

THE EVOLUTION OF THE DIRECTLY PRODUCING HYBRID GRAPEVINE VARIETY *ISABELLA*, CULTIVATED *IN VITRO*

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Abstract

The *in vitro* regenerating ability of the *Isabella* grapevine variety, directly producing hybrid, on media with cytochinine (BAP) in different concentration, combined with auxine (AIB), also in larger (3 mg/l) and smaller (0.5 mg/l) concentrations, evolves according to the hormonal balance. The nodal tissue of the HDP *Isabella* grapevine variety has inferior regenerative ability compared to other hybrid varieties and further more, this ability is absent on a medium without hormones (V_1). The highest *in vitro* node regeneration percentage (circa 85%) was recorded on the V_6 medium (MS + 2.0mg/lBAP + 2.0mg/lAIB), and the highest number of small, rooted plantlets (circa 4 plantlets / node) was obtained on the V_5 medium (MS + 2.0mg/lBAP + 1.0mg/lAIB). On the medium without hormones, on which no plantlets differentiated themselves, (V_4 = MS + 0.5mg/AIB + 3.0mg/lBAP), risogenic callus was formed, with particular evolution. We recommend the attentive future observation of the *Isabella* variety, taking into account other aspects as well, in order to precisely establish the evolution directions according to the hormonal balance of the medium. Generally *in vitro* HDP evolution can constitute a particular model, which can be compared with the evolution of the other cultivated grapevine varieties.

Key words: HDP, directly producing hybrid, *Isabella*, regenerative ability, behavior, organ genesis, callus formation.

INTRODUCTION

Our interest for directly producing hybrids was born out of the necessity to know multiple of their *in vitro* behavioral aspects and to find a way to exploit the qualities these varieties display in resisting disease and pests. There are countries which are preoccupied with the necessity of grapevine germoplasm conservation, even of varieties from the directly producing hybrid group, varieties with special biological and physiological qualities (Boursiquot, J., 1997). The grapevine variety classification, according to production direction, includes HPD varieties; *Isabella*, *Jaquez*, *Lidia* etc. varieties are known to be of American descent (Oșlobeanu et al., 1980). There is another group of HPDs, created in Romania, containing varieties such as Negru tinctorial (Tinctorial Black) and Purpuriu (Purple), also known as "old varieties" (Olteanu et al. 2002). Although both in the past (I. C. Teodorescu, 1964) and in the present (UE Law 83/2007) the mandatory deforestation of existing HDP variety areas was encouraged, we believe that the HDP group, which also includes the *Isabella* variety, which we studied, can be interesting for researchers in the biotechnology field, just like any other wild variety.

The cultivated Romanian grapevine varieties were studied *in vitro*, observing their behavior and the effect of a balanced hormonal balance on their micro-propagation (Butiuc-Keul et al., 2008; Laslo, V., et al., 2010). Romanian researchers have also observed the evolution of grapevine embryo-cultures (Ionescu et al., 2003) and the production of grapevine seedlings (Vișoiu and Teodorescu, 2001). Afterwards, the role of the genotype according to the hormonal balance and the *in vitro* maintenance of some Romanian varieties was researched (Vișoiu, 1998; Vișoiu et al. 2008). In the process of *in vitro* micro-multiplication of grapevine varieties (Gray and Benton, C.M., 1992), ideal hormonal balances were established and a micro-multiplication protocol was initiated (Mahatre and Bapat, 2007). Original studies are also known, with the goal of replacing some medium components, such as sucrose with sugar or honey (Pătru and Cachișă, 2007), with remarkable results. The biotechnology field regarding grapevine has shown that through the H.D.P. resistance to disease and pests, varieties in this group can constitute valuable research material for gene transfer and amelioration of cultivated varieties (Mhatre and Bapat, 2007; Guță et al., 2007).

MATERIALS AND METHODS

The experiment was initiated in *spring*, after obtaining juvenile vegetal material by forcing the HDP vine cords, *Isabella* variety. *Nodes* were removed from young shoots, which were cultured *in vitro* on BAP and AIB mediums, in variants listed in Table 1. The base medium was MS (Murashige-Skoog, 1962), considered a witness sample V₁; in V₂, V₃ and V₄ variants, different concentrations of BAP (1, 2 and 3 mg/l) were used, with a unique dose of AIB (0.5 mg/l), and in the case of V₅, V₆ and V₇ variants, a single concentration of cytochinine (2mg/l BAP), and varied doses of auxines (1, 2 and 3 mg/l AIB) were used. The mediums were conceived in the above-mentioned formulas in order to observe the effects of large doses combined with smaller doses of phytohormones on the *in vitro* organ genesis of the *Isabella* grapevine variety.

Table 1.

The composition of culture mediums for ISABELLA

Var.	Base medium	Medium composition (mg/l)
V ₁	MS	Witness medium - after Murashige Skoog 1962
V ₂	MS	MS + 0.5 mg/l AIB + 1.0mg/l BAP
V ₃	MS	MS + 0.5 mg/l AIB + 2.0mg/l BAP
V ₄	MS	MS + 0.5 mg/l AIB + 3.0mg/l BAP
V ₅	MS	MS + 2.0 mg/l BAP + 1.0 mg/l AIB
V ₆	MS	MS + 2.0 mg/l BAP + 2.0 mg/l AIB
V ₇	MS	MS + 2.0 mg/l BAP + 3.0 mg/l AIB

(BAP=benzylaminopurine; AIB= β indolil acetic acid)

The grapevine nodes were incubated on the medium variants listed in Table 1, after circa one month, followed by observations regarding the *in vitro* tissue behavior and its evolution direction.

RESULTS AND DISCUSSION

After 30 days of *in vitro* culturing of the nodal tissue detached from the Isabella variety, observations were made regarding: the percentage of regeneration of explant on cultivated medium variants, then the number of formed plantlets, the initiation of the root system, with additional observations regarding an eventual particular behavior of the tissue, according to case (callus genesis or risogenesis). The results of measurements and the observations regarding the evolution direction of the nodal explant are consigned to Table 2.

Table 2.

Results regarding the behavior of the ISABELLA hybrid (after 30 days)

Medium	% Regen.	Nr. pl./ L (cm)	Root system	Observations regarding the evolution direction
V ₁	0	-	-	Stationary explant
V ₂	30	1-2pl./ 4 cm l	-	Regeneration with modest root system (Photo. 1)
V ₃	35	1 pl/ 0.5 cm l	-	Necrotic base
V ₄	60	-	-	Callus, well developed roots, risogen (Photo. 2)
V ₅	65	4-5 pl/ 0.5 cm l	2rād./ 2cm l	Small plants with a callus sleeve around them (Photo. 3)
V ₆	85	1-2 pl/ 0.8 cm l	3-4rād./ 1,5 cm l	Necrotic callus at the plantlets' base, well developed root system, thick, white roots. (Photo.4)
V ₇	76	1-2 pl./ 1cm l.	4 rād./ 1,5 cm l	Regeneration and multiplication, multiple secondary roots (Photo. 5 and 6)

The regenerating ability of the HDP grapevine variety Isabella. The regenerating ability generally depends on the nature of the variety and hormonal balance of the medium, with stimulating effect to this end. In the case of the Isabella variety, regeneration was not observed on the base

medium MS (V_1), which brings us to the supposition that the variety absolutely needs stimulating substances to achieve *in vitro* regeneration. The values of the regeneration percentage are generally smaller than other grapevine varieties, but they rise above 85% on V_6 , medium with the following composition: MS+2.0mg/lBAP+2.0mg/l AIB, whereas on the other media, it sits between 30-76%, in relation to the medium composition. (Fig. 1).

Another supervised aspect is the *formation of neo-plantlets*, as a phenomenon which can be observed on almost all medium variants which contain phytohormones, except V_4 (MB+0.5mg/lAIB+3.0mg/lBAP); probably the high dose of cytochinine, combined with a moderate dose of auxine stimulates other processes (callus generation, particular root differentiation). Generally, 1 or 2 plants 0.5 – 4 cm in height were initiated, according to the hormonal balance; V_5 generates a small number of plantlets (see Table 1) with a callus sleeve around them.

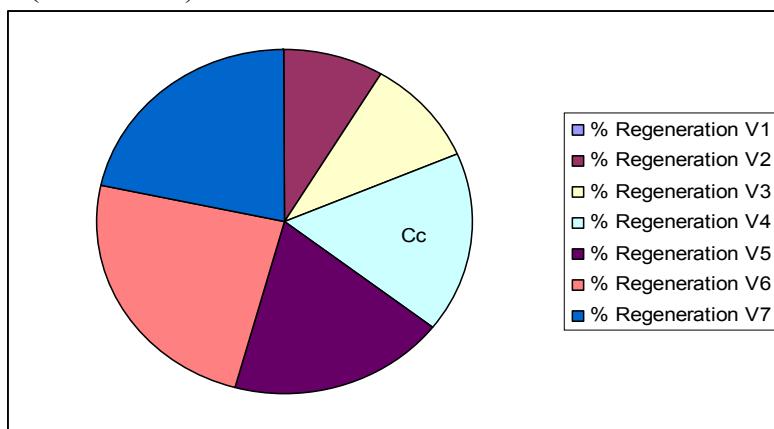


Fig. 1 Regeneration percentage for Isabella variety, after 30 days

The aspect of the *root* system is entirely unique, with the majority of the roots being thick, long and white, developed at the base of a callus tissue – hence we are dealing with risogen callus (V_4), which can generate plants given certain medium compositions. The evolution of the nodal explant of Isabella grapevine, regarding the *regeneration direction* (see Table 2), allows us to conclude that this HDP variety has a particular evolution, dependent on the hormonal balance, on the nature of the phytohormone and on its concentration. We also point out callus formation at the root system level on V_4 , well developed root system at the base of the differentiated neo-plantlets (V_6), then a very rich secondary root system, differentiated on main, thick roots (V_7), as seen in Photo 5.



Photo. 1 Variant V₂



Photo. 2 Variant V₄



Photo 3. Variant V₅



Photo 4 .Variant V₆



Photo. 5 Variant V₇



Photo. 6 Variant V₆

CONCLUSIONS

After 30 days of *in vitro* culturing, the apex of the Isabella grapevine variety regenerated plantlets (circa 30-85), on all phytohormone environments, which did not occur on the basic environment (MS), acting as a control environment. The neo-plantlet regeneration is dependent on the presence of phytohormones in the environment; the mean number of regenerated plantlets is 1-2 of circa 1 cm each, except in the case of the environment which didn't have hormones (V₁) and in the case of the environment with a high dose of cytochinine (V₄), where plantlet regeneration could not be achieved. The root system maintains the characteristics of the variety, with thick, white roots, differentiated at the base of the callus tissue; this is the so-called risogen callus. We believe that the *in vitro* behavior of the Isabella variety warrants close inspection on other types of base mediums, with different phytohormone contents.

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