Comparing aryltetralin lignans production by adventitious roots from three *Linum* species

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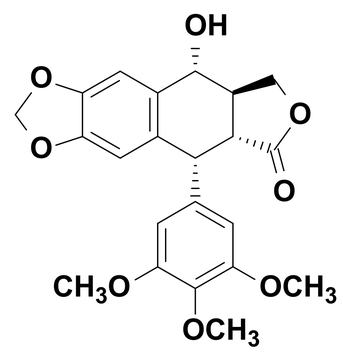
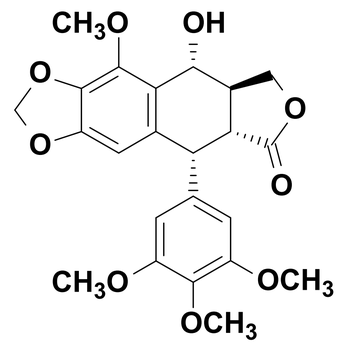
Introduction

Plant secondary metabolism represents a network of chemical signals which connect the plant with its environment. Among the secondary metabolites, lignans are plant phenols deriving from phenylpropanoid pathway and play an important role in plant defense, conferring protection against herbivores and microorganisms, and also showing antibacterial, antiviral, and antifungal properties. Moreover, they display a wide range of applications as components of food, textiles, and medicine. These compounds have attracted the attention for their potent antiviral and antineoplastic properties (1). In particular, the aryltetralin-lignans (ATLs) have received much attention since the discovery of podophyllotoxin, the most representative compounds of this class, well-known for its anticancer properties and used as lead compound for the semi-synthesis of the anticancer drugs (etoposide, teniposide). It is currently produced largely from *Podophyllum hexandrum* and *P. peltatum*, slow-growing endangered species. Although several plant species are known to synthetize podophyllotoxin and its derivatives, *Linum* species seem to be particularly promising because they can accumulate high amounts of these compounds and can be easily grown in fields or cultivated as cell cultures.

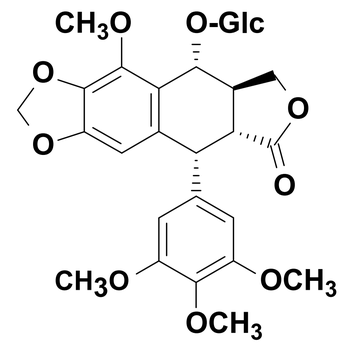


PTOX

In this work, a biotechnological approach was used, based on in vitro tissue cultures, to enhance lignans production. In particular, adventitious roots cultures (ARc) from ***L. mucronatum***, ***L. flavum*** and ***L. dolomiticum*** were obtained and different elicitation strategies were applied to improve ATLs production. Total phenolic content, total flavonoid content and antioxidant activity were also assessed. Additionally, structural investigations by HPLC and NMR analysis were performed to compare the production of **podophyllotoxin (PTOX)**, **6-methoxy podophyllotoxin (MPTOX)** and **6-methoxy podophyllotoxin 4-β-D glucoside (MPTOX-Glc)** among the different species.



MPTOX



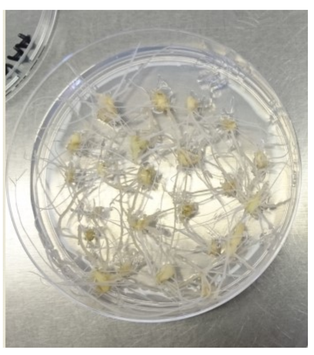
MPTOX-Glc

Results

The presence of ATLs was previously reported in different tissue cultures of *Linum* species belonging to the *Sillinum* section, in particular in cell and hairy roots cultures. In this study the ARc of ***L. mucronatum*** and ***L. dolomiticum*** were developed and described for the first time. Moreover, the elicitors **methyl jasmonate** and **coronatine**, never used on ARc of these species, were able to improve ATLs content. These elicitors caused a significant inhibition of growth for ***L. flavum*** and ***mucronatum***, but do not affect the growth of ***L. dolomiticum***. The elicitors have a strong effect to increase the total phenolic content in the three species, whereas enhance the content of flavonoids and antioxidant activity only in ***L. flavum*** and ***L. mucronatum*** ARc. Two ATLs (**MPTOX** and **MPTOX-Glc**) were identified using ^{1}H-NMR and quantified in the control and elicitor treated samples in addition to **PTOX.** The results showed that ***L. flavum*** and ***L. dolomiticum*** ARc produced, after elicitation, a good amount of **PTOX**, as compared to ***L. flavum*** hairy roots previous studied. On the contrary, **PTOX** was not detected in ***L. mucronatum*** ARc. However, the most abundant compounds present in the ARc of the three *Linum* species were **MPTOX** and **MPTOX-Glc**. For these molecules, ***L. dolomiticum*** was the highest productive species, after treatment with **methyl jasmonate** (for **MPTOX**) and **coronatine** (for **MPTOX-Glc**). ***L. mucronatum*** ARc is less suitable to yield ATLs, producing more antioxidant compounds such as phenols and flavonoids, whereas ***L. dolomiticum*** and ***L. flavum*** ARc seemed to be more performing. Overall, ARc represents a very good system to be used for large-scale production of secondary metabolites because of their biosynthetic ability, great stability and they are simpler to obtain and safer than hairy roots.

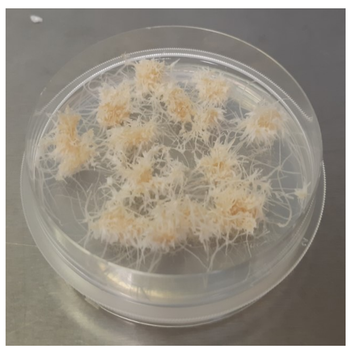
Conclusions

The results of this study indicate that ***L. dolomiticum*** ARc, described for the first time, is the ATLs best producer among the three species investigated. Moreover, the two elicitors used were effective in enhancing secondary metabolism of these tissues, but the effect of each molecule could be different in relation to the species tested (2).



L. flavum

L. mucronatum



L. dolomiticum

Reference

1) Mascheretti, Iride; Alfieri, Michela; Lauria, Massimiliano; Locatelli, Franca; Consonni, Roberto; Cusano, Erica; Dougué Kentsop, Roméo A.; Laura, Marina; Ottolina, Gianluca; Faoro, Franco; Mattana, Monica. 2021. "New Insight into Justicidin B Pathway and Production in Linum austriacum" Int. J. Mol. Sci. 22, no. 5: 2507. https://doi.org/10.3390/ijms22052507

2) Manuscript in preparation

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