EVOLUTION OF *IN VITRO* MULTIPLICATION OF BIRD'S FOOT TREFOIL SUBCULTURES

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Abstract

This study focused on increasing the efficiency of in vitro multiplication of bird's foot trefoil. Besides the tested bird's foot trefoil cultivars Alina and Nico, the local ones Suceava I, Suceava II, Suceava III and Suceava IV were also included in the study. Axillary bud branch terminals with one-two levels of leaflets were harvested and passed on a substrate for multiplication in subcultures.

Explants that are inoculated into aseptic media and cultivated in conditions that are appropriate to their growth will develop in time and result in cultures with certain characteristics. In accordance with the aim pursued, and the nature of meristematic explants, after in vitro inoculation they develop stemlets with roots at their ends, that is, vitro plants are born.

The differences observed during the three cycles of subcultures showed differential genotypic reactions of the bird's foot trefoil cultivars. Of the two tested cultivars, Alina showed a higher proliferative capacity in subcultures.

Key words: bird's foot trefoil, explants, inoculum, minicuttings, subcultures, vitro plants.

INTRODUCTION

In the case of bird's foot trefoil, a subculture is obtained by splitting stemlets generated from an inoculum into micro- and minicuttings. The separation and passaging of cultures, as well as their pricking out, are performed by cultivating them on the same type of medium, with the aim of obtaining high clonal multiplication rates in bird's foot trefoil (Savatti M. et al, 2003, Köteles N. et al, 2014, Dragomir N., 1998).

MATERIAL AND METHOD

Axillary bud branch terminals with one-two levels of leaflets were harvested and passed on a substrate for multiplication in subcultures.

RESULTS AND DISSCUSIONS

Explants that are inoculated into aseptic media and cultivated in conditions that are appropriate to their growth will develop in time and result in cultures with certain characteristics. In accordance with the aim pursued, and the nature of meristematic explants, after *in vitro* inoculation they develop stemlets with roots at their ends, that is, vitro plants are born.

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A differentiation between the initial culture and the consecutive ones is required. The evolution of *in vitro* multiplication of bird's-foot trefoil in subculture I is shown in Table 1.

Table1

Evolution of in vitro multiplication in subculture I for Bird's-foot trefoil

Cultivars	Initial culture		Subculture I		
	Stage I plantlets	Plantlets with proliferations	Offshoot fragments	Plantlets with proliferation	
				No.	%
Alina	50	without proliferation	45	32	71.1
Nico	50	without proliferation	45	38	84.4
Suceava I	57	without proliferation	50	30	60.0
Suceava II	50	without proliferation	44	28	63.6
Suceava II	58	without proliferation	48	32	66.7
Suceava IV	55	without proliferation	50	30	66.0
Total /media	320/53.3		282/45.3	190/31.7	68.6

The evolution of *in vitro* multiplication of bird's foot trefoil in subcultures II and III is shown in Table 2.

Table2

Evolution of in vitro multiplication in subcultures II and III for Bird's-foot trefoil

Cultivars	Subculture II			Subculture III		
	Detached offshoots	Plantlets with proliferation		Detached offshoots	Plantlets with proliferation	
		Nr.	%		Nr.	%
Alina	100	57	57.2	130	38	29.2
Nico	100	60	60.1	120	40	33.3
Suceava I	100	48	48.0	130	30	23.1
Suceava II	100	50	50.4	120	35	29.2
Suceava II	100	36	36.5	115	32	27.8
Suceava IV	100	32	32.0	115	28	24.3
Total /media	600/100	283/47.5	47.4	730/122	203/34	27.8

The replications derived from the initial culture and the development of inocula in subculture will bear the „print" of the initial culture. This „print" will be present in the first stages of subcultivation. As a matter of fact, the stages covered in the subcultivation of the inocula can also imprint on them reaction particularities.

From the results obtained it can be concluded that the differences occurring during the three subculture cycles show differential genotypic reactions of the bird's foot trefoil cultivars. Looking at all three subcultures,

it can be noticed that at the end of subculture I, out of the 282 offshoot fragments resulting from the plantlets of the initial culture 190 plantlets (approximately 68.6%) proliferated offshoots, which made it possible to detach 600 offshoots for subculture II, out of which 283 proliferated (approximately 47.4%), while in subculture III the proliferation percentage was 27.8%.

CONCLUSIONS

It can be concluded that while advancing from one subculture to another (in this case subculture III), a process of senescence can be noticed, which is shown by a drop in the proliferation percentage.

It can also be noticed that Alina and Nico cultivars show clearly higher proliferation rates than the local cultivars. Of the two tested cultivars, Alina shows a higher proliferation capacity in subcultures.

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