ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume LX 16 Number 8, 2012

THE EFFECT OF MEDIA COMPOSITION ON MULTIPLICATION OF GRAPE ROOTSTOCKS IN VITRO

B. Křižan, E. Ondrušiková, J. Moudrá

Received: September 13, 2012

Abstract

KŘIŽAN, B., ONDRUŠIKOVÁ, E., MOUDRÁ, J.: *The effect of media composition on multiplication of grape rootstocks in vitro.* Acta univ. agric. et silvic. Mendel. Brun., 2012, LX, No. 8, pp. 141–144

The current demand for in vitro cultures of grape rootstocks, not only for mass production of plants, but also for genetic engineering is evident. The study on micropropagation of grape rootstock genotypes namely Kober 5BB, Kober 125AA and Teleki 5C was performed. The aim of the study was to develop an optimized protocol to obtain large quantity of plant material. Protocol is based on regeneration via organogenesis, considering that grape embryogenic calluses are laborious to establish and the genotype of the regenerated plants can be altered.
Using of Driver and Kuniyuki Walnut media for the establishing of proliferating cultures gave better results than Murashige Skoog media in case of all used rootstocks. Subsequent cultivation on modified Murashige Skoog media with 1-naphtalene acetic acid and increased concentration of cytokynin was characterized by multiplication of cultures and formation of clusters with high multiplication capability. The clusters obtained from rootstock genotypes were suitable for mass propagation as well as for genetic transformation due to their high ability of regeneration.

Vitis, in vitro propagation, plant growth regulators, rootstocks, regeneration

Virus free (or virus tested) plants are preferred material to material with unknown phytosanitary status. Rootstock plants for *Vitis vinifera* are used not only for plantation, but also for study of resistance induced by gene manipulation. High multiplication ability of used plants in vitro conditions is required for the large-scale clonal micropropagation as well as for genetic manipulations. Micropropagation of grape cultivars was described by authors Martinez, Tizio (1989). Previous and recent works concerned to micropropagation or virus elimination of grape rootstocks present different requirements of individual rootstocks on type and concentration of plant growth regulators and also on media formulations. Media used for grape rootstocks are: MS Murashige, Skoog (1962), WPM Lloyd, McCown (1981) and B5 Gamborg *et al*., 1968. MS medium is commonly used. Alizadeh *et al.* (2010) published protocol for micropropagation of four genetically different rootstocks: Dogridge (*Vitis champini*), SO4 (*V. berlandieri* × *V. riparia*), H-144 (*V. vinifera* × *V. labrusca*) a 3309 C (*V. riparia* × *V. rupestris*) using MS media. MS medium was also used in work of authors Sajid and Ahmed, (2008) for propagation of new genotype of grape Sunder Khani. Using MS media was also recommended for propagation of four rootstock genotypes (1103 Paulsen, Ru 140, Freedom and SO4) in another work (El-Agamy *et al*., 2009). Using of MS media in this case increased average weight of plants. But cultivation on WPM media increased number and length of roots. Using of media B5 was unsuitable for rootstock propagation in this study. Bhor *et al*., 2009 used MS media with 1mg.l^{-1} BA for propagation of *Vitis champini*. It is also possible to find contradictory information from published articles. For example Lu (2005) published finding, that WPM medium is more suitable for cultivation of *Vitis thunbergii* than MS or NN (Nitsch and Nitsch, 1969). Media formulation and plant growth regulators have an important influence on multiplication of grape rootstock. Therefore were tested 3 grape rootstock used in Czech Republic, trying to optimize protocol

for large-scale clonal micropropagation to increase efficiency of propagation and thus to increase also number of plants produced.

MATERIALS AND METHODS

Plant material

Kober 5BB (*V. berlandieri* × *V. riparia*), Teleki 5C (*V. berlandieri* × *V. riparia*) and Kober 125 AA (*V. berlandieri* × *V. riparia*) were used for the culture establishment.

Mother plants cultivated in insect proof screen-house were used for collection of shoots. Mother plants were tested by ELISA and RT-PCR methods and absence of Grapevine fanleaf virus (GFLV), Grapevine fleck virus (GFkV), Arabis mosaic virus (ArMV), Grapevine *virus* A (GVA) and Grapevine leafroll associated viruses –1 and –3 (GLRaV-1 and –3) ware confirmed.

Culture establishment and growing conditions

The cultures were established from young actively growing shoots. Collected shoots were divided into one-node segments and disinfected with 0.2% $HgCl_2$ aqueous solution for 7 min and finally washed three times with sterilized distilled water for 10 min each. Nodal explants were inoculated on four media compositions: MS (Murashige Skoog, 1962), WPM Lloyd, McCown (1981), B5 Gamborg *et al.* (1968) and DKW Driver, Kuniyuki (1984). All media were supplemented with their respective vitamins, sucrose and 0.75 mg.l^{-1} BA (6-benzylaminopurine) and 0.1 mg.l^{-1} IAA (indole-3-acetic acid). The clusters of shoots that regenerated from nodal segments were subcultured by separating individual shoots after 4 weeks and transferred to fresh medium. The experiment was performed with 720 plants in total, 30 plants of each rootstock were inoculated for each media composition. Experiment was repeated twice. Cultures were incubated in culture room conditions maintained at 24 ± 2 °C with a 16/8 h photoperiod at a light intensity of 36 µmol.m^{-2}.s^{-1}. Clusters were divided after 4 weeks and transferred to fresh medium. Proliferation (no. of new shoot per explant inoculated) was recorded in the third subculture.

In the next experiment, nodal explants from cultures cultivated on MS media were used to optimize the type and the concentration of growth regulators for shoot growth and proliferation. Concentrations of plant growth regulators used in experiment were: BA (6-benzylaminopurine) 0.75, 1.0, 2.0 mg.l^{-1} in combination with NAA (α naphtalene acetic acid) 0.01, 0.05 mg.l^{-1} or with IAA (indole-3-acetic acid) 0.05, 0.1 mg.l^{-1}. In this experiment, the data were collected after one subculture (4 weeks). For the statistical analysis Duncan's multiple range test was used.

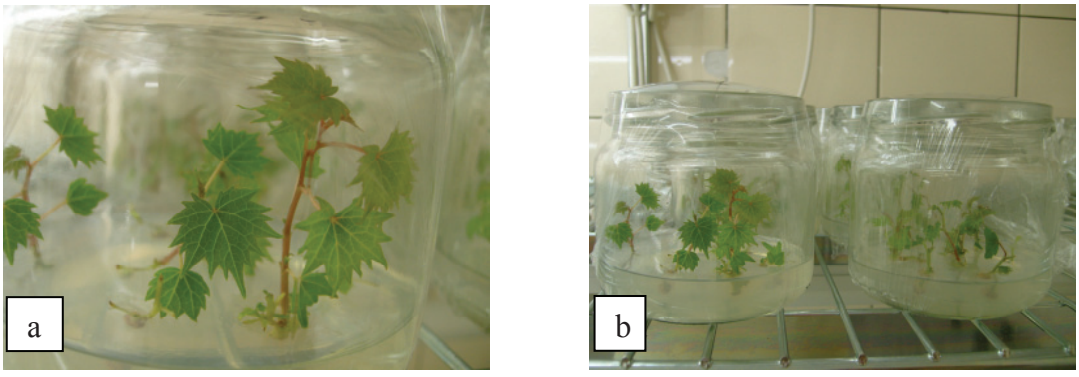
RESULTS AND DISCUSSION

The experiment with media formulation, when four different media were tested (MS, WPM, B5 and DKW), displayed strong effect on the shoot proliferation. Comparison of the four media composition (Tab. I) revealed that DKW was superior to MS, WPM and B5. Using DKW medium, the average number of new shoots produced per explant was 2.33 and was significantly higher than that using other media (Tab. I). On MS media and WPM media, the average numbers of new shoots produced per explant were 1.39 and 1.35 respectively. The lower average number of new shoots produced per explant 1.09 showed plants cultivated on B5. During the experiment neither callus formation nor hyperhydricity was observed.

I: *Effect of media composition on number of new shoots per explant after 28 days of culture*

Media composition	Kober 5 BB	Kober 125 AA	Teleki 5C
MS	1,28	1,35	1,55
DKW	2,14	3,26	2,45
WPM	1,32	1,41	1,25
B5	1,12	1,5	1,32

Although we found no data about using DKW media for grape rootstock in literature, this medium shoved the best results for establishing cultures and multiplication in our experiments. In case of other media except DKW we confirm results obtained by El-Agamy *et al.*, 2009. Also in their work using MS and WPM was beneficial than using media B5



1: *Kober 5BB (a – detail of plant cultivated on MS, b – plants grown in vitro, 4 weeks after establishing cultures)*

for cultivation of four grape rootstocks. Different requirements to media formulation could be also related to genotype. In vitro multiplication of shoots was strongly influenced by the type and concentration of plant growth regulators (PGR) used. PGR-free medium was the least effective medium for shoot proliferation as compared with all other PGR-supplemented media. Use of 2.0 mg.l^{-1} BA resulted in production of on an average 3.62 shoots per explant inoculated. However, the same concentration of BA with 0.01 NAA helped in produced 8.77 new shoots, which is significantly higher, than that produced by using BA separately or in combination with IAA (Tab. II).

II: *The effect of PGRs (in mg.l^{-1}) on shoots proliferation after 28 days of culture. (x data not evaluated, due to callus production and insufficient replication in experiment)*

BA	IAA	NAA	Kober 5 BB	Kober 125 AA	Teleki 5C
0.00	0.00	0.00	0.53	0.65	0.84
0.75			1.28	1.38	1.55
1.00			2.14	1.85	1.77
2.00			4.70	3.25	2.18
1.00		0.01	2.28	2.04	1.76
2.00		0.01	7.70	12.27	8.21
1.00		0.05	5.26	6.12	3.45
2.00		0.05	x	x	x
1.00	0.05		2.18	2.64	1.93
2.00	0.05		3.92	3.32	2.15
1.00	0.1		1.32	1.81	1.64

While, use of 0.75 mg.l^{-1} BA, 1.0 mg.l^{-1} BA + 0.01 mg.l^{-1} NAA and 1.0 mg.l^{-1} BA + 0.05 mg.l^{-1} NAA medium did not show any significantly different effect of production new shots. Concentration of 2.0 mg.l^{-1} BA together with 0.01 NAA induced the higher number of new shoots in comparison with other concentrations. This PGRs combination was found to be optimum for shoot propagation of rootstocks tested. Using of BA (in concentrations 1.0 mg.l^{-1} and 2.0 mg.l^{-1}) and BA in combination with 0.1 IAA evoke hyperhydricity effect on plants. Strong callus formation was observed in plants cultivated on 2.0 mg.l^{-1} BA + 0.05 mg.l^{-1} NAA. Obtained results confirm the information published earlier. El-Agamy *et al*., 2009 used for propagation of four rootstock genotypes BA in concentration 2.0 mg.l^{-1}. Bhor *et al*., 2009 used MS media with lower concentration of BA (1mg.l^{-1}) for propagation of *Vitis champini*. In another work, authors found that hyperhydricity effect could be reduced by reducing BA concentration in medium from 2 to 1 mg.l^{-1} (Heloir *et al*., 1997). In our work the hyperhydricity effect depend on type of auxin used for cultivation. In case of using 0.01 mg.l^{-1} NAA, no hyperhydric plant was found even when 2.0 mg.l^{-1} BA was used. Authors Alizadeh *et al.* (2010) published protocol for micropropagation of four genetically different rootstocks: Dogridge (*Vitis champini*), SO4 (*V. berlandieri* × *V. riparia*), H-144 (*V. vinifera* × *V. labrusca*) a 3309 C (*V. riparia* × *V. rupestris*). They recommended using of MS medium with 2.0 mg.l^{-1} BA and 0.2 mg.l^{-1} NAA, to enhance the culture establishment and reducing time to grow. We did not confirm information that B5 media is suitable for grape multiplication as published by Lu (2005).

CONCLUSION

The experiment with media formulation, when four different media were tested (MS, WPM, B5 and DKW) displayed strong effect on the shoot proliferation. Using DKW medium, the average number of new shoots produced per explant was significantly higher than in the case of other media. Subsequent cultivation on modified MS media with 0.01 mg.l^{-1} NAA and 2.0 mg.l^{-1} BA was characterized by multiplication of cultures and formation of clusters with high multiplication capability. This PGRs combination was found to be optimum for shoot propagation of rootstocks tested and the average number of new shoots produced per explant was significantly higher than that produced by using BA separately or in combination with IAA. Using of BA separately in both concentrations) and also in combination with 0.1 IAA caused hyperhydricity effect on plants. Strong callus formation was observed in plants cultivated on 2.0 mg.l^{-1} BA + 0.05 mg.l^{-1} NAA.

SUMMARY

The present study describes the in vitro propagation system for three rootstocks of grape with a high shoot propagation rate. Using of Driver and Kuniyuki Walnut media for the establishing of proliferating cultures gave better results than Murashige Skoog media in case of all used rootstocks. Subsequent cultivation on modified Murashige Skoog media with 0.01 mg.l^{-1} NAA and 2.0 mg.l^{-1} BA was characterized by multiplication of cultures and formation of clusters with high multiplication capability (8.77 new shoots per explant in average). The protocol described here could be used for large-scale clonal micropropagation of tested rootstocks suitable for Czech Republic conditions.

Acknowledgement

The financial support of the project NAZV QH91214 of the Czech Ministry of Agriculture is gratefully acknowledged.

REFERENCES

ALIZADEH, M., SINGH, S. K., PATEL, V. B., 2010: Comparative performance of in vitro multiplication in four grape (*Vitis* spp.) rootstock genotypes. International Journal of Plant Production 4 (1), pp. 41–50.

BHOR, R. P., AHIRE, D. B., BAN, Y. G., BORSE, S. N. 2009: Study on micropropagation in grape (*Vitis champini*). Ecology, Environment and Conservation 15 (1), pp. 41–44.

CELIK, H., SÖYLEMEZOGLU, G., ERTUNC, F., CAKIR, A., DURSUNOGLU, S., AKBAS, B., 2009: Clonal micropropagation of main grape and rootstock varieties of Turkish viticulture for obtaining virus-free basic nursery stocks. Acta Horticulturae 827, pp. 421–424.

DRIVER, J. A., KUNIYUKI, A. H., 1984: In vitro propagation of paradox walnut rootstock. Hort. Sci. 19: 507–709.

EL-AGAMY, S. Z., EL-MAHDY, T. K., MOHAMED, A. A., 2009: In vitro propagation of some grape rootstocks. Acta Horticulturae 839, pp. 125–131.

GAMBORG, O. L., MILLER, A., OJIMA, K., 1968: Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. 50, 151–158.

HELOIR, M. C., FOURNIOUX, J. C., OZIOL, L., BESSIS, R., 1997: An improved procedure for the propagation in vitro of grapevine (*Vitis vinifera*) using axillary bud microcuttings. Plant Cell Tissue Org. Cult. 49, 223–225.

LLOYD G., MCCOWN B., 1981: Commercially-feasible micro-propagation of Mountain laurel, *Kalmia latifolia*, by use of shoot tip culture. Int. Plant Prop. Soc. Proc. 30: 421–427.

LU, M. C., 2005: Micropropagation of Vitis thunbergii Sieb. et Zucc., a medicinal herb, through high-frequency shoot tip culture. Scientia Horticulturae 107: 64–69.

MARTINEZ, E. A., TIZIO, R., 1989: Grapevine micropropagation through shoot tips and minicuttings from in vitro cultured one-node cuttings. HortScience 24(3): 513.

MURASHIGE T., SKOOG F., 1962: A revised medium for rapid growth and bio-assays with tobaco tissue cultures. Physiol. Plant. 15: 473–497.

NITSCH, J. P., NITSCH, C., 1969: Haploid plants from pollen grains. Science 163, 85–87.

SAJID, G. M., AHMED, Z., 2008: Evaluation of various levels of mineral nutrients and plant growth regulators for In vitro culture of grape. Pakistan Journal of Botany 40 (1), pp. 329–336.

Address

Ing. Břetislav Křižan, Ph.D., Ing. Eva Ondrušiková, CSc., Ing. Jana Moudrá, Mendeleum – Ústav genetiky, Mendelova univerzita v Brně, Valtická 334, 691 44 Lednice, Česká republika, e-mail: bretislav.krizan@seznam.cz