PARTICULAR REGENERATIVE ASPECTS OF THE HPD 1001 GRAPEVINE VARIETY, CULTIVATED *IN VITRO*

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Abstract

The 1001 directly producing hybrid (HPD) grapevine variety was experimented upon, cultivated in vitro from apex, detached from shoots obtained after forcing the grapevine cords in growing chamber conditions. Two basic mediums were used: MS (after Murashige-Skoog) and G (after Gamborg). On these two environments, the apex showed regeneration and differentiation of 1-2 un-rooted plantlets. Two variants with the following compositions were experimented with afterwards: ***HPDV$_1$*** *= G +0.5 mg/l TDZ (tidiazuron) +0.5 AIB (β indolil acetic acid); and* ***HPDV$_2$*** *= G+0.5mg/l TDZ + 1.0mg/l AIB. 50 days after the culturing of the apex on these mediums, the tidiazuron proved its effect on the differentiation of the callus on grapevine variety 1001. In combination with a small dose of auxine ((HPDV$_1$), a mass of callus is obtained, containing antocyans, friable, lacking plant regeneration ability. On the variant with a double dose of AIB (HPDV$_2$), callus is formed on a high percentage of apexes (over 50%), proves to be regenerative, differentiating neo-plantlets. We believe the mix of auxines in higher concentration with a smaller dose of tidiazuron (substance considered to be in the cytochinine class), favors the formation of embryoids on the callus and the differentiation of plantlets. We also believe that the inhibition of the differentiation of roots (even in the presence of an auxine) is due to the powerful cytochemical effect of the tidiazuron.*

Key words: Tidiazuron, β indolil acetic acid, apex, HPD, 1001 variety, embryogenic callus, embryoids, neo-plantlet differentiation

INTRODUCTION

Historically, there is little data on directly producing grapevine varieties (Constantinescu G., 1971). Regarding the systemization of grapevine varieties, a classification was made based on their production directions, classification which includes the directly producing hybrid grapevine varieties (HPD). This group holds the Isabella, Jacquez, Lidia, Noah, Othello(1001) etc. varieties, of which it is known that they have an American descent (Olteanu et al. 2002). At the middle of the last century, C. I Constantinescu (who endeavored to study the ancient vineyards of the Dacians) supported the removal from culture of HPDs, because of some unwanted effects they might have on the young human organism. Thus, in 1943, the "Law for the imposition and deforestation of directly producing hybrid plantations" is promulgated (Oşlobeanu, 1991). To this end, not the European Union, after the admittance of Romania, modified the Law concerning grapevine and wine nr. 244/2002, turning it into law 83/2007, which stipulates that until 2014, all HDP plantations must be eliminated.

However, in other countries (ex. France), studies are being conducted on the necessity of conserving the grapevine germoplasm, including even some varieties from the HDP group (Boursiquot, J., 1997). Recent research in the field of biotechnology concerning grapevine have shown that the HDP resistance to disease and pests can constitute valuable material for the transfer of the virus resistant to phytosanitary attack (*Pathriana and Meckenzie*, 2007; *Guţă e*t al., 2007).

The grapevine varieties were studied *in vitro,* for gene transfer, somatic embryo-genesis (*Popescu* et al. 2003), amelioration work (*Cong Linh Le*, 1987; Ionescu and Brânduşe, 1994) and the production of vineyard seedlings (Vişoiu and Teodorescu, 2001). Grapevine germoplasm collections constitute a valuable reproductive material which limits the risk which can occur with the *in vitro* storage of varieties (*Gray, and Benton, C.M.,* 1992). The micro-multiplication method of grapevine *in vitro* established the optimal hormonal balances for the grapevine varieties cultivated here (Butiuc-Keul et al., 2008; Laslo, V., et al., 2010), also establishing a micro-multiplication protocol (Mahatre and Bapat, 2007). *In vitro* propagation not only depends on the basic medium and the hormonal balance used (*Vişoiu, E*., et al., 2008), but also on the genotype, in correlation with the ambient environment (*Vişoiu, E*., et al., 2003), in the obtainment of virus-free plantlets (*Brezeanu, A*., et al., 1994).

MATERIALS AND METHODS

The 1001 variety, which we studied, belongs in the HDP group. In order to initiate the culture, we started from young shoots, obtained by forcing the growth of cords from which *apexes* were then harvested. The *in vitro* culture was initiated in the month of May, and the apexes were placed on medium variants specified in Table 1. Two basic medium types were used (MB), after Murashige-Skoog, 1962 (MS) and after Gamborg, 1968 (G). Afterwards, the Gamborg medium was used as basic medium for two variants: $HPDV_1$ and $HPDV_2$. These variants were conceived in order to stimulate the formation of callus, the implication of this substance in the formation of the embryogenic callus and the differentiation of neo-plantlets from callus (Srangsam and Kanchanapoom, 2002) being documented.

Table 1.

Culture environments used for the *in vitro* culture of HPD, 1001

Variants	Medium composition + Hormonal balance (mg/l)
Mt_1	MS (basic medium after Murashige- Skoog-MS)
Mt_2	G (basic medium after Gamborg –G)
$HPDV_1$	G + 0.5 mg/l tidiazuron (TDZ) + 0.5 mg/l AIB
$HPDV_2$	G + 0.5 mg/l tidiazuron (TDZ) + 1.0 mg/l AIB

A moderate dose of tidiazuron was used (0.5 mg/l), combined with an auxine AIB (β indolil acetic acid), in two concentrations, of 0.5 mg/l and 1 mg/l respectively, combination which proved beneficial for the initiation of callus on the 1001 (HPD) variety, but surprisingly leading to the inhibition of root system formation, an effect probably due to the powerful cytochemical effect manifested by the tidiazuron.

RESULTS AND DISCUSSION

Observations regarding the evolution of explants formed out of the apex were made 50 days after inoculation, following the formation of neo-plantules, the evolution of the root system and the initiation of callus on the Gamborg medium in the presence of tidiazuron (TDZ) and auxine AIB. Observations are listen in Table 2, based on which we can conclude that the two variants with only the basic medium (MS and G), differentiation of 1-2 rootless plantlets takes place, so we are dealing with a simple *in vitro* regeneration, the Gamborg medium proving to be superior.

Table 2

Regarding the evolution of the grapevine hybrid 1001
(after circa 50 days)

Medium	No. pl./ L (cm)	Root Sys.	Callus mass	Observations
Mt_1(MS)	1pl/2 cm	-	-	A simple regeneration (Fig. 1)
Mt_2(G)	2 pl/ 1cm			Multiplication (2 plants, one small)
$HPDV_1$	-	-	1,8-2.0 cm Ø	Uniform evolution, consistent callus differentiation, intense red color (antocyan presence (Fig. 2)
$HPDV_2$	1pl./1,5cm	-	1-1,5 cm Ø	Explants generate embryogenic callus, out of which circa 50% regenerates new, un-rooted plantlets out of this callus tissue (Fig. 3 and 4)

On the **$HPDV_1$**(G+0,5mg/l TDZ+0.5mg/l AIB) variant, after circa 50 days, the apex of the 1001 variety evolves uniformly, differentiating a callus mass which envelops the surface of the flask (Fig. 2), with a diameter of circa 1.5-2.0 cm, of red color, which requires the formation of antocyans. On the second phytohormone variety **$HPDV_2$** (G+0,5mg/l TDZ+1.0mg/l AIB), the test initially generates an embryogenic callus, with lightly colored cones (protuberances, bumps), visible, embryoids, out of which new, un-rooted grapevine plantlets shortly develop (Fig. 4). We consider the experiment of interest because it proves that TDZ behaves similarly to a cytochinine, determining the differentiation of neo-plantlets from callus, and also the stimulation of the production of antocyans on the callus mass, tissue which will constitute valuable biochemical analysis material in the future.

Fig. 1 Plant regeneration (Mt)

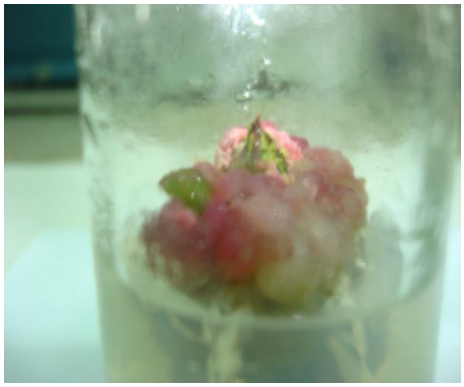

Fig. 2 Calus regeneration on $HPDV_1$

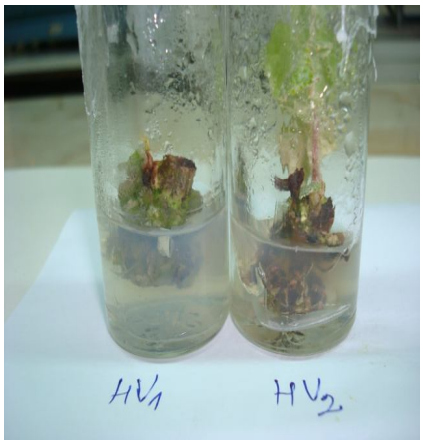

Fig. 3 Plant regeneration on $HPDV_2$ callus on $HPDV_2$

Fig. 4 Plant regenerated from

CONCLUSIONS

We can conclude that the presence of tidiazuron (TDZ) in the Gamborg basic medium has an effect on callus formation, when combined with an auxine, AIB in this case. The small auxine dose (var. $HPDV_1$) stimulates only the differentiation of red colored callus from all apical explants cultivated *in vitro*, material which will be biochemically analyzed from a pigment forming standpoint, also observing the peroxydase values, the dehydrogenases in the tissue etc. On the variant with a large concentration of AIB (1 mg/l) and TDZ (var. $HPDV_2$), embryogenetic callus is obtained in circa 50% of the tissues, from which after a further 20 days of culturing, grapevine plantlets become differentiated, lacking a root system. In order to establish rooting, mini-cuttings are severed from a node of the

resulting plantlets, which are then transfered on an MS medium with added auxine only, in slightly larger concentration.

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