Romanian Biotechnological Letters *Vol. 15, No.3, 2010*
Copyright © 2010 University of Bucharest Printed in Romania. All rights reserved
ORIGINAL PAPER

Effect of some growth regulators on "in vitro" culture of two *Vitis vinifera* L. cultivars

Received for publicationAugust 29, 2009
Accepted, May 10, 2010

M.A. AAZAMI[1]
[1]*Department of Horticulture, Faculty of Agriculture, University of Maragheh, Maragheh 55181-83111, Iran, Email: Aazami58@gmail.com*

Abstract

Shoot apical meristems were used for in vitro regeneration in 2 grape cultivars -"Soltanin" and "Sahebi". Meristems were taken from 10 cm length shoots. The culture media used in this study was MS (Murashige & Skoog, 1962), with hormonal supplements consisting of: A (1 mgL^{-1} BA), B (1.5 mgL^{-1} BA), C (1 mgL^{-1} IBA+ 1.5 mgL^{-1} BA) and D (1 mgL^{-1} TDZ). Results indicated that B and C variants of culture media produced the highest average number of shoots per cultured apex (3.8-5.4). Consequently, the shoots were twice subcultured on a medium variant supplemented with 1 mg/L TDZ+1.5 mg/L GA. The "in vitro" derived shoots were pretreated with 1 mgL^{-1} IBA, and then directly potted, which caused significant enhancement in root number per shoot. The good rooting achieved under "in vivo" conditions resulted in the reduction of the time spent for vitroplant acclimatization compared with "in vitro" rooting and, therefore, the time needed for plantlet regeneration was shortened. There was no significant difference between the two cultivars regarding the measured traits.

Keywords: *Vitis vinifera*, BA, IBA, TDZ, meristems, "in vitro" culture

Introduction

Grapevine (*Vitis vinifera* L.) is one of the most widely grown fruit crops in the world [1]. Genetic improvement of the classic cultivars in order to obtain high quality wine and table grape varieties through conventional hybridization methods does not appears to be practical and therefore non-conventional approaches have been proposed [1,2,3]. A prerequisite to develop such approaches for grapevine is the ability to perform efficient "in vitro" regeneration techniques [1], but in some instances the genetic variability of the material has to be taken into account [2,3,4]. Micropropagation in grapevine was first performed by "in vitro" culture of micro-cuttings [5] but more recently introduction of bud proliferation has been shown to provide an alternative pathway to grapevine micropropagation [6,7]. Micropropagation represents an efficient method of plant regeneration and rapid propagation through organogenesis and embryogenesis of any valuable genotype obtained by non-conventional methods [2] and therefore regeneration of whole plants by somatic embryogenesis and organogenesis has been intensively studied [3,8]. According to some authors, combinations of different cytokinins such as, thidiazuron and kinetin were more effective for establishing morphogenetic cultures in some *V.vinifera* cultivars whereas BA was the best in *V.rotundifolia* [9]. Lee & Wetzstein [7] obtained up to 250 shoots per axillary bud in the cultivar 'Summit' by maintaining the undivided tissue mass through 16 weeks of culture and produced greenhouse acclimated potted plants following "in vitro" rooting. Thus, the objective of this experiment was to study for the first time the regeneration potential of "in vitro" cultivated meristems and in vivo/in vitro rooting and potting capacity in two major Iranian grapevine cultivars, "Soltanin'" and "Sahebi".

Materials and methods

Plant materials: Cuttings obtained from 10-15 years-old vines belonging to the "Soltanin" and "Sahebi" grape cultivars were forced in greenhouse and 10 cm elongated shoots were used as explant sources. The shoot segments with apical and axillary buds were surface-disinfected for 15 min by continuous agitation in 5% commercial bleach and the buds were aseptically dissected further more to remove all extraneous leaves except for the small appendages directly enclosing the shoot apical meristem.

Morphogenetic cultures initiation and establishment: Shoot tips were transferred to the initiation culture medium variants, consisting of MS [10] basal medium supplemented with 30 gL^{-1} sucrose, 6 gL^{-1} agar and with four hormone types and combinations: A [1mgL^{-1} BA (6-benzylaminopurine)], B (1.5mgL^{-1} BA), C [1mgL^{-1} IBA (indole-3-butyric acid) + 1.5mgL^{-1} BA] and D [1mgL^{-1} TDZ (Thidiazuron]. After 2-4 weeks, the resulted number of shoots produced per meristem was counted. Following multiple shoot proliferation, the cultures were transferred to fresh culture media for shoot elongation and further growth. The resulting shoots were twice subcultured on MS medium supplemented with 1mgL^{-1} TDZ+ 1.5mgL^{-1} GA_3 (gibberellic acid). Finally the shoots were detached and transferred to the rooting media.

Rooting stage: The shoots obtained from B and C culture media were tested for rooting capacity due to the high regeneration potential of these variants compared with A and D. For "in vitro" rooting the shoots were cultured in MS medium supplemented with 0.5mgL^{-1} NAA (α – naphthalene-acetic acid).

"In vitro" derived rooted plantlets were acclimatized under controlled environment, especially high relative humidity conditions. Rooting percentage and root length were determined after 4 weeks.

"In vitro" derived shoots, excised and pretreated with 1mgL^{-1} IBA, were directly potted in potting mix (2/3 Peat + 1/3 Perlite). The pots were covered with a plastic house inside greenhouse under misting system for 4 weeks. Afterward, the rooted plantlets were transferred to the ambient greenhouse conditions without plastic cover and mist system.

Data analysis: Data were analyzed using SAS software (Ver. 8.02) and means differentiated by Duncan's multiple range tests at 0.05% probability level.

Results

Shoot production on MS medium supplemented with the four different hormone types and combinations was evaluated. Mean shoot number of 3.8-5.4 per apex was obtained on B and C variants of culture media. There was no significant difference regarding shoot production between the two cultivars. However "Soltanin" showed slightly better shoot production compared with "Sahebi" (Table 1). The study of the effects of the four hormonal treatments on the shoot production in the 2 cultivars showed that BA at 1.5mgL^{-1} concentration was the best for shoot proliferation in both 'Soltanin' and 'Sahebi' cultivars. (Table 1).

Table1. Effects of different hormonal treatments on "in vitro" shoot production in the 2 grape cultivars.

Factors		Shoots per apex
Culture medium	A	1.6d
	B	3.8b
	C	5.4a
	D	2.5c
Cultivar	'Soltanin'	4.1a
	'Sahebi'	3.8a
Culture medium (CM)		**
Cultivar (Cv)		NS
CM× Cv		*

A (1 mgL^{-1} BA), B (1.5 mgL^{-1} BA),
C (1 mgL^{-1} IBA+ 1.5 mgL^{-1} BA) and D (1 mgL^{-1} TDZ)
** Significant at $p\geq0.01$; * Significant at $p\geq0.05$; NS: Non significant

The shoots produced on TDZ-containing medium were stunted and distorted compared with those on BA variants. Before rooting, the shoots were maintained for 2 subcultures on the culture medium variant supplemented by 1 mgL^{-1} TDZ+1.5 mgL^{-1} GA. For "in vivo" rooting, the elongated shoots were detached, pretreated with 1mgL^{-1} of IBA, and then were potted in the potting mix.
Rooting was also performed under "in vitro" condition. In this case the shoots were harvested and transferred on MS medium containing 0.5 mgL^{-1} NAA. The mean rooting response of the two cultivars was 54% and 86% for "in vivo" and "in vitro" conditions, respectively (Table2).

Table2. "In vivo" and "in vitro" rooting of grape shoots.

		Rooting (%)	Root length (mm)
In-vivo	'Soltanin'	65b	22a
	'Sahebi'	53c	18bc
In-vitro	'Soltanin'	92a	19b
	'Sahebi'	81ab	13c
Culture kind (CK)		**	**
Cultivar (Cv)		*	**
CK× Cv		NS	*

** Significant at p≥0.01; * Significant at p≥0.05; NS: Non significant

Rooting percentage and root length of both "in vivo" and "in vitro" conditions were significant (p≥0.01) for both cultivars (Table2), with "in vitro" rooting percentage being significantly higher than that of "in vivo" condition. As regards the root length that obtained from "in vitro" derived shoots was significantly lower than that of "in vivo" ones. More than that, the time needed for plantlet acclimatization under "in vivo" root production system was significantly lower compared with that of "in vitro" condition, and therefore the overall plant production time was shortened.

Discussion

Apical meristems contain less endophytic contamination and they are most vigorous during initial rapid growth [11]. Support for this hypothesis comes from our preliminary experiments with some slow growing, potted grapes, where "in vitro" proliferation of shoot tip cultures was difficult [7]. Our methodology is based on the reports of other authors about using apical meristems as explants for "in vitro" grapevine culture establishment [12,13,14,15], and differs from the protocol described by Lee & Wetzstein [7], where axillary buds were used as initial explants.. The shoots developed within 2-4 weeks and the average number of shoots per apex was 3.8-5.4. The shoots produced on B and C culture media had a normal morphology compared to those from other variants. In a series of previous studies BA has been used for micropropagation in Muscadinia and Euvitis species, hybrids and cultivars [12, 16, 17]. In the present experiment the BA treatment produced rooted shoots with significantly more roots per shoot compared with others but percentage of rooted shoots and average root length were unaffected.

"In vitro" rooting studies demonstrated that medium with 1mgL^{-1} NAA significantly increased percentage of rooted shoots, but root lengths were smaller compared with "in vivo" conditions. The stimulatory effects of NAA on adventitious rooting of "in vitro" produced grape shoots have been previously described [13, 18, 19]. In *V. vinifera* cv. Perlette up to 95% rooting of micro-cuttings were obtained on MS medium supplemented with IBA and NAA [19,20,21]. In our experiment the best shoot proliferation medium (MS with 1.5 mgL^{-1} BA) resulted in shoots that were successfully rooted "in vivo", and subsequently decreased plantlet acclimatization period. Similar to our results an optimum rate of shoot proliferation

was also reported in different grape cultivars by other researchers [22, 23]. However, following our studies, a clonal propagation protocol, characterized by higher regeneration efficiency, was simply achieved by “in vitro” manipulations.

References

1. S. MEDEROS-MOLINA, *Culture medium requirements for micropropagation of Vitis vinifera L. cv. Listan Blanco.* Acta Hort., 754 (1), 265-271 (2007).
2. J.A. STAMP, S.M. COLBY, C.P. MEREDITH, *Direct shoot organogenesis and plant regeneration from leaves of grape (Vitis spp.).* Plant Cell Tiss. Org. Cult., 22, 127-133 (1990).
3. L. TORREGROSA, A. BOUQUET, *Adventitious bud formation and shoot development from in vitro leaves of Vitis×Muscadinia hybrids.* Plant Cell Tiss. Org. Cult., 45, 245-252 (1996).
4. P. THOMAS, G.S. PRAKASH, *Sanitizing long-term micropropagated grapes from covert and endophytic bacteria and preliminary field testing of plants after 8 years in vitro.* In Vitro Cell. Deve. Bio.-Plant, 40 (6), 603-607 (2004).
5. S.G. TANGOLAR, K. ERCIK, S. TANGOLAR, *Obtaining plants using in vitro micrografting method in some grapevine varieties (Vitis vinifera L.).* Biotechnol. & Biotechnol. Eq., 17 (2), 50-55 (2003).
6. R. JONA, K.J. WEBB, *Callus and axillary-bud culture of Vitis vinifera ‘Sylvaner Riesling’.* Sci. Hort., 9, 55-60 (1978).
7. N. LEE, H.Y. WETZSTEIN, *In vitro propagation of muscadine grape by axillary shoot proliferation.* J. Amer. Soc. Hort. Sci., 115, 324-329 (1990).
8. L. MEI-CHUN, *Micropropagation of Vitis thunbergii Sieb. Et Zucc., a medicinal herb, through high-frequency shoot tip culture.* Sci. Hort., 107 (1), 64-69 (2005).
9. R. SUDARSONO, R.G. GOLDY, *Effect of some growth regulators on in vitro culture of three Vitis rotundifolia cultivars.* HortScience, 23,757 (1988).
10. T. MURASHIGE, F. SKOOG, *A revised medium for rapid growth and bioassays with tobacco tissue cultures.* Physiol. Plant., 15, 473-497 (1962).
11. M.J. JASKANI, H. ABBAS, R. SULTANA, M.M. KHAN, M. QASIM, I.A. KHAN, *Effect of growth hormones on micropropagation of Vitis vinifera L. cv. Perlette.* Pak. J. Bot., 40 (1), 105-109 (2008).
12. D.J. GRAY, L.C. FISHER, *In vitro shoot propagation of grape species, hybrids and cultivars.* Proc. Fla. State Hort. Soc., 98, 172-174 (1985).
13. D.J. GRAY, C.M. KLEIN, *In vitro shoot micropropagation and plant establishment of 'Orlando Seedless' grape and 'Tampa' rootstock.* Proc. Fla. State Hort. Soc., 100, 308- 309 (1987).
14. D.J. GRAY C.M. KLEIN, *In vitro micropropagation and plant establishment of 'Blanc du Bois' grape.* Proc. Fla. State. Hort. Soc., 102,221-223 (1989).
15. S.A. SALAMI, A. EBADI, Z. ZAMANI, *Commercial mass clonal propagation of grapevine cv. ‘Bidaneh sefid’; in vitro fragmented shoot apex culture of an elite germplasm.* Acta Hort., 829 (1), 88-94 (2009).
16. D.S. HAN, Y. NIIMI, J.Y. WU, *Micropropagation of Vitis amurensis Rupr.; improved control.* Vitis, 42 (3), 163-164 (2003).
17. N. ALASANIA, S. MANJGLADZE, N. LOMTATIDZE, S. TSKVITINIDZE, *Effect of different growth media on in vitro propagation of grapevine cultivar ‘Chkhaveri’.* Bull. Georg. Natl. Acad. Sci., 175 (3), 94-96 (2007).
18. R. CHEE, R.M. POOL, D.A. Bucher, *Method for large scale in vitro propagation of Vitis.* New York Food Life Sci. Bul. 109:1-9 (1984).
19. M.S. BARRETO, A. NOOKARAJU, Effect *of auxin types on in vitro and ex vitro rooting and acclimatization of grapevine as influenced by substrates.* India J. Hort., 64 (1), 11-17 (2007).
20. V.T. LEWANDOWSKI, Rooting *and acclimatization of micropropagated Vitis labrusca Delaware.* HortScience, 26, 586-589 (1991).
21. A.L. BUTIUC-KEUL, A. COSTE, A. HALMAGYI, DELIU, M. FARAGO, M. ILIESCU, R. LUORAS, *In vitro micropropagation of several grapevine cultivars from Romania*. Acta Hort., 812 (1), 133-138 (2009).
22. M. BARLASS, K.G.M. SKENE, *In vitro propagation of grapevine (Vitis vinifera L.) from fragmented shoot apices.* Vitis, 17, 335-340 (1978).
23. S. NAMLI, F. ADIYAMAN, E. AYAZ, *Comparative studies on the proliferation of lateral buds of Vitis vinifera L. cv. Cardinal during different periods of six months of the year at in vitro condition.* Int. J. Agri. Biol., 9 (1), 38-40 (2007).