IN VITRO RESPONS OF SEVERAL CULTIVARS OF *VITIS VINIFERA* L ON MEDIA WITH BALANCED PHYTOHORMONE RATIO

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Abstract: *In vitro preservation of several cultivars of Vitis vinifera L as Cabernet Sauvignon and Riesling Italian by in vitro regeneration and multiplication on MS medium supplemented with balanced phytohormone ratio and supplement of NH_4NO_3 was achieved in this work. In vitro culture was initiated from shoot tip and nodal explants. Plant regeneration, complete organogenesis and multiplication were achieved on media V_2 supplemented with BA–5.0 mg/l+IAA–0.5 mg/l and V_3 supplemented with BA-5.0 mg/l+IAA-0.5 mg/l+825mg/l NH_4NO. The media supplemented with zeatine (V_4,V_5,V_6) ensure a lower multiplication. The medium supplemented with activated charcoal (V_1) stimulated plant regeneration from shoot tip explants but not from nodal explants and the multiplication was low. The cultivar Cabernet Sauvignon showed the highest percent of regeneration (70-75%) and Riesling Italian cultivar showed only 60% regeneration. In vitro plant regeneration was obtained after 4 months, depending on the explant and medium composition. Rooting was induced only after microcutting and the transfer of microcuttings on fresh medium supplemented with IBA. Acclimatization of in vitro plantlets depends on the radiculary system, the conditions of plant protection under glass globe in the first hours after ex vitro transfer and also on the stage of organogenesis*

Key words: *shoot tip, node, Cabernet Sauvignon, Riesling Italian, regeneration, organogenesis, multiplication, acclimatization*

INTRODUCTION

Preservation of *Vitis vinifera* L species makes possible the maintenance and continuous improvement of valuable cultivars in collections from our contry. Germplasm collections provide valuable source for grapevine breeding and ensure the maintenance of cultivars in case of climate change or accidents, pathogen attack (BOURSIQUOT et al., 1997), limits these risks and preserve biological material (GRAY et al., 1992; LAROUSSE, 1995). Beside cryopreservation, *in vitro* multiplication suppose periodical subculture of explants on fresh media (MHATRE et al., 2007; VIȘOIU et al., 1989; VIȘOIU et al., 1994; VIȘOIU & TEODORESCU, 2001; VIȘOIU et al., 2008). Our country has a tradition in grapevine breeding, but during the time, several problems arissed as pathogen attack with viruses, mycoplasma, bacteria, wich have produced significant damages and even losing of several cultivars from collections (PATHRIANA et al., 2007). Thus, prophylaxis activities, analysis and selection of material for breeding are compulsory (VIȘOIU et al., 2008). *In vitro* culture of *Vitis vinifera* species was accomplished in 1961 on culture media without growth regulators by GALZY (1961), later on, HARRIS & STEVENSON (1982) suggested that supplementation of culture media with cytokins improves the multiplication (BUTIUC-KEUL et al., 2008). In vitro multiplication of grapevine depends on the culture media, the growth regulators (BUTIUC-KEUL et al., 2008; BUTIUC-KEUL et al., 2009a; BUTIUC-KEUL et al., 2009b;CRĂCIUNAȘ et al., 2009) and also on the genotype and enviromental conditions (VIȘOIU et al., 1989). *In vitro* culture of grapevine was also used for production of virus free plantlets (BANU et al., 1995), (BREZEANU et al., 1994) and somatic embryogenesis as well (BARBA et al., 1992).

The aim of our work was to study the respons of some explants of grapevine belonging to *Cabernet Sauvignon* and *Riesling Italian* cultivars, prelevated from Crăciunel-

Blaj vineyard, *in vitro* multiplication and production of virus free plantlets. *Cabernet Sauvignon* is a french cultivar highly spread in most of the countries (Larousse, 1995) and also in romanian vineyards. In Romania, there are about 11500 ha cultivated with *Cabernet Sauvignon* (OŞLOBEANU et al., 1991). *Riesling Italian* was introduced in culture in Romania because of their ecological particularities, about 20000 ha are cultivated with this cultivar (OŞLOBEANU et al., 1991) and it is used for production of sparkling wines in Transilvania and Crişana (OLTEANU, 2002).

MATERIAL AND METHODS

Plant material

Diferent explants as shoot tips and nodes were prelevated from shoots. The explants were sterilized with 0.1% $HgCl_2$ solution, 10 minutes, washed three times with sterile water and then inoculated on culture media.

Culture media and conditions

Bazal medium MS (MURASHIGE &SKOOGE, 1962), solidified with 3% agar-agar. The pH was established at 6.1 prior autoclavation at 120 °C, 1 atm, 20 min. Several growth regulator were tested in diferent combinations and concentration as it could be seen in Table 1.

Cultures were grown under 25-26 °C, at photoperiod regime of 16 h (cool-white fluorescent lights, 30 µmol s^{-1} m^{-2}). The results were evaluated after 4 months of culture. The percent of regeneration (%) depending on the culture media and the type of explant, *in vitro* organogenesis (number of plantlets and roots/explant), multiplication and several aspects of aclimatization were followed in this study.

RESULTS AND DISCUSSIONS

In vitro culture and multiplication of these cultivars of grapevine was accomplished in order to obtain valuable material for long term preservation and also for cultivar IMPROVEMENT and selection of new varieties by induction of somaclonal variations.

Table 1

Culture media used for *in vitro* culture of *Vitis vinifera* L cultivars

Variant	Bazal medium	BA mg/l	Z mg/l	IAA mg/l	Activarted charcoal (g/l)	NH_4NO_3 (mg/l)
V_o	MS	-	-	-	3,5	-
V_1	MS	1.0	-	0.5	-	-
V_2	MS	5.0	-	0.5	-	
V_3	MS	5.0	-	0.5	-	825
V_4	MS	-	1.0	0.5	-	-
V_5	MS	-	5.0	0.5	-	-
V_6	MS		5.0	0.5	-	825

(MS = Murashige – Skoog medium; BA = Benzyladenine; Z =zeatine; AIA = indoliyl acetic acid)

The percent of plant regeneration from shoot tip explant was followed after 80 days of *in vitro* culture on diferent media. The highest percent of regeneration was obtained on MS medium supplemented with 5.0 mg/l BA and 825 mg/l NH_4NO_3 (V_3). The percent of stationary explants (S), necrotic explants (N) and calus induction (C) on explants was also followed. Thus, the percent of regeneration in case of *Cabernet Sauvignon* cultivar was 48%, 20% were necrotic explants and 17% of explants generated calus with 1.2-2.0 mm in diameter (Figure 1). On the other variant of culture media, the percent of regeneration was inferior (V_2). On media supplemented with zeatine (V_4, V_5, V_6), regeneration was not obtained.

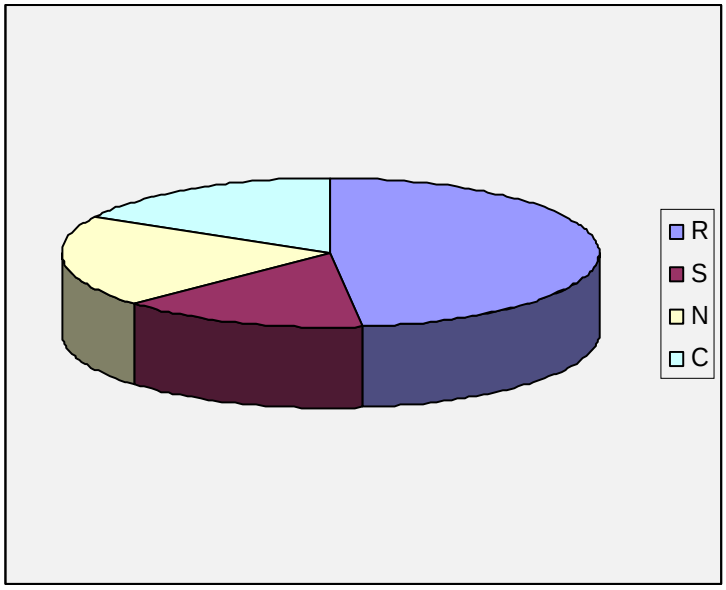

Figure 1: The percent of regeneration of grapevine shoot tip explants of *Cabernet Sauvignon*, after 2 months of *in vitro* culture (R=regeerated explants, S=stationary explants, N=necrotic explants, C=calus induction on explants)

After 2 months of *in vitro* culture, the explants of *Riesling Italian* had a weak evolution, significant evolution was observed after 4 months of culture only on media supplemented with 5.0 mg/l BA (V_2 and V_3).

In vitro organogenesis in case of *Cabernet Sauvignon* cultivar was obtained after 150 days of culture only on media supplemented with BA (V_2 and V_3), in average 5.0 -6.0 plantlets/shoot tip explant were obtained, having in average 4.0-4.5 cm length and 5-6 pairs of leaves. On the other culture media, *Cabernet Sauvignon* cultivar shows an inferior organogenesis, only 1-2 plantlets/shoot tip explant of about 1.5 cm length, on control medium. On media supplemented with zeatine, *in vitro* organogenesis is inferior even to control. It is well known that zeatine is not favourable for *in vitro* organogenesis and multiplication of grapevine (CONG, 1987).

The cultivar *Riesling Italian* shows an inferior organogenesis in comparison with *Cabernet Sauvignon* cultivar. *In vitro* organogenesis was good on medium supplemented with 5.0 mg/l BA and NH_4NO_3, in average 3.0-3.2 plantlets/shoot tip explant were obtained, having 2.0-2.5 cm length and 2-3 pairs of leaves (Figure 2).

After 150-180 days of *in vitro* culture, the multiplication of *Cabernet Sauvignon* was successfully obtained: on V_o medium, 1.8 plantlets/explant were obtained, on V_1 medium, 2 plantlets/explant were obtained, on V_2 medium, 6.5 plantlets/explant, on V_3 medium, 7.5 plantlets/explant, on V_4 medium, 1.2 plantlets/explant were obtained and on V_5 and V_6 media, calus was obtained (Figure 3).

In vitro multiplication of *Reisling Italian* was inferior in comparison to *Cabernet Sauvignon.*

In vitro cultures of grapevine were preserved 20 months, the proliferation rate being in average 3.8 shoots/explant in 6-7 weeks, eighter from shoot tip or node explants.

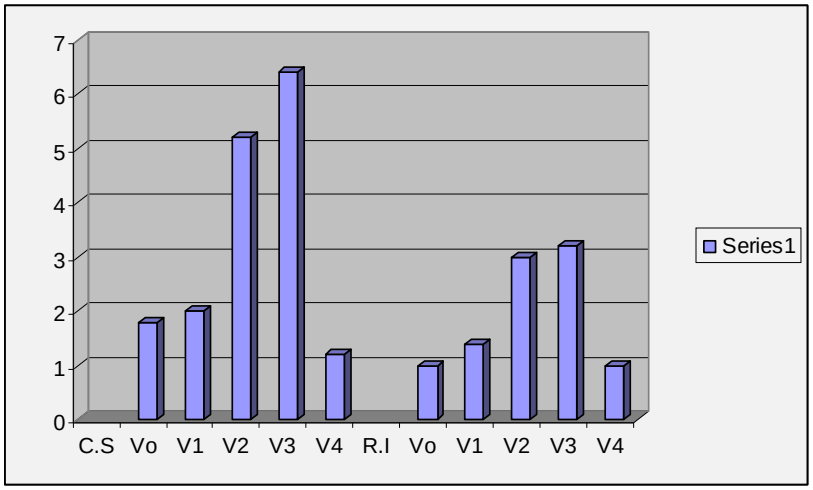

Figure 2: The plant multiplication from shoot tip explant on the two cultivars of grapevine (C.S.= *Cabernet Sauvignon*; R.I.= *Riesling Italian*).

After 4.5 months, root induction was weak, in average 1-2 roots/explant were obtained, having 2-3 cm length. After that, microcuttings from *in vitro* plantlets were transferred on fresh medium supplemented with IBA, which has favourable effect on root induction of ex vitro or *in vitro* plants. Several variants of media were tested: R_o =MB+3.0 g/l activated charcoal; R_1=MB+1.5 mg/l IBA; R_2=2.0 mg/l IBA. Even on the first 10 days, small roots were observed, but after 30 days a well formed radicular system was obtained. The percent of rooting was 90% in case of *Cabernet Sauvignon* cultivar and 69% in case o *Riesling Italian* cultivar (Figure 4.).

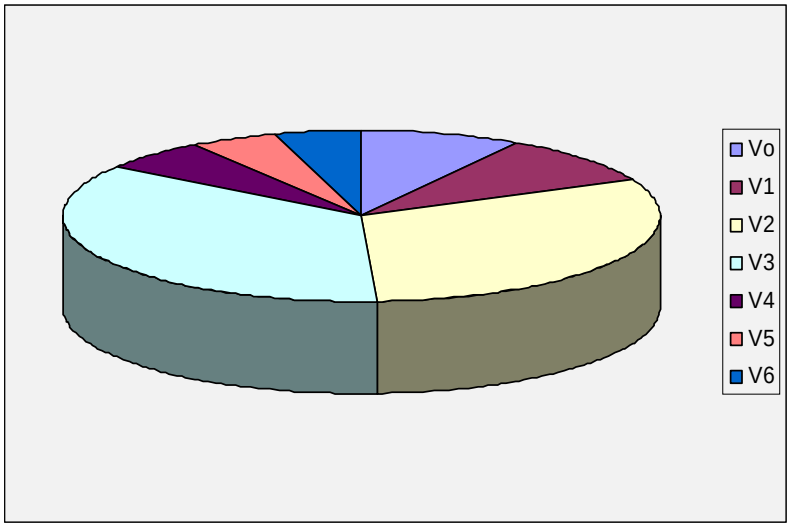

Figure 3: *In vitro* multiplication of *Cabernet Sauvignon* cultivar on diferent culture media

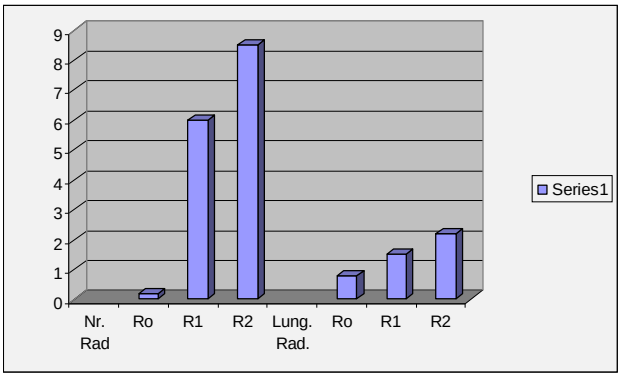

Figure 4: *In vitro* root induction on *Cabernet Sauvignon* and *Riesling Italian*, after 30 days of culture

In vitro rooting of plantlets demonstrated the role of IBA in this process, 2.0 mg/l of IBA increased the number of rooted microcuttings, the number of roots and their vigurosity. Rooting of microcuttings was obtained in the first 10 days after IBA treatment (media R_1 and R_2), wich is important requirement for acclimatization of *in vitro* plantlets.

Acclimatization of *in vitro* plants was achieved with any problems if the atmospheric and substrate humidity was continuously controled. In the frst 70 h, plantlets were covered with glass globe. The percent of acclimatization was up to 90%. *In vitro* plantlets of grapevine are very sensitive to ex vitro conditions, the succes of acclimatization depends on the reduction of light intensity, temperature and increases of humidity in the first days of acclimatization and then, the parameters could be adapted to the field conditions.

CONCLUSIONS

1. *Cabernet Sauvigon* cultivar showed high capacity of *in vitro* regeneration in comparison with *Riesling Italian* cultivar, wich showed regeneration capacity only on media supplemented with high concentration of BA and supplement of NH_4NO_3.
2. *In vitro* multiplication from shoot tip explants is higher then the multiplication from node explants.
3. *In vitro* organogenesis-caulogenesis and multiplication is higher on media supplemented with 5.0 mg/l BA, in average 6-7 neoplantlets/shot tip explant were obtained in *Cabernet Sauvignon* and only 3 neoplantlets/shoot tip explant in *Riesling Italian*.
4. The presence of BA in culure medium have favourable effect on grapevine proliferation, even in combination with supplement of NH_4NO_3.
5. Rooting of *in vitro* plantlets was achieved only after microcutting and culture of microcuttings on fresh medium supplemented with IBA in high concentration. After 30 days a well formed radiculary system was obtained.
6. The capacity of acclimatization of *in vitro* plantlets of grapevine depends on the vigurosity of radiculary system, the complet development of neoplantlets and the conditions and stages of acclimatization.

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