gabr et al. 2012). Further studies have shown rapid growth of transformed hairy roots of *Nepeta catariain vitro* cultures in comparison to non-transformed (Nermeen et al. 2015) and accumulation of metabolites, typical for the progressive development of tissues as in case with lignan accumulation in hairy root cultures of flax (Gabr et al. 2016). Moreover, numerous hairy root cultures tend to accumulate metabolites in higher concentration as plant tissues, as it has been shown for *Fagopyrum esculentum* Moench culture, accumulating phenolic compounds (Gabr et al. 2012).

*Rol*A, *rol*B and *rol*C genes are essential for the induction of the hairy root phenotype and activate secondary metabolism in transformed cells in different plant families (Bulgakov 2008; Bulgakov et al. 2012; Chandra 2012). Despite most of the research performed over the years, the biochemical and molecular functions of these genes remain poorly understood. Transformation with *A. rhizogenes* increased numerous lignans, particularly justicidin-B, podophyllotoxin and hinokinin, in different *Linum* species such as *L. perenne*, *L. corymbulosum*, *L. album*, *L. tauricum* and *L. narbonense* (Fuss 2003; Hemmati et al. 2007; Ionkova et al. 2013). However, hairy root cultures of *L. usitatissimum, L. bienne* and *L. grandiflorum* were not able to accumulate all individual lignans, which were found in plant seeds (Schmidt et al. 2006). In our recent studies Gabr et al. (2016), transformed hairy root culture of *L. usitatissimum* efficiently accumulating plant lignans (SDG, SECO and MAT) was successfully established from callus culture.

Nowadays, many researchers pay great attention on study the accumulation of lignans indifferent *in vitro* cultures of *L. usitatissimum* (Abbasi et al. 2017; Anjum et al. 2017). Therefore, in this study, we aimed to investigate the accumulation of SDG, SECO and MAT lignans in *A. rhizogenes* transformed root culture of *L. usitatissimum*.

2. Results and discussion

2.1. Establishment of hairy root cultures

Hairy roots of flax (*L. usitatissimum*) were initiated through inoculation of plant roots with *A. rhizogenes* A4 strain. After 10–14 days, transformed roots began to grow more rapidly as non-transformed control roots (Figure S1).

2.2. PCR detection

PCR amplification (Figure S2) confirmed that the *rol*B gene was presented in transformed roots. The *rol*B gene has been identified as a key player in the formation of hairy roots during the plant-*A. rhizogenes* interaction (Bulgakov et al. 2012).

2.3. Lignans HPLC analysis

SDG, SECO and MAT have been quantified and determined by HPLC in transformed and non-transformed (control) root cultures (Table 1, Figures S3 and S4). SECO concentration was twofold higher in transformed root culture compared to the non-transformed root culture. SDG and MAT were exclusively detected in transformed root cultures and didn't detect in the non-transformed root culture. Total lignans concentration in transformed root cultures was 55.5% higher than in the control cultures. Recently, Abbasi et al. (2017) reported that *L. usitatissimum* L. hairy root culture was found to be more efficient in accumulation of total phenolics, lignans (SDG and LDG) and neolignans in comparison to callus culture.

2.4. Phenolic HPLC analysis

In the present study, 4-hydroxybenzoic acid, vanillic acid, chlorogenic acid, caffeic acid *p*-coumaric acid, sinapic acid, ferulic acid, trans-3-hydroxy-4-methoxycinnamic acid, *p*-anisic acid and trans-*o*-hydroxycinnamic acid were identified in the control and in transformed root cultures of flax *L. usitatissimum* by HPLC (Table 2). Inductions of sinapic and ferulic acids has shown that they can be further metabolised as precursors of lignans and influence biosynthetic pathway for lignans in transformed roots. The total phenolic acid content in transformed root cultures was approximately 3.5-fold higher than control. *Rol*B could act as a transcription activator/ mediator for up regulation of genes involved in phenylpropanoid pathway such as phenylalanine ammonia-lyase (Kiselev et al. 2007).

2.5. Antioxidant capacity

The free radical scavenging properties were evaluated against $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ radicals. The results of $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ free radical scavenging assay showed that the antioxidant capacity of the transformed root cultures in both cases was significantly ($p \leq 0.001$ and $p \leq 0.05$, respectively) higher than control (Figure S5). According to Bulgakov et al. (2012), *rol*B genes enhance expression of genes, responsible for ROS answer and consequently synthesis of antioxidants, they also play a role not only in cell differentiation processes and in reactive oxygen species metabolism.

2.6. Comparative evaluation between hairy root transformation from callus and hairy root transformation from roots

In the current study, hairy root cultures, obtained through transformation of plant roots (HRR) could more efficiently accumulate total phenolic acids as compared to hairy roots

Table 1. Lignans concentration (SDG, SECO and MAT) in transformed root culture and non-transformed root culture.

Lignans (µmol/g DM)	Non-transformed root culture	Transformed root culture
SDG	ND	0.130
SECO	1.099	2.107
MAT	ND	0.236
Total lignans	1.099	2.473

ND: not-detected.

Table 2. Phenolic acids content of flax hairy root culture and non-transformed root culture, µmol/g DM.

Phenolic acid, µmol/g DM	Non-transformed root culture	Transformed root culture
4-hydroxybenzoic acid	3.218	3.394
Vanillic acid	1.102	2.898
Chlorogenic acid	0.938	2.789
Caffeic acid	0.242	0.390
Syringic acid	0.022	ND
p-coumaric acid	0.497	0.186
Sinapic acid	4.162	21.786
Ferulic acid	0.367	1.541
trans-3-hydroxy-4-methoxycinnamic acid	0.113	2.968
p-Anisic acid	0.184	0.068
Trans-*o*-hydroxycinnamic	0.595	4.239
Total phenolic acid	11.440	40.259

obtained from transformed callus (HRC) (Gabr et al. 2016). Phenolic acids increased in HRR more than in HRC by 56.95%. Likewise, total lignans was increased in HRR more than HRC by 42.74%. There were also significant differences between HRR and HRC in the percentage of antioxidant capacity measured by DPPH and ABTS ($p \leq 0.001$ and $p \leq 0.01$, respectively).

HRR and HRC are recommended as useful tools for accumulating total lignans and phenolic acid. However, HRR accumulate total lignans and phenolic acids in higher concentrations than HRC.

3. Conclusions

In this study, we successfully established an efficient protocol for hairy root transformation of *L. usitatissimum* form plant roots as explant material. In addition, this study provides a useful system for further study on hairy root culture as potential source of valuable lignans. Further knowledge of biosynthetic pathways of lignans in plants and hairy root cultures can open possibilities to regulate the production of phytochemicals. The strategies for regulation of biosynthesis through selection of cultivation media, phytohormone composition, feeding with precursors and application of elicitors for optimisation of the synthesis of the desired products will play also an important role.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Abbasi BH, Anjum S, Hano C. 2017. Differential effects of *in vitro* cultures of *Linum usitatissimum* L. (Flax) on biosynthesis, stability, antibacterial and antileishmanial activities of zinc oxide nanoparticles: a mechanistic approach. RSC Adv. 7:15931.

Anjum S, Abbasi BH, Doussot J, Favre-Réguillon A, Hano C. 2017. Effects of photoperiod regimes and ultraviolet-C radiations on biosynthesis of industrially important lignans and neolignans in cell cultures of *Linum usitatissimum* L. (Flax). J Photochem Photobiol, B. 167:216–227.

Bulgakov VP. 2008. Functions of *rol* genes in plant secondary metabolism. Biotechnol Adv. 26:318–324.

Bulgakov VP, Tatiana YG, Galina NV, Yuri NS, Galina KT, Dmitry VB, Dmitry LA, Yuri NZ. 2012. The *rol*B gene suppresses reactive oxygen species in transformed plant cells through the sustained activation of antioxidant defense. Am Soc Plant Biol. 158:1371–1371.

Chandra S. 2012. Natural plant genetic engineer *Agrobacterium rhizogenes*: role of T-DNA in plant secondary metabolism. Biotechnol Lett. 34:407–415.

Fuss E. 2003. Lignans in plant cell and organ cultures: an overview. Phytochem Rev. 2:307–320.

Gabr A, Sytar O, Abdelrahman A, Smetanska I. 2012. Production of phenolic acids and antioxidant activity in hairy root cultures of different explant sources of common buckwheat (*Fagopyrum esculentum* M).. Aust J Basic Appl Sci. 6:577–586. ISSN 1991-8178

Gabr AMM, Mabrok HB, Ghanem KZ, Blaut M, Smetanska I. 2016. Lignan accumulation in callus and *Agrobacterium rhizogenes*-mediated hairy root cultures of flax (*Linum usitatissimum*). Plant Cell Tiss Organ Cult. 126:255–267.

Hemmati S, Schmidt TJ, Fuss E. 2007. (+)-Pinoresinol/(-)-lariciresinol reductase from *Linum perenne* Himmelszelt involved in the biosynthesis of justicidin B. FEBS Lett. 581:603–610.

Ionkova I, Sasheva P, Ionkov T, Momekov G. 2013. *Linum narbonense*: a new valuable tool for biotechnological production of a potent anticancer lignan Justicidine B. Pharmacogn Mag. 9:39–44.

Kiselev kV, Dubrovina AS, Veselova MV, Bulgakov VP, Fedoreyev SA, Zhuravlev YN. 2007. The *rol*B gene-induced overproduction of resveratrol in *Vitis amurensis* transformed cells. J Biotechnol. 128:681–692.

Linnaeus C. 1857. Species plantarum. London: The Royal Society of London; p. 300.

Mabrok HB, Klopfleisch R, Ghanem KZ, Clavel T, Blaut M, Loh G. 2012. Lignan transformation by gut bacteria lowers tumor burden in a gnotobiotic rat model of breast cancer. Carcinogenesis. 33:203–208.

Mazur W, Adlercreutz H. 1998. Naturally occurring oestrogens in food. Pure Appl Chem. 70:1759–1776.

Nermeen MA, Gabr AMM, Ibrahim NM, Shevchenko Y, Smetanska I. 2015. Study the effect of hairy root transformation on rapid growth (growth morphology) of *Nepeta cataria in vitro* cultures. J Innovations Pharm Biol Sci. ISSN: 2349-2759:440–450.

Papandreou D, Zujaja TN, Maitha R. 2015. The role of soluble, insoluble fibers and their bioactive compounds in cancer: a mini review. Food Nutr Sci. 6:1–11.

Schmidt TJ, Hemmati S, Fuss E, Alfermann AW. 2006. A combined HPLC-UV and HPLC-MS method for the identification of lignans and its application to the lignans of *Linum usitatissimum* L. and L. bienne Mill. Phytochem Anal. 17:299–311.