INFLUENŢA MEDIILOR DE CULTURĂ ASUPRA ÎNMULŢIRII "*IN VITRO*" A UNOR PORTALTOI DE CIREŞ ŞI VIŞIN
CULTURE MEDIA INFLUENCE ON "*IN VITRO*" PROPAGATION OF SOME SWEET AND SOUR CHERRY ROOTSTOCKS

Catiţa Plopa, Gheorghe Mladin, Valentina Isac, Sergiu Ancu
Research Institute for Fruit Growing Pitesti, Romania

Abstract:

Improved *in vitro* clonal propagation methods are valuable tools for nurseries. The present study reports the trials undertaken to optimise the in vitro clonal propagation ability of three important cherry rootstocks: IPC 2, IPC 3 and IPC 5. Axillary buds explants were grown in axenic culture on defined culture media Murashige & Skoog (MS), Lee & Fossard (LF), Quoirin &Lepoivre (QL) including auxins, giberrellic acid, and cytokinins. Cherry rootstocks responded differently to the same medium and environmental conditions during establishment, proliferation and rooting *in vitro*. The best results during in all propagation phases was recorded with IPC 3 rootstock. The regenerated shoots of all the three rootstocks species tested grew well on the shoots proliferation medium Lee & Fossard supplemented with 1.0mg l^{-1} benzyl adenine and 0.2mg l^{-1} naphthalene acetic acid. Micropropagated shoots of IPC 3 were rooted with good results (73.33 % rooted plants) using 1.5 mg l^{-1} indolilbutyric acid. In the same conditions the results for IPC 5 was 46.66 % rooted plants and for IPC 2 only 33.33 % rooted plants.

Cuvinte cheie: in vitro, cireş, mediu de cultura,
Keywords: in vitro, cherry, rootstocks, culture media

1. Introduction

The IPC series of rootstocks was selected at the Research Institute for Fruit Growing Pitesti-Maracineni. IPC 2 and IPC 5 rootstocks are compatible with most sweet cherry cultivars and IPC 3 are compatible with most sweet cherry and sour cherry (Mladin and colab. 2007). Tissue culture techniques offer the oportunity for rapid propagation and distribution of plant materials. These methods hold promise for higher production of plant material in a shorter period of time with less labor and at lower production costs*. In vitro* morphogenesis and at requires the inducing of some autoregulate phenomena that are autonomous and depend on new heterothrophe conditions that were created. In conformity with literature (Boxus and Druart, 1989) the explants have minim (limit) size for manifested cells totipotentiality under *in vitro* conditions. Other factor for successful of *in vitro* culture is represented by media compounds. This is typical for every species or cultivars, the requests are different even depending on the explants used (Isac, 1983). Together with basic culture media, the current methods used to *in vitro* culture in order to induct and sustain of organogenesis have as practical and theoretical base the hormonal balance concept. This study was initiated to optimize routine tissue culture methods to establish, proliferate and root plants IPC 2, IPC3 and IPC 5 rootstocks.

2. Material and methods

Biological material: was represented by meristem from – meristematic dom with 0.8 -0.9 mm size, obtained from apically buds from annual branches and – meristematic dom with 0.2 - 0.3 mm size from IPC 2, IPC 3 and IPC 5 rootstocks**.**

Disinfections of biological material consists of washing with water + liquid detergent + Tween 80 for 5 min; immersion in alcohol 96^{o} for 15 min and immersion in calcium hypochlorite for 10 min, washing with distilled and sterile water 3 x 10 min.

Culture media were represented of M &S (1962), Lee & Fossard (1977), Quoirin & Lepoivre (1977) and The organic carbon source was assured using sacharose (30 g/l) and agar like gelifiant agent (6 g /l). So resulted the variants:

-for the differentiation phase
V1= Lee & Fossard + 0.01 mg/l IBA + 0.1 mg/l GA_3
V2 = M&S + 0.01 mg/l IBA + 0.1 mg/l GA_3
V3 = Quoirin &Lepoivre + 0.01 mg/l IBA + 0.1 mg/l GA_3
-for the multiplication phase
V1 = Lee & Fossard + 1mg/lBAP + 0.1 mg/l GA_3 + 0.2 mg/l ANA
V2 = M&S + 1mg/lBAP + 0.1 mg/l GA_3 + 0.2 mg/l ANA

V3 = Quoirin &Lepoivre + 1mg/lBAP + 0.1 mg/l GA_3 + 0.2 mg/l ANA
- *for rooting phase*
V1 = Lee & Fossard + 1,5 mg/l IBA
V2 = M&S + 1mg/lBAP + 1,5 mg/l IBA
V3 = Quoirin &Lepoivre + 1,5 mg/l IBA
V4 = Lee & Fossard + 1.5 mg/l NNA
V5 = M&S + 1.5 mg/l NNA
V6 = Quoirin &Lepoivre + 1.5 mg/l NNA

The explants were put in glasses to laminar air hood. Culture conditions consisted of temperature of 22-23^0 C photoperiod: 16 hours light (2,000-2,500 lx) and 8 hours dark.

The observations were made for 30 viable explants for every treatment.

3. Results and discussions

In vitro explants evolution ***in differentiation phase*** has been different depending on the size of the explant, the type of culture media and variety (Table 1):
- *variety*: the ability of regeneration depending on variety was different. Thus, under the same given conditions: variant of the culture media (V1), explant size (0.8-0.9 mm) the recorded values ranged between 100% differention plants of the IPC 3 rootstock and only improved 33.3 % differentiation explants the IPC 2 .

- *explant size* had a great influence in the regenerative capacity with significant differences on the same type of culture medium (V1), IPC 3 rootstock the recorded 100 % regeneration , when explants size was 0.8-0.9 mm versus 0.2-0.3 mm, regeneration was 26.6 %. There have been situations when the explants with the size of 0.2-0.3 mm have not differentiated: IPC 2, IPC 5 both on V2 and IPC 2 on V3.

- *culture media*: the best results were obtained on the culture media V1 with an average of 57.7% explants differentiated and 51% explants differentiated on V3. The culture media V2 has provided inadequate conditions for most varieties of range except IPC 3 variety which differentiated in proportion of 66.6% for the explants of 0.8-0.9 mm.

In multiplication phase

The multiplication rate depended on:
- *basal medium*, (table 2). The best multiplication medium for IPC 3 and IPC 5 rootstocks was V1 (Lee Fossard) resulting in 7,8 and 5 shoots/explant and for IPC 2 resulted 3.5 shoots/explant. The assortment media was 5.4 . The multiplication rate on MS and Q & L medium was with media/assortment between 2.1 and 2.5 shoots/explants.
- *variety* (table 2). The multiplication ability recorded from IPC 3 that had a higher media 4.4, compared with IPC 5 with 3.1 media /culture media and IPC 2 with 2.5.media/culture media.

After 4 subcultures, some plants were transferred to **rooting media** .

The data presented in the figure 1 shows that the studied rootstocks had an different response to the growing media composition.
The best rooting medium for all rootstocks was Lee Fossard with 1.5 mg/l IBA (V1) that recorded 73.33 % did not rooted for IPC 3 and 33,33 % rooted plants for IPC 2 and IPC 5 ,46.66 % rooted plants (figure 1). The smaller capacity for rooting was noticed in the case of V5 and V 6 when the media compound did not assured rooting conditions.

4. Conclusions

For in vitro propagation explants a complex of factors has operated: culture media through its components and hormonal balance, explants size and variety.

-the culture media which provided the best result in regeneration, multiplication and rooting phase was Lee &Fossard .

-the use of the explants with 0.2-0.3 mm size to obtain a differentiation slower compared with the explants with 0.8-0.9 mm size as a result of lower regenerative capacity.

-The most difficult in vitro rooting was for IPC 2 an IPC 5.

-IPC 3 rootstock has a good behaviour in *in vitro* condition.

References

1. Adams A.N. –The detection of Plum pox virus in prunus species in enzyme-linked immunosorbent assay (ELISA) Annals of Applied Biology, 90:215-221, 1978.
2. Boxus Ph., Druart Ph., - The production of fruit and vegetable plant by *in vitro* culture. Actual possibilities and perspectives. 49 année – n 396 – 4 – trimester, 1989.
3. 3.Clark, M.F., Adams A. N. –Characteristics of a microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J.Gen.Virol. 34:475-483, 1977.
4. De Fossard R.A. –Tissue culture in horticulture – a perspectives. Acta Horticulturae, Grand, 78, p. 455-459, 1977
5. 5.Lloyd G.B., McCown B.H. – 1981 – Commercially feasible micropropagation of mountain laurel (Kalmia latifolia)by use of shoottip culture. Proc. Inter. Plant. Prop. Soc. 29: 216-229, 1981.
6. Isac Maria –Comportarea unor soiuri de prun în procesul de microînmulţire. Lucrările celui de al II-lea Simpozion Naţional de culturi de ţesuturi vegetale *in vitro* , vol. II, 1983
7. Mladin Gh. Parnia P, Ancu S. Petrescu Silvia – 2007 – Comportarea in livada a unor portaltoi vegetative de cires. 40 de ani de cercetare in pomicultura 1967-2007. Editura INVEL Multimedia.
8. Murashige T., Skoog F. –A revised for rapid growth and bioassay with tabacco tissue cultures; Physiol Plant., 15: 473 – 497, 1962.
9. Quoirin M., Lepoivre P. – Etude de mileux adaptes aux cultures *in vitro* de Prunus. Acta Horticulturae, 1977.

Tables and Figures

Table 1. **Explants differentiated level (%) after 3 weeks of culture**

No	Rootstocks	Size explant (mm)	Culture media variants		
			V1	V2	V3
1	IPC 2	0.2-0.3	6.6	-	-
		0.8-0.9	33.3	26.6	30
2	IPC 3	0.2-0.3	26.6	6.6	16.6
		0.8-0.9	100	66.6	86.6
3	IPC 5	0.2-0.3	3.3	-	3.3
		0.8-0.9	40	33.3	36.6
	Differentiation media/assortment	0.2-0.3	**57.7**	**42.1**	**51**
	Differentiation media/assortment	0.8-0.9	**26.4**	**2.2**	**6.6**

Table 2. **The evolution of the rootstocks in the multiplication phase**

Rootstocks	V1	V2	V3	**Media / culture media**
	Media shoots/explant S1+S2+S3+S4	Media shoots/explant S1+S2+S3+S4	Media shoots/explant S1+S2+S3+S4	
IPC 2	3.5	2	2	**2.5**
IPC 3	7.8	2.5	3	**4.4**
IPC 5	5	2	2.5	**3.1**
Media /assortment	**5.4**	**2.1**	**2.5**	

S1+S2+S3 +S4 = subculture 1+subculture 2+subculture 3+subculture 4

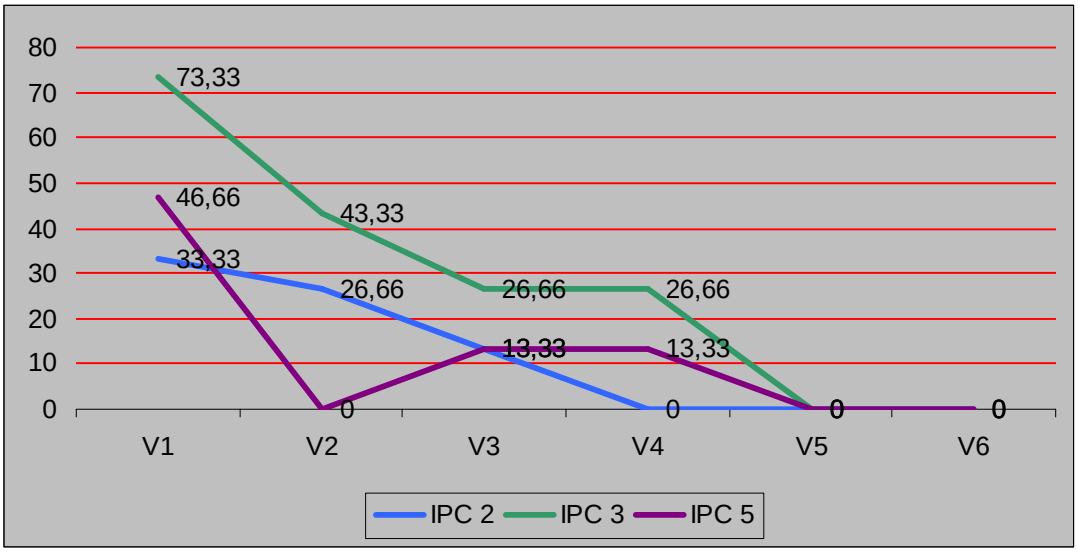

Figure 1. Rooting percentage of IPC 2, IPC 3 and IPC 5 on different culture media variant