REGENERATION OF GRAPEVINE PLANTS FROM FOLIARY EXPLANTS UNDER *IN VITRO* CULTURE CONDITIONS

REGENERAREA DE PLANTE DE VIȚĂ DE VIE ÎN CONDIȚII DE CULTURĂ *IN VITRO* DIN EXPLANTE FOLIARE

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Abstract. *Leaf explants were excised from in vitro developed shoots, Xenia and Cabernet 33 Vl. cultivars and cultured on Murashige & Skoog (1962) added with 1.5 mg/l Benzyl aminopurinã, 0.2 mg/l Acid indolil acetic, 40 mg/l adenine sulphate and 3% (w/v) sucrose. Adventitious buds developed after about 2 – 3 weeks of culture at the petiolar stub and with low frequency from lamina tissues. 80% - 85% from explans exhibited further shoots growth after several adventitious buds subculture on medium with similar composition except Indolyl acetic acid which was replace with Thidiazuron. The greatest regeneration capacity was showed by the youngest leaves situated in the apical bud.*

Rezumat. *Explante foliare prelevate de la lăstari diferentiati in vitro, genotipurile Xenia si Cabernet 33 Vl., au fost cultivate pe mediul Murashige & Skoog (1962) aditionat cu 1.5 mg/l Benzyl aminopurinã, 0.2 mg/l Acid indolil acetic, 40 mg/l adenine sulphate si 3% (w/v) zaharozã. Dupã 2 – 3 sãptãmâni de culturã la baza petiolului, si, în mãsurã mai micã pe suprafata laminei foliare, s-au dezvoltat muguri adventivi. 80% - 85% din mugurii diferentiati au evoluat în plante, dupã subcultura repetatã a mugurilor adventivi pe un mediu cu o compozitie asemãnãtoare cu a mediului initial, cu exceptia Acidului indolil acetic care a fost înlocuit cu Thidiazuron. Capacitatea cea mai mare de regenerare au manifestat-o frunzele cele mai tinere situate în mugurele apical.*

Like many woody crops, grape has been relatively recalcitrant to in vitro adventitious regeneration. Adventitious shoot organogenesis has been achieved from fragmented shoot apices, internode segments and leaves. Successful regeneration has been obtained with some cultivars, there is no reliable regeneration procedure thai may be applicable to all major cultivars. The most important factors involved in the regeneration process are the genotype, explant source, the media culture composition and culture conditions (Cheng and Reisch, 1989; Clog at al., 1990; Martinelli et al., 1993, Reisch at al., 1989; Tang and Mullins, 1990; Torregrosa, 1994, 1995). The methods were applied with good results in the obtaining of transgenic plants with modified characters (Martinelli and Mandolino, 1994; Kikkert at al., 1996). This paper describes a new method providing a high regeneration frequency of adventitious shoots in case of two *Vitis vinifera* varieties.

MATERIAL AND METHOD

The biological material used in the experiment consisted in herbaceous shoots of Vitis vinifera cultivars Italia and Cabernet 33 VI. produced from dormant cuttings planted in hydroponic culture conditions. Bud cultures were initiated from single axillary bud microcuttings without leaves and cultivated on MS basal medium supplemented with 2.25 mg/l Benzyl aminopurine. Foliary tissues obtained from newly developed shoots were used as initial explants for the regeneration protocol. Five leaves of various sizes (1.2 – 6.5 mm) were removed from the apical and the first axillary bud by a single cut made near the junction of petiole and lamina. The smallest leaves were removed with the aid of a stereomicroscope. Thirty explants were used for each culture medium or treatment.

The in vitro regeneration procedure involved three steps: induction of adventitious buds from foliary explants, regeneration and elongation of shoots from adventitious buds and rooting of shoots.

The composition of the culture media used in this study differed as regards the culture stage but always using the MS medium as base. The induction of adventitious buds was achieved on medium supplemented with 1.5 mg/l Benzyl aminopurine, 0.2 mg/l Indolyl acetic acid, 40 mg/l adenine sulphate and 3% (W/V) sucrose. Adventitious buds were transferred on two media: medium A, added with 2.0 mg/l Benzyl aminopurine and 0.5 mg/l Indolyl acetic acid and medium B, added with 2.25 mg/l Benzyl aminopurine and 0.1 mg/l Indolyl acetic acid. For the propagation phase involving successive subcultures every 30 – 45 days of adventitious buds microcuttings obtained from the proliferating shoots only Indolyl acetic acid was substituted with Thidiazuron. All culture media were sterilized by autoclaving 20 minutes at 120°C and 1 atm.

The culture was incubated in a controlled climate chamber at 25 ± 2°C, with a photoperiod of 16 hours. The light was provided by fluorescent tubes of white light.

RESULTS AND DISCUSSIONS

After about 5 – 7 days under culture, the leaves began to grow, showing a slight swelling. After 10 – 15 days, explants had swollen petiolar stubs, associated with petiole elongation. Adventitious buds began to differentiate at the buttom of the petiole or on the lamina surface after 20 – 25 days of culture. Buds continued to develop and after one month adventitious shoots had grown. At this time the greatest part of the regenerating explants showed a mass of adventitious leaves, buds and shoots up (Figure 1).

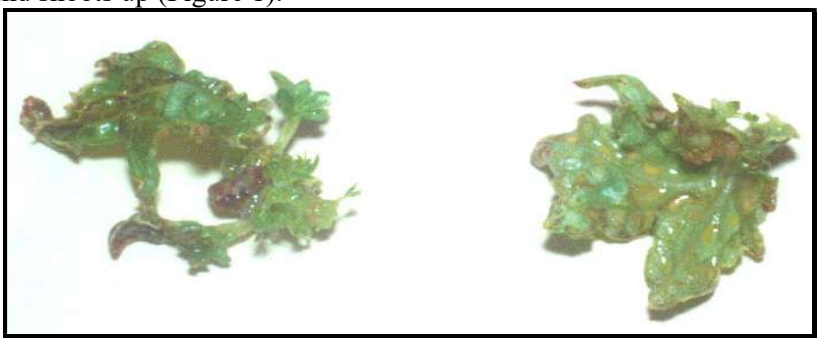

Figure 1 Adventitious buds formation from petiolar stubs and on surface lamina

The size of the leaves affected the capacity of explants to produse adventitious buds and shoots. The best results were observed in case of the smallest leaves (1.2 mm), the procent of explants producing adventitious shoots decreased with increasing leaf size (Figure 2).

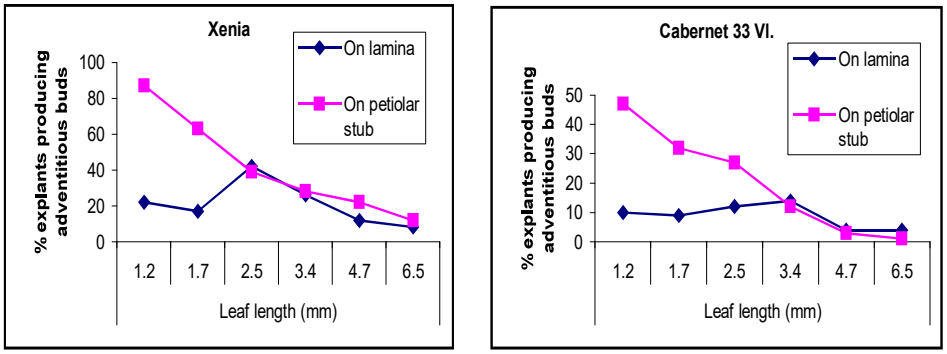

Figure 2 Effect of leaf size on adventitious buds organogenesis. Each value is a mean ± SE of 30 replicates.

The transfer of explants with adventitious buds to fresh regeneration medium at 4 weeks and at 8 weeks, promoted further shoot development. The percentage of explants forming shoots was dependent on the cultivar, the medium composition and the origin of shoots. The best regeneration occurred from explants represented by petiolar stubs with adventitious buds on A medium in case of both cultivars but, Cabernet 33 VI. variety was less responsive than Xenia. The percent of explants forming shoots on the surface lamina decreased with increasing the period of subculture from 4 to 8 weeks because of the necrosis of foliary lamina (Table 1).

Table 1

Adventitious shoots formation on regeneration media

Cultivar	Culture media	% Explants forming shoots			
		4 weeks		8 weeks	
		Petiolar stubs with adventitious buds	Surface lamina with adventitious buds	Petiolar stubs with adventitious buds	Surface lamina with adventitious buds
Xenia	A	33 ± 8	8 ± 4	42 ± 5	5 ± 2
	B	18 ± 2	5 ± 2	25 ± 3	3 ± 5
Cabernet 33 VI.	A	19 ± 5	7 ± 2	22 ± 3	3 ± 3
	B	14 ± 3	3 ± 3	16 ± 5	2 ± 2

At the time of the transfer of explants to fresh regeneration medium adventitious shoots were up to 3 mm long. Shoots continued to grow and after 6 weeks individual cultures had from 5 – 18 shoots greater than 4 mm long (Figure 3).

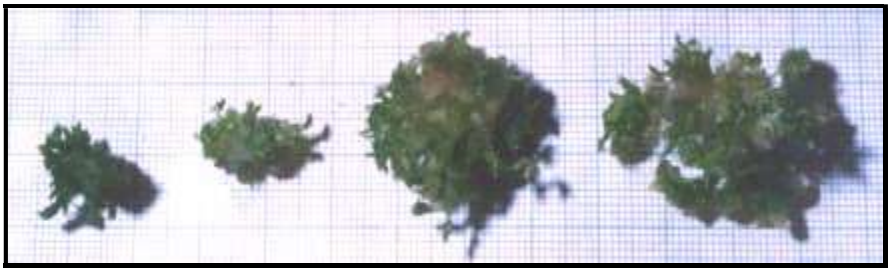

Figure 3 Adventitious shoots development during 6 weeks

The regeneration of shoots from adventitious buds situated at petiolar stubs resulted in superior shoots development in comparison with that obtained from adventitious buds situated on lamina surface (Table 2)

Table 2

Shoots development from different sites after 6 weeks

Cultivar	Site	Explants with shoots > 4 mm long (%)	Mean no. shoots >4 mm/explant
Xenia	Petiolar stubs with adventitious buds	76 ± 4	6.8 ± 2
	Surface lamina with adventitious buds	12 ± 2	3.0 ± 1.0
Cabernet 33 Vl.	Petiolar stubs with adventitious buds	38 ± 5	4.2 ± 0.8
	Surface lamina with adventitious buds	5 ± 1.2	2.0 ± 1.0

The propagation was continued by realizing successive subcultures of adventitious buds micro cuttings on fresh medium with similar composition in order to obtain a large number of shoots without morphologic and quality affected. The sprouting rate of buds was relative high during the four subcultures (92% - 72%) in case of both genotypes. The vitrification affected 7 % – 12% of the explants in the third subculture and 16% - 28% in the fourth subculture (Figure 4).

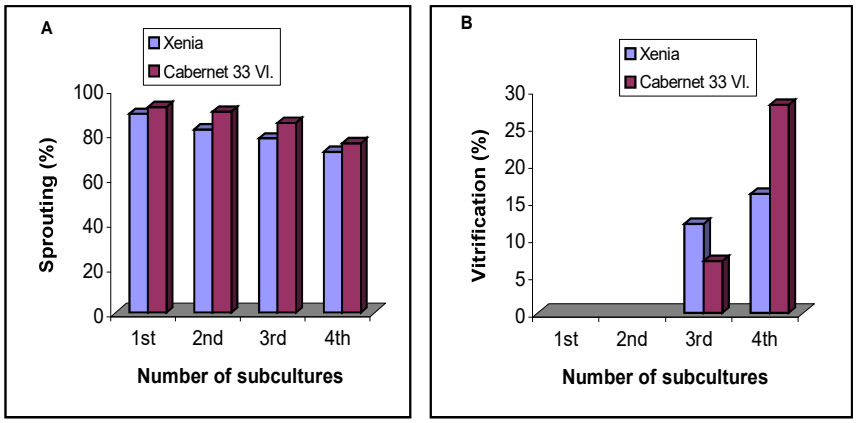

Figure 4 Percentage of sprouting (A) and vitrification (B) during four subcultures on the propagation medium

Rooting and roots growth occurred from more than 95% of adventitious shoots. After 25 – 30 days from shoots transfer into the rooting medium the plants were placed into presterilized peat pots and maintained in high humidity (90% - 95%).

When shoots reached 4 – 5 cm in height with 6 – 7 developed leaves the grapevines were acclimated to growth chamber conditions by lowering the humidity to 65 – 70%. The plants resembled normal young grapevine seedlings with spiral phyllotaxy and no tendrils (Figure 5). Root tip squashes of several regenerated plants reveled the diploid chromosome number (2n = 38). The regeneration process, from leaf to pots-grown adventitious plants occurred within 24 weeks.

Figure 5 Grapevine plants acclimated to in vivo conditions

CONCLUSIONS

Regeneretion from the petiole or on the lamina surface did not involve an intermediary callus stage. The youngest and the smallest leaves were the most organogenic at all regeneration sites. Regeneration declined with increasing leaf size. The percentage of explants forming shoots was dependent on the cultivar, the medium composition and the origin of shoots. The best regeneration occurred from explants represented by petiolar stubs with adventitious buds in case of both cultivars but, Cabernet 33 Vl. variety was less responsive than Xenia.

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