

## 7.30 – Flax tissue cultures as biofactory for lignan production

### Background

Lignans are the main secondary metabolites synthesized by *Linum* species as plant defense compounds. The demand for these metabolites has increased in recent years thanks to their bioactivities (from antioxidant to anticancer) for application in pharmaceutical and nutraceutical industries [1]. *Linum* species (more than 200) are specialized in the production of specific class of **lignans: aryltetralin-type (ATLs), arylnaphtalene-type (ANLs) and dibenzylbutyrolactone-type (DBBLs)**. In this work were developed:

1. tissue cultures from different flax species for the production of ATLs and ANLs lignans
2. elicitation strategies to enhance lignans production: 100µM MeJA (methyljasmonate), 10µM COR (coronatine)
3. citolocalization of justicidin B
4. small-scale bioreactor for scale-up production



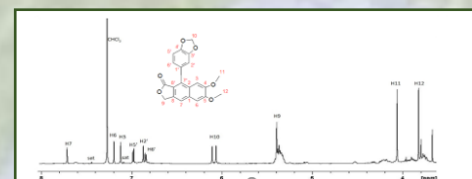
I. Mascheretti<sup>1</sup>, R. Dougué Kentsop<sup>1</sup>, M. Alfieri<sup>2</sup>, M. Laura<sup>3</sup>, G. Ottolina<sup>2</sup>, R. Consonni<sup>2</sup>, F. Faoro<sup>4</sup>, M. Lauria<sup>1</sup>, M. Mattana<sup>1</sup>  
<sup>1</sup> IBBA-CNR, Milano; <sup>2</sup> SCITEC-CNR, Milano; <sup>3</sup> CREA-OF, Sanremo; <sup>4</sup> UniMI, Milano

### Results

#### a) ANLs lignans

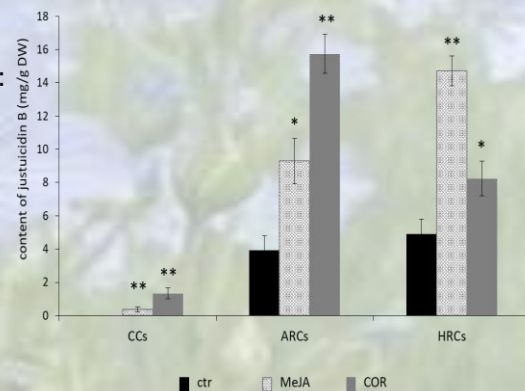
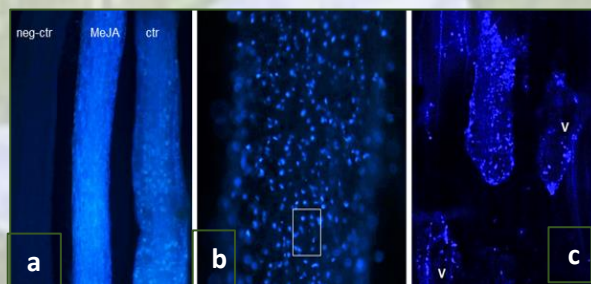
**justicidin B** production from *Linum austriacum* cultures: cell (CC), adventitious roots (ARC) and hairyroots (HRC).  
Treatments: **ctr, 100µM MeJA, 10µM COR**

**Isolation and identification** of justicidin B by TLC and NMR



Justicidin B **localization** in ARC:

- a) Autofluorescence of justicidin B
- b) Picture Enlargement
- c) Spots located in cell periphery, likely in cytoplasm

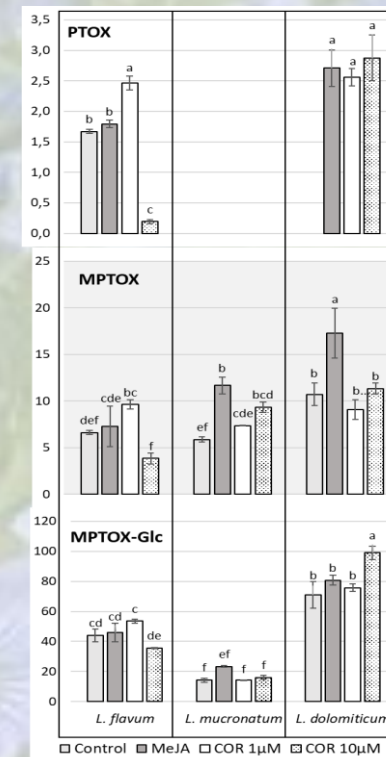
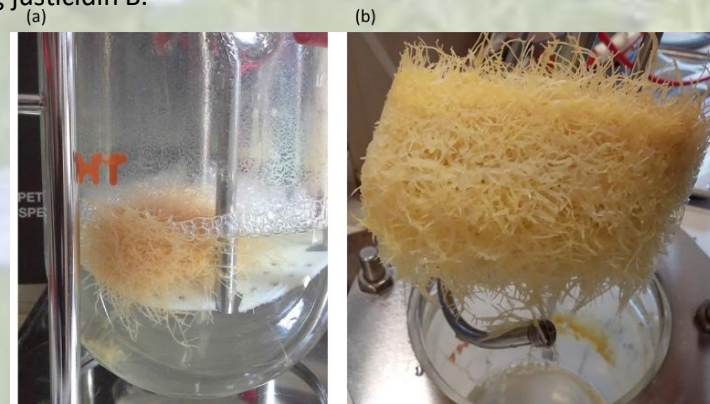


#### b) ATLs lignans

Production of the 3 main ATLs lignans podophyllotoxin (**PTOX**), 6-methoxypodophyllotoxin (**MPTOX**) and 6-methoxypodophyllotoxin -7-O-β-glucoside (**MPTOX-Glc**) by elicited or not ARC from *L. flavum*, *L. mucronatum*, *L. dolomiticum*.

#### Bioreactor:

Set-up of a Pilot bioreactor of ARC from *L. austriacum* for a scale-up of Justicidin B production. Biomass obtained after three Weeks of growth. Start: 5gr, end; 48gr (FW) with total production of 35mg justicidin B.



### Conclusions

1. The production of lignans was different in different tissue cultures being **ARC** and **HRC** more productive.
2. **Elicitor treatments** triggered different effects depending on the species analyzed
3. Cellular **localization** of justicidin B in *L. austriacum* ARC is reported for the first time
4. Successful set-up of a small scale **bioreactor** to grow ARC and HRC for secondary metabolites production.

#### MATERIALS AND METHODS

See references [2] and [3]

#### REFERENCES

- [1] De Silva et al., *Pharmaceuticals*, 2019;
- [2] Mascheretti et al., *Int. J. Mol. Sci.* 2021; 22(5), 2507;
- [3] Alfieri et al., *Molecules* 2022; 27(9), 2732