technische universität dortmund

Fakultät Bio- und Chemieingenieurwesen

Initiation of Callus Culture of *Juniperus communis* L. Horstman

Anna Galkin[a], Sayed Farag[a], Oliver Kayser[a]

[a] Technische Biochemie, Lehrstuhl Technische Biochemie Fachbereich Bio- und Chemieingenieurwesen, Universität Dortmund, Emil-Figge-Str. 66, 44227 Dortmund, Germany

Introduction

Juniperus communis, belonging to the family of Cupressaceae, is an evergreen tree with needle –like leaves. It grows like a shrub or small tree and it is distributed widely globally from cold northern areas to the warm southern areas. Juniperus species are known to produce podophyllotoxin, a lignan, which is valuable precursor of anticancer and antiviral medicines (Canel et al. 2000, Eyeberg et al. 2006). Podophyllotoxin itself is too toxic for the therapeutic use but its semisynthetic derivatives etoposide, teniposide and etophos are important antitumor medicines used in the treatment of various cancers such as lung cancer, testicular cancer and lymphomas (Giri and Narasu 2000, Botta et al. 2001). Since natural sources of podophyllotoxin become scarce and the demand continuously increases alternative ways to obtain podophyllotoxin are needed. Lignans, produced by terrestrial plants, are biosynthetically derived from the phenylpropanoid pathway. However, the biosynthetic formation of podophyllotoxin is not fully known. The first steps of the lignan biosynthesis are known but the later steps leading to the podophyllotoxin formation are still to be discovered.

Methods

In order to achieve an continuous known source of podophyllotoxin, to study biochemical pathways of podophyllotoxin formation, we initiated a callus culture of twigs of *Juniperus communis* L. Horstmann growing in Rombergpark, Dortmund, Germany (Picture 1.).

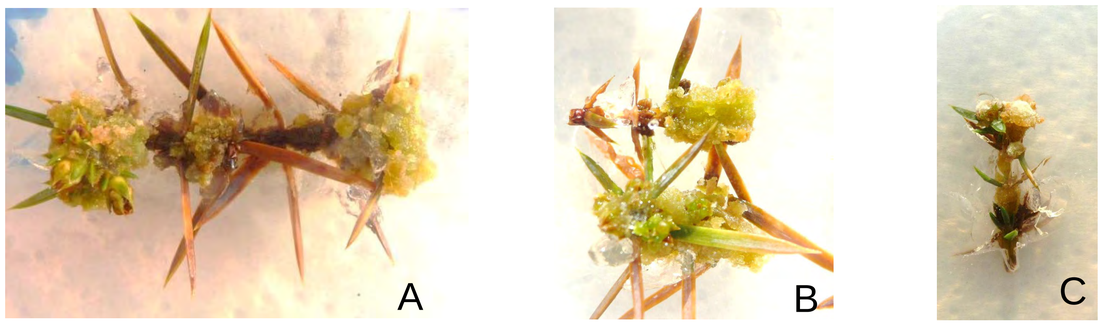
To optimize the callus formation we cultivated sterilized twigs of the Juniperus on agar plates supplemented with various growth hormones and with or without activated charcoal (Table 1). These plates were then incubated in 25ºC and 20ºC in 6000 lux with light cycle of 16 h light and 8 hours dark, and one set of the plates were kept all the time in the dark.

Picture 1. *Juniperus communis* L. Horstman growing in Rombergpark, Dortmund, Germany.

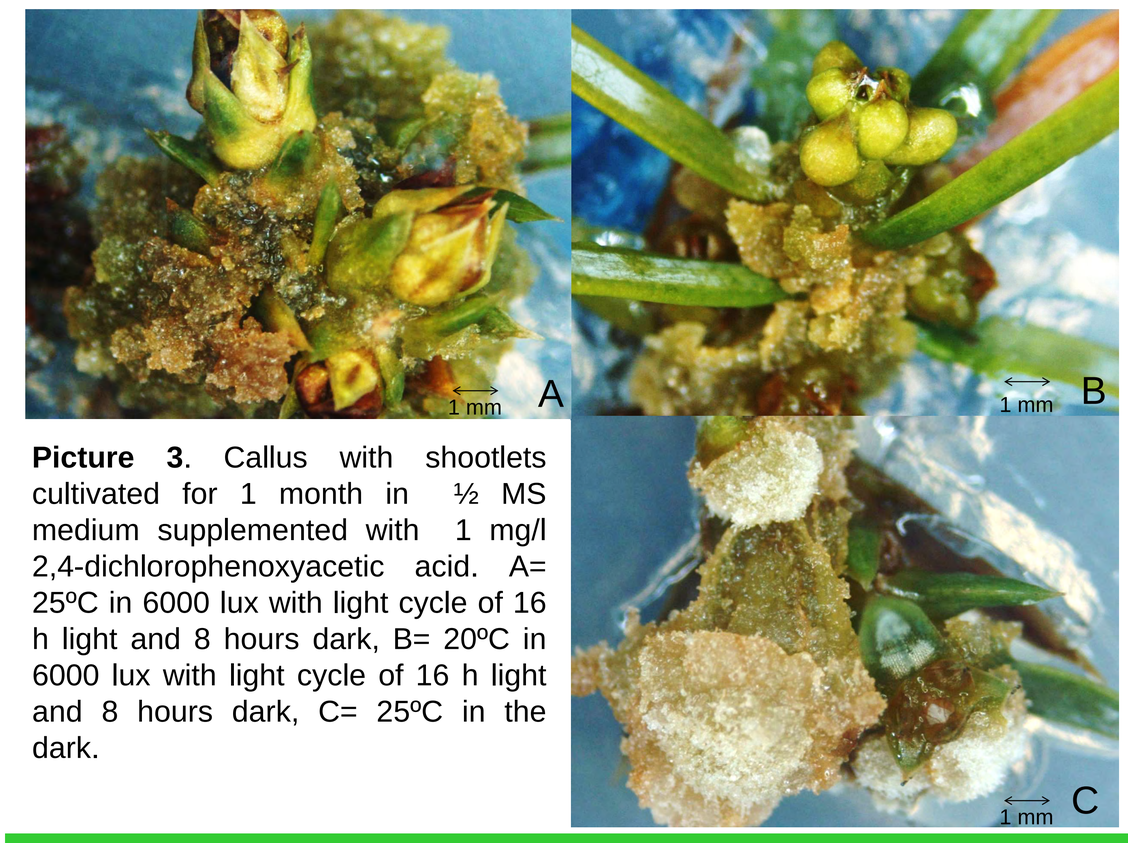
Table 1. Medium compositions used for initiation of the callus cultures. MS=Murashigae and Skoog medium(1962), B5= Gamborg's Media(1968), 2,4D=2,4-dichlorophenoxyacetic acid, NAA=naphthalene acetic acid, Kin=kinetin, BA=6-Benzylaminopurine, coal= activated charcoal. The amount of growth hormones is presented as mg/l.

MS	B5	2,4 D	NAA	Kin	Ba	coal
1/2		0,2				
1/2		0,4				
1/2		0,6				
1/2		1				
1						
	1					
1			1		1	
1			1		3	
1/2		0,2				x
1/2		0,4				x
1/2			1		1	x
1/2			1		1	x
	1/2	0,2				x

Results



Picture 2. Callus cultivated for 1 month in ½ MS medium supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid. A= 25ºC in 6000 lux with light cycle of 16 h light and 8 hours dark, B= 20ºC in 6000 lux with light cycle of 16 h light and 8 hours dark, C= 25ºC in the dark.



Picture 3. Callus with shootlets cultivated for 1 month in ½ MS medium supplemented with 1 mg/l 2,4-dichlorophenoxyacetic acid. A= 25ºC in 6000 lux with light cycle of 16 h light and 8 hours dark, B= 20ºC in 6000 lux with light cycle of 16 h light and 8 hours dark, C= 25ºC in the dark.

Conclusions

- In general callus formation was slow. First signs of the callus could be seen after two weeks of cultivation and the growth continued slow.
- 2,4-dichlorophenoxyacetic acid induced callus formation best. The concentration didn't have major effect between 0,2-1,0 mg/l. Also micropropaganation could be noticed.
- In media supplemented with naphthalene acetic acid and kinetin no callus was formed.
- Activated charcoal reduced the callus formation.
- Callus was formed fastest at 25ºC 6000 lux with light cycle of 16 h light and 8 hours dark. In 20ºC with light cycle or at 25ºC in total darkness callus formation was much slower. Initiation of callus started one week later than in 25ºC with light cycle and the growth rate was slower.
- The callus formed in 25ºC in total darkness was whitish while in the light it was brown-greenish.

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Fakultät Bio- und Chemieingenieurwesen
Prof. Dr. Oliver Kayser
Lehrstuhl Technische Biochemie
Emil-Figge-Str. 66
D-44227 Dortmund

+49 (0)231 / 755 - 7487
+49 (0)231 / 755 - 7489
Oliver.Kayser@bci.tu-dortmund.de
http://www.tb.bci.tu-dortmund.de

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