Meristem culture for clonal micropropagation of grapevines

H. ÇELIK and M. BATUR

Department of Horticulture, Faculty of Agriculture, University of Ankara, 06110 Ankara, Turkey

Summary: Experiments were carried out to determine the effects of MS medium concentrations (4/4 MS, 3/4 MS, 2/4 MS) combined with GA_3 at 0.0, 0.1 and 0.5 mg/l, and vitamin formulations (MS, Morel, thiamine-inositol) on success of meristem culture and of 15 auxin (IBA) x cytokinin (BAP) combinations on shoot and root formation for clonal micropropagation of the wine grape cv. Kalecik Karasi and the rootstock cv. 41 B M.G.

Best results were obtained from standard MS mineral composition and vitamin formulation combined with 2.5 mg/l BAP and 0.5 mg/l GA_3 for primary meristem cultures of both genotypes; 0.0 mg/l IBA x 1.0 mg/l BAP for shoot subculture and 1.0 mg/l IBA x 0.0 mg/l BAP for root subculture of Kalecik Karasi; 0.0 mg/l IBA x 1.0 mg/l BAP for shoot subculture and 5.0 mg/l IBA x 0.0 mg/l BAP for root subculture of 41 B, considering shoot, explant, root, callus and particularly entire plant formation in the cultures of both stages.

Key words: tissue culture, micro-propagation, growth, meristem, root, shoot, culture medium, vitamin, growth regulator.

Introduction

Meristem-tip culture has been widely used in clonal micro-propagation of higher plants during the last three decades. The most important application of meristem culture is in the production of virus-free plants, rapid multiplication and also in the long-term storage of such virus-free clonal germplasm through cryopreservation techniques (QUAK 1977; KARTHA 1981).

Although the theoretical aspects of virus eradication from plants by meristem-tip culture is not clear yet, ever since the pioneering work of LIMASSET and CORNUET (1949) this technique has been the most efficient method of eliminating viral pathogens from crop plants.

Several workers reported that grapevines have been fully freed of certain wide-spread viral pathogens such as fan leaf, leaf roll and yellow speckle, either by meristem culture alone or meristem culture in combination with thermotherapy (BARLASS *et al.* 1982; GRENAN 1984; BLAICH 1985).

In the past decade, numerous studies were carried out to explore the reactions of *Vitis* species to meristem culture for the above-mentioned purposes (FAVRE 1978; BARLASS and SKENE 1980, 1981, 1982; CHÉE and POOL 1983, 1985; GRAY and FISHER 1987; FANIZZA 1987; ALLEWELDT and HARST-LANGENBUCHER 1987).

Materials and methods

As the basic plant material, apical meristems that are 0.5 mm in length with two or three leaf primordia, isolated from growing tips of current shoots of Kalecik Karasi (*V. vinifera* L.) which is a superior red wine variety, and 41 B M.G. (Chasselas x *V. berlandieri*) which is a difficult-to-root and lime resistant rootstock variety, were used in the experiments which were carried out in three consecutive steps:

Experiment I: Effects of various concentrations (4/4 MS, 3/4 MS, 2/4 MS) of agar-solidified (0.8 %) standard MURASHIGE and SKOOG (1962) medium supplemented with sucrose (3.0 %) and BAP (2.5 mg/l) (CHÉE and POOL 1985) with three combinations of Ga_3 at 0.0, 0.1 and 0.5 mg/l on success of the meristem cultures in establishment medium.

Table 1: Effects of MS concentrations, GA_3 and vitamin formulations on success of the cultures in establishment medium

MS	GA_3 (mg/l)	Survival rate (%) K.Karasi	Survival rate (%) 41 B	Growth (%) K.Karasi	Growth (%) 41 B
	0.0	57.1	71.4	21.4	3.6
4/4	0.1	53.6	54.3	17.9	37.1
	0.5	75.0	67.9	64.3	53.6
	0.0	67.9	51.4	42.9	17.1
3/4	0.1	71.4	64.3	25.0	7.1
	0.5	60.7	28.6	32.1	17.9
	0.0	67.9	64.3	32.1	3.6
2/4	0.1	53.6	39.3	39.3	-
	0.5	57.1	20.0	50.0	-
	Vit. Formul.				
	MS Vit.	100.0	92.8	100.0	83.3
4/4	Morel Vit.	100.0	72.5	100.0	60.7
	Thia.-Mes.In.	100.0	93.8	100.0	79.6

Experiment II: Effects of three vitamin formulations (MS vitamin, Morel vitamin and thiamine + meso-inositol) supplemented in standard MS medium on success of the meristem cultures in establishment stage.

Experiment III: Effects of 15 auxin (IBA) x cytokinin (BAP) combinations in the above-mentioned MS medium supplemented with 0.5 mg/l GA_3, designed with a predominance of BAP in shoot proliferation medium and IBA in rooting medium, on shoot and root formation and development of the cultures.

Cultures were incubated at constant temperature of 25 °C, 16 h photoperiod (4000 lux) and 70 % relative humidity during establishment (3 weeks), shoot proliferation and rooting subcultures (5 weeks in each stage) (WETHERELL 1982; HARRIS and STEVENSON 1982).

Results and discussion

Experiment I

Highest survival rates of the meristem cultures for both Kalecik Karasi (75.0 %) and 41 B M.G. (67.9 %) were obtained from 4/4 MS x 0.5 mg/l GA_3. Similarly, the same combination also gave better results in the development of survival meristems for both genotypes (Table 1, Fig. 1).

Experiment II

As shown in Table 1, all meristems of Kalecik Karasi which were cultured in standard MS medium with the combination of the three vitamin formulations were still alive. However, survival rates of 41 B meristems were above 90.0 % in MS vitamin and thiamine + meso-inositol, but 72.5 % in Morel vitamin. The rates of growing meristems are also similar to that survival data.

Experiment III

For both genotypes, 0.0 mg/l IBA x 1.0 mg/l BAP and 0.5 mg/l IBA x 1.0 mg/l BAP combinations gave better shoot proliferation and development in shoot medium (Table 2, Fig. 2). On the other hand, satisfactory rooting rates (above 50.0 %) were obtained with those combinations in Kalecik Karasi. The combination containing only BAP at 1.0 mg/l also produces entire plants at the rate of 42.9 % in the same genotype. These data are in agreement with the reports of SKENE and BARLASS (1980), HARRIS and STEVENSON (1982), MASAHIKO *et al.* (1982). Although the data concerning shoot development in 41 B were generally similar to those of Kalecik Karasi, as a result of inefficient rooting, an entire plant production rate of 18.8 % was only achieved in the combination containing 0.5 mg/l IBA and 0.5 mg/l BAP.

Results on rooting and entire plant formation in rooting medium were found to be rather different according to the genotypes. Highest root formation was obtained in the combinations of 1.0 mg/l IBA x 0.0 mg/l BAP (86.7 %) and 1.0 mg/l IBA x 0.5 mg/l BAP (85.7 %) for Kalecik Karasi; 5.0 mg/l IBA x 0.0 mg/l BAP (100.0 %), followed by 2.5 mg/l IBA x 0.0 mg/l BAP (90.0 %) and 5.0 mg/l IBA x 0.5 mg/l BAP (90.0 %) for 41 B. In entire plant formation, similar combinations as above gave the best results: 1.0 mg/l IBA x 0.5 mg/l BAP (71.4 %) and 1.0 mg/l IBA x 0.0 mg/l BAP (66.7 %) for Kalecik Karasi; 5.0 mg/l IBA x 0.0 mg/l BAP (80.0 %) and 5.0 mg/l IBA x 0.5 mg/l BAP (70.0 %) for 41 B (Table 3, Fig. 3).

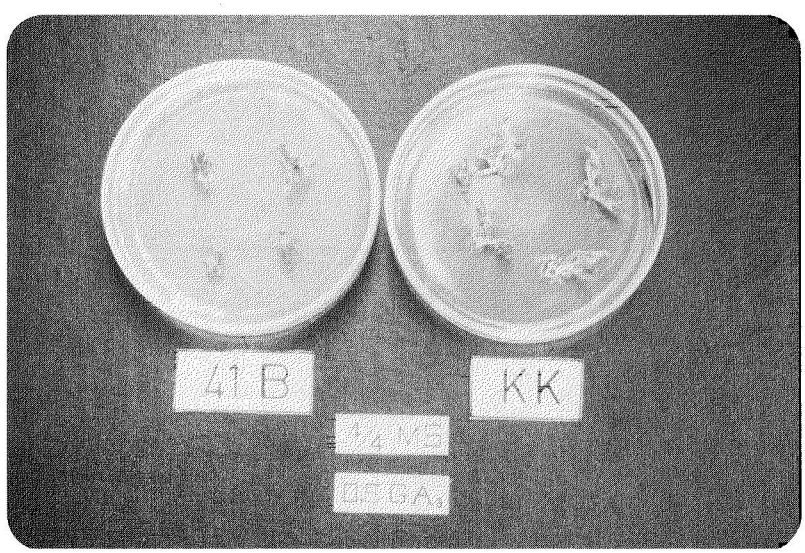

Fig. 1: Development of apical meristems in the establishment medium of MS supplemented with 0.5 mg/l GA_3.

Table 2: Effects of IBA x BAP combinations on growth of the apical meristems of wine grape cv. Kalecik Karasi and rootstock cv. 41 B M.G. in shoot medium

IBA (mg/l)	BAP (mg/l)	Shoot Formation(%) K.K.	41 B	No.of Shoots/ Culture K.K.	41 B	No.of explants/ Culture K.K.	41 B	Total wt.of culture(mg) K.K.	41 B	Entire plant(%) K.K.	41 B
0.0	0.0	-	-	-	-	1.66	2.00	537.7	239.3	-	-
0.0	0.5	20.0	25.0	0.20	0.25	2.53	2.56	911.3	528.8	20.0	-
0.5	0.5	13.3	18.8	0.13	0.18	2.93	2.31	930.5	1174.3	13.0	18.8
0.0	1.0	42.9	43.8	0.57	0.43	3.64	3.12	2439.0	909.4	42.9	-
0.5	1.0	42.9	40.0	0.78	0.60	4.07	3.00	1039.7	1731.9	28.6	-
1.0	1.0	33.3	18.8	0.33	0.18	3.16	2.43	1793.5	1857.6	33.3	-
0.0	2.5	-	18.8	-	0.25	4.18	3.68	2643.2	1207.8	-	-
0.5	2.5	6.7	31.3	0.06	0.31	3.80	2.62	1702.4	1407.6	6.7	-
1.0	2.5	13.3	31.3	0.13	0.31	3.06	2.68	2180.3	1968.5	6.7	-
2.5	2.5	18.7	25.0	0.18	0.25	3.75	1.68	1718.6	1984.5	-	-
0.0	5.0	-	-	-	-	2.50	2.56	1071.6	778.0	-	-
0.5	5.0	13.3	-	0.13	-	4.06	2.62	1785.3	1173.0	-	-
1.0	5.0	-	-	-	-	3.53	2.25	2265.7	1301.5	-	-
2.5	5.0	-	-	-	-	3.56	2.43	2318.9	1752.1	-	-
5.0	5.0	7.2	-	0.07	-	3.28	1.86	2516.2	514.5	-	-

Table 3: Effects of IBA x BAP combinations on root and shoot growth of the apical meristems of wine grape cv. Kalecik Karasi and rootstock cv. 41 B M.G. in rooting medium

IBA (mg/l)	BAP (mg/l)	Root formation(%)		No.of roots/ culture		Total wt.of culture(mg)		Entire plant(%)	
		K.K.	41 B	K.K.	41 B	K.K.	41 B	K.K.	41 B
0.0	0.0	66.7	33.3	1.58	0.75	1012.5	321.4	13.3	25.0
0.5	0.0	76.9	53.8	2.23	1.92	1040.6	617.9	53.8	38.5
0.5	0.5	69.2	54.5	0.07	1.72	1786.6	1468.0	53.8	45.5
1.0	0.0	86.7	63.6	2.86	2.81	1957.2	722.0	66.7	36.4
1.0	0.5	85.7	70.0	1.92	1.70	1206.1	1515.0	71.4	40.0
1.0	1.0	50.0	25.0	0.90	0.33	1624.9	1336.8	30.0	16.7
2.5	0.0	63.6	90.0	2.00	5.30	1367.6	916.2	54.5	60.0
2.5	0.5	78.6	75.0	3.14	2.83	1796.5	2008.9	57.1	66.7
2.5	1.0	66.7	41.7	1.73	1.33	1767.2	1897.2	46.7	41.7
2.5	2.5	41.7	33.3	1.07	0.58	2671.0	2175.4	33.3	16.7
5.0	0.0	64.3	100.0	1.42	4.60	1612.1	1002.9	50.0	80.0
5.0	0.5	84.6	90.0	2.53	3.90	1926.0	2107.8	61.5	70.0
5.0	1.0	83.3	30.0	2.50	1.10	2536.1	1669.9	58.3	30.0
5.0	2.5	72.7	10.0	2.09	0.50	2253.3	1525.1	27.3	10.0
5.0	5.0	30.8	-	0.50	-	3296.9	509.6	-	-

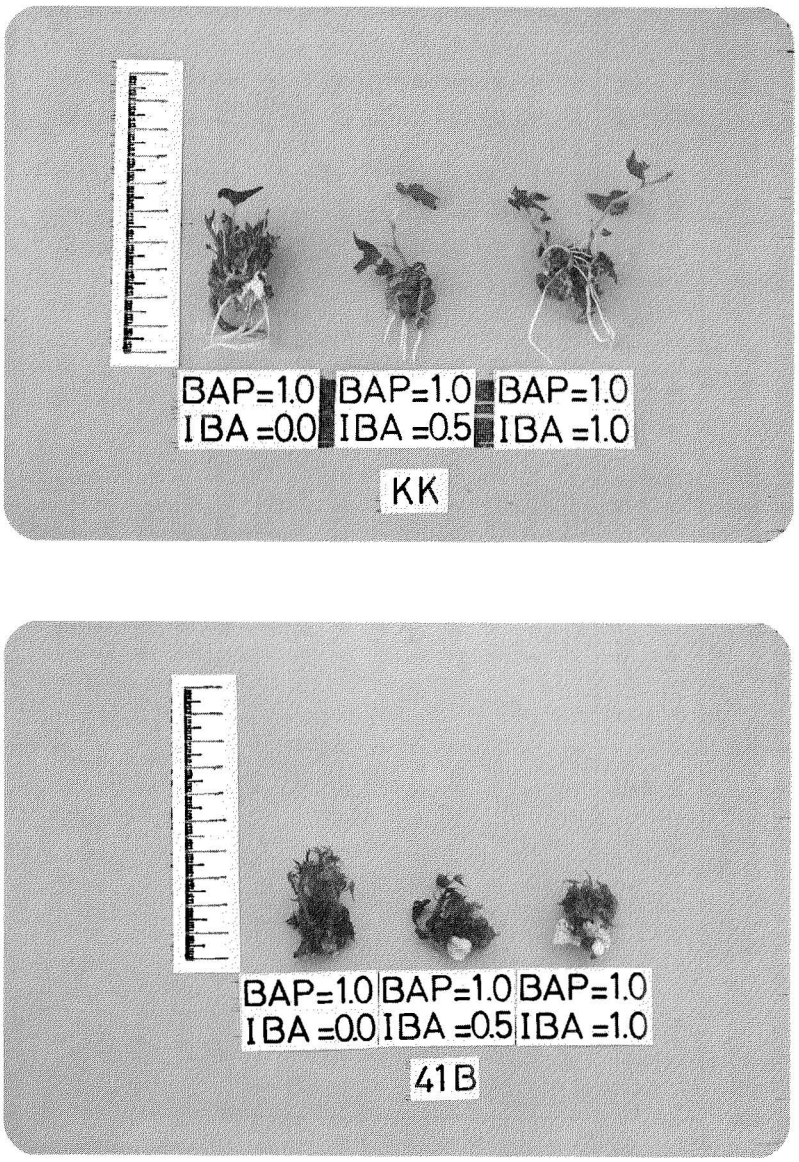

Fig. 2: Growth patterns of Kalecik Karasi and 41 B M.G. apical meristems in shoot subculture.

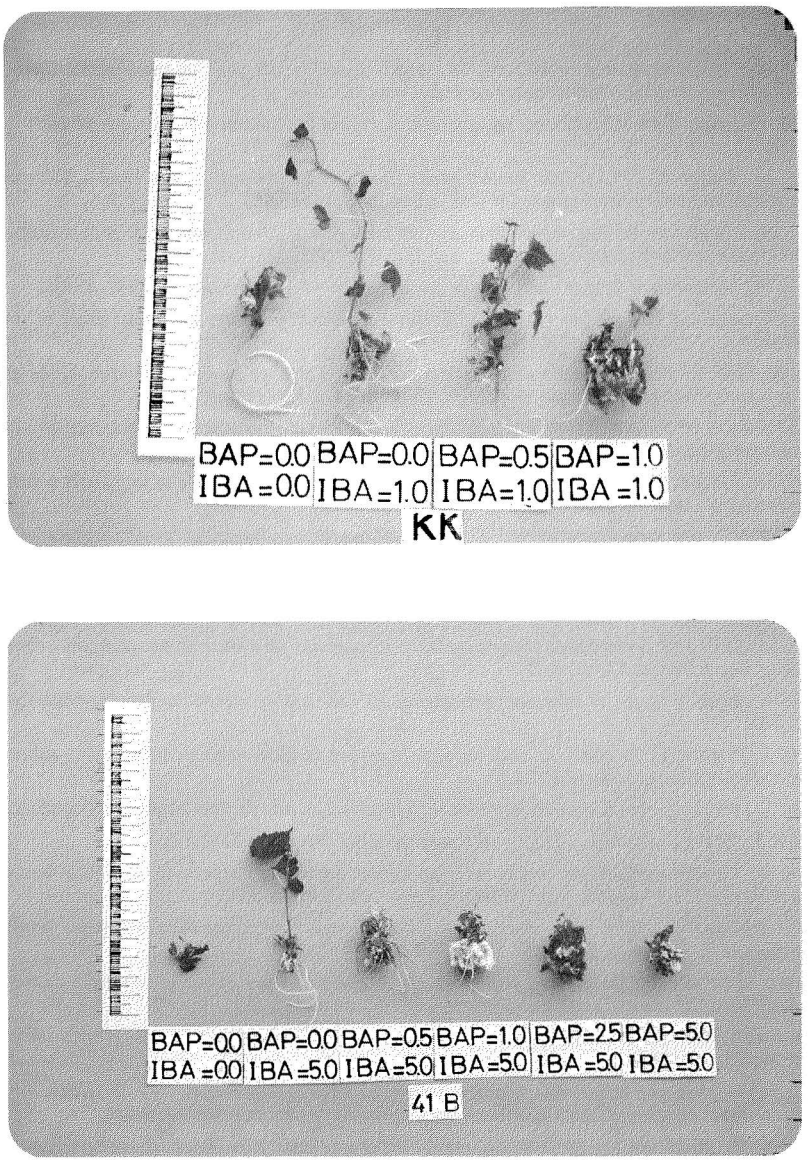

Fig. 3: Growth patterns of Kalecik Karasi and 41 B M.G. apical meristems in root subculture.

Literature

ALLEWELDT, G.; HARST-LANGENBUCHER, M.; 1987: Der Einfluß von Wachstumsinhibitoren auf die Langzeitlagerung von *in-vitro*-Kulturen der Rebe. Vitis **26**, 57-64.

– – ; 1987: The effect of growth inhibitors on long-term storage of *in vitro* cultures of grapevine. Hort. Abstr. **57**, 9309.

BARLASS, M.; SKENE, K. G. M.; 1980: Studies on the fragmented shoot apex of grapevine. I. The regenerative capacity of leaf primordial fragments *in vitro*. J. Exp. Bot. **31**, 483-488.

– – ; – – ; 1981: Studies on the fragmented shoot apex of grapevine. III. A scanning electron microscope study of adventitious bud formation *in vitro*. J. Exp. Bot. **32**, 1079-1083.

– – ; – – ; 1982: Virus-free vines from tissue culture. Austral. Grapegrower Winemaker (224), 40-41.

– – ; – – ; WOODHAM, R. C.; KRAKE, L. R.; 1982: Regeneration of virus-free grapevines using *in vitro* apical culture. Ann. Appl. Biol. **101**, 291-295.

BLAICH, R.; 1985: Recherches sur les cultures de méristèmes et d'organes de vigne *in vitro* en vue de la sélection et de la conservation de génotypes. Bull. O.I.V. **58** (650/651), 391-395.

– – ; 1985: Studies on *in vitro* cultures of grape organs and meristems with the aim of selecting and preserving genotypes. Hort. Abstr. **55**, 8502.

CHÉE, R.; POOL, r. M.; 1983: *In vitro* vegetative propagation of *Vitis*: Application of previously defined culture conditions to a selection of genotypes. Vitis **22**, 363-374.

– – ; – – ; 1985: *In vitro* propagation of *Vitis*: The effects of organic substances on shoot multiplication. Vitis **24**, 106-118.

FANIZZA, G.; 1986: BAP induces rooting *in vitro* shoot tip culture in *Vitis*: genotypic variation. Genet. Agrar. **40**, 448-449.

FAVRE, J. M.; 1977: Premiers résultats concernant l'obtention *in vitro* de néoformations caulinaires chez la vigne. Ann. Amélior. Plantes **27**, 151-169.

– – ; 1978: Preliminary results on obtaining spontaneous *in vitro* shoot production in the grapevine. Hort. Abstr. **48**, 240.

GRAY, D. J.; FISHER, L. C.; 1987: *In vitro* shoot propagation of grape species, hybrids and cultivars. Proc. Florida State Hort. Soc. **98**, 172-174.

GRENAN, S.; 1984: Polymorphisme foliaire consécutif à la culture *in vitro* de *Vitis vinifera* L. Vitis **23**, 159-174.

HARRIS, R. E.; STEVENSON, J. H.; 1982: *In vitro* propagation of *Vitis*. Vitis **21**, 22-32.

KARTHA, K. K.; 1981: Meristem culture and cryopreservation. In: THORPE, T. A. (Ed.): Methods and Applications. Plant Tissue Culture. Academic Press, New York.

LIMASSET, P.; CORNUET, P.; 1949: Recherche du virus de la mosaïque du tabac dans les méristèmes des plantes infectées. C. R. Acad. Sci., Paris, **288**, 1971-1972.

MASAHIKO, I.; TOMIO, S.; HIDEO, T.; KATSUYASU, U.; 1982: Elimination of grapevine viruses by meristem tip culture. Proc. 5th Intern. Congr. Plant Tissue and Cell Culture, 409-413.

MURASHIGE, T.; SKOOG, F.; 1962: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**, 473-497.

QUAK, F.; 1977: Meristem culture and virus-free plants. In: REINERT, J.; BAJAS, Y. P. S. (Eds.): Applied and Fundamental Aspects of Plant Cell, Tissue and Organ Culture. Springer-Verlag, Berlin.

SKENE, K. G. M.; BARLASS, M.; 1980: Micropropagation of grapevine. Proc. Intern. Plant Propagators Soc. **30**, 564-570.

WETHERELL, D. F.; 1982: Introduction to *in vitro* propagation, 1-85, University of Connecticut. Avery's Plant Tissue Culture Series, New Jersey.