Micropropagation of Grape Cultivars (*Vitis vinifera* L.) on Different Basal Media Supplemented with Benzyl Adenine

Ali-akbar MOZAFARI (✉)
Ommar GHORAISHI
Nasser GHADERI
Teimoor JAVADI

Summary

In vitro propagation is a commercial technique that is used for plant propagation around the world. Therefore, the development of *in vitro* techniques is a sure way for rapid propagation of many plant species. In this study, effect of Woody Plant Medium (WPM) and Murashighe and Skoog (MS) media supplemented with 0.0, 2.2, 4.4, 6.6 and 8.8 µM benzyladenine (BA) on regeneration of three grape cultivars ('Bidaneh Sefid', 'Farkhi' and 'Khoshnav') were investigated. In proliferation stage, length of shoots, number of shoots, number of leaves and the final status of explants were evaluated. In rooting stage, effect of indole-3-butyric acid (IBA) in three concentrations (0.0, 0.5 and 1.0 µM) on root initiation, number of rooted explants, root length and number of roots were evaluated. The results showed that the longest shoots were obtained in MS medium supplemented with 2.2 µM BA in the three cultivars, while most adequate shoots were observed in MS medium containing 4.4 µM BA in all studied cultivars. In rooting stage, the best results were obtained by 0.5 µM IBA. The results of this study showed that grape regeneration potential *in vitro* conditions depend to cultivar, culture medium and concentration of growth regulators.

Key words

grape, regeneration, basal medium, *in vitro*

Department of Horticultural Sciences, Faculty of Agriculture, University of Kurdistan, Sanandaj, Iran. P. O. Box: 416, Postal code: 66177-15175
✉ e-mail: a.mozafari@uok.ac.ir

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Introduction

Grape is one of the most important fruits in the world and it is very important to national economies. The tissue culture is an important method for clonal propagation of herbaceous and woody plant species (Mhatra et al., 2000). Use of various organs such as shoot tip and node for *in vitro* regeneration of grapevine (*Vitis vinifera* L.) spices and cultivars is reported (Bigger, 2010). Thidiazuron (TDZ) was found to be more effective than benzylaminopurine (BAP) for muscandine (*V. rotundifolia*) grapes micropropagation (Sudarsono and Goldy, 1988). Whereas, Gray and Benton (1991) reported that BAP was found to be better for the same species. Also, Mhatre et al. (2000) reported that for shoot multiplication of *V. vinifera*, BAP was better in comparison with other plant growth regulators.

Studies have shown that *in vitro* propagation of grape is affected by various factors such as genotype and culture conditions (Monette, 1988). Different media for grape *in vitro* propagation has been used (Mukherjee et al., 2010). The effect of media such as Murashige and Skoog (MS) (Ghulm, et al., 2006) and diluted MS with macro salts at a half of the concentration (Yahyoui et al., 1998) on different grape cultivars micropropagation has been reported. The effect of MS medium and BA on 'Perlette' grape propagation was studied and the best shooting was observed in MS medium with 1.5 mg l^{-1} BA (Muhammad et al., 2008).

For *in vitro* rooting of shoots, IAA at 0.2 mg l^{-1} was found to be the optimum to produce the highest results and 2.03 roots per shoot were obtained, whereas use of IBA or NAA resulted in rooting with high frequency of callus formation (Mukherjee et al., 2010).

In the present study, *in vitro* propagation of three commercial grape cultivars ('Khoshnav', 'Bidaneh sefid' and 'Farkhi') was investigated. 'Khoshnav' is very important black seeded cultivar that is cultivated in rain feed area in west of Iran. 'Bidaneh Sefid' is a seedless (Stenospermocarp), but 'Farkhi' is a seeded cultivar. Color of both cultivars is white. *In vitro* propagation of 'Farkhi' and 'Khoshnav' still has not been studied. Therefore, the aim of this study was to test the effect of two media (Woody Plant Medium, 1981 (WPM) and Murashighe and Skoog, 1962 (MS)) and five concentrations of BA (0.0 (control), 2.2, 4.4, 6.6 and 8.8 µM) on node explants proliferation of these cultivars.

Materials and methods

Plant materials preparation

Young and actively growing shoots of grape cultivars were collected from *ex vitro* plants. The collected shoots were stripped off all leaves and explants were stored in cold and moist place until culture. Fourth and third nodes from the end of shoot were used as initial explants for regeneration.

Explants sterilization

The explants were washed in running tap for 30 min for surface sterilization. Then, under aseptic conditions, the explants were immersed in 70% ethanol for 10-20 seconds, and were sterilized by 1000 ppm nanosilver for 20 minutes (Gouran et al., 2014). Finally, explants were washed three times with sterilized distilled water for 10 min. Nodal segments (1cm) were dissected and were placed horizontally on the culture medium.

Proliferation stage

In this stage individual nodal cutting (1 cm long) was inoculated in MS and WPM media supplemented with 30 mg l^{-1} sucrose, 7 mg l^{-1} agar- agar and 0.0 (control), 2.2, 4.4, 6.6, and 8.8 µM of BA. The culture medium pH was adjusted to 5.8 before autoclaving. Explants on culture medium were incubated in growth chamber at 23 ± 1 °C with a 16 h photoperiod at a light intensity of 32 µmol photons m^{-2} s^{-1} and 60 ± 5 percent relative humidity. Two months after planting, proliferation (number of new shoot per explant), shoot length (cm) and leaf number were recorded.

Leaf and shoot statues were performed according Ebadi et al. (2011) methods. Leaves status (ratings scale of 1 to 4) and the final status of the shootlets (ratings scale of 1 to 5) were studied. Status of the leaves was determined based on the following: 1) Tiny leaves or very tiny leaves (proliferation), brown or drying out: 2) Small leaves, leaves color was bright green: 3) Moderate leaf size, almost opening and widespread, in some cases vitrfied: 4) leaves completely broad and widespread, common green color and without any complications. The final status of shoots based on the ratings score was determined as follows: 1) Tiny or very tiny leaves, very short shoots, dried and brown explants: 2) Tiny leaves, shoots longer than no. 1 but with a very thin stem: 3) The leaves are small, sometimes edges of leaves was jagged, shoots and stems were thin: 4) Leaves size larger than no. 3, desirable shoots with appropriate diameter and stem thickness greater than no. 3: 5) Completely expanded leaves, shoots with appropriate length and diameter.

Shoot rooting

Shoots obtained from the proliferation stage were divided into smaller pieces (length of 1-1.5 cm long) and subcultured on 1/2 MS medium supplemented with three levels of indole-3-butyric acid (IBA) (0.0, 0.5, and 1.0 µM). After four weeks, percent of rooted explants, number of roots per explant and root length (cm) were recorded. Also, times of root development beginning were investigated at different stages of testing.

Plantlets hardening and acclimatization

The shoot cultures after growth on rooting medium were washed thoroughly and transferred to small pots filled with loamy soil, coco peat, vermiculite and perlite (1:1:1:1 ratio). The plantlets were fertilized by 1/2 MS nutrient solution weekly. After four weeks, the acclimated plants were transferred to greenhouse.

Statistical analysis

A two way analysis of variance (ANOVA) was performed for cultivar (C) and benzyl adenin concentration (BA) for each of the medium used (MS and WPM). Means were compared using Dancan's multiple tests in SAS (SAS Institute, Cary, NC, USA, ver. 9.1) (at $\alpha = 0.05$ significance level) within each cultivar. Charts and curve fittings were performed using Microsoft Excel software 2007.

Results and discussion

Shoot length

The results showed that different concentrations of BA had a different effect on shoot length in all three cultivars (Tables 1 and 2). The highest and the shortest shoots at a concentration of 2.2 BA and 8.8 µM BA, respectively were observed on both

MS basal medium (Table 1) and WPM (Table 2). The plantlets height decreased with increasied concentrations of BA (Tables 1 and 2). Also, all three cultivars showed a similar response toward different concentrations of BA on both MS and WPM media. In this experiment, a significant difference (at the 5% level) of the adventitious shoots height at different concentrations of BA in both MS and WPM basal media was observed (Table 1 and 2). There was no significant intraction between cultivar (C) and BA, so additional analysis of variance was not required for factors separately.

Use of 2.2 and 4.4 µM BAP in MS medium showed higher efficiency in production of shoots per explant in three cultivars. BA superiority on *in vitro* proliferation of grape shoots have been reported (Banez et al., 2003; Singh et al., 2004). In present study, nutritional needs of grape were better provided in MS medium in comparison to WPM. Results showed that grape has higher nutritional requirements for growth and development under *in vitro* conditions that may vary among cultivars. One possible explanation for this result is the difference in nitrogen and potassium content between the MS and WPM medium. The nitrogen and potassium content of the MS medium was approximately two-fold higher than that of the WPM medium (Mei-Chun Lu, 2005).

Number of shoot

The lowest number of adventitious shoots was observed on basal MS (Table 3) and WPM (Table 4) medium without BA. Results showed that the number of adventitious shoots is improved with the increasing of BA concentrations. All cultivars on MS medium produced the highest number of adventitious shoots at 8.8 µM BA (Table 3 and Figure 8). The highest number of adventitious shoots was produced on WPM medium with 6.6 µM BA (Table 4). All studied cultivars have shown uniform response to different concentrations of BA on both media (Tables 3 and 4).

The presence of cytokinins in culture medium has been considered essential for proliferation of lateral and terminal buds (Mhatra et al., 2000). Similar results were also reported by Skiada et al. (2010) and Poudel et al. (2005). Ibáñez et al. (2003) reported that the optimum concentration of BA varies slightly between cultivars and should be determined in each individual case. The present study revealed that MS medium was superior for proliferation of grape shoots in comparison with WPM medium. For some plant taxa, a major causative factor appears to be the high ammonium ion content of the MS medium (Leonhardt and Kandeler, 1987). The results showed that MS medium was more effective compared to WPM media for adventitious shoots formation. This result may be explained by the fact that MS is full strength medium and contain higher macro elements concentration than WPM. There are differences in micronutrient composition between MS and WPM media (Murashige and Skoog, 1962; Lloyd and Mc Cown, 1981) which might cause differences in shoot number production in different species. The difference in microelement compositions among MS and WPM media might cause differences in proliferation rate and chlorophyll content (Mei-Chun Lu, 2005).

Number of leaf

There was no significant difference in the leave number per adventitious shoot between different cultivars, but significant difference was observed between different BA concentrations

Table 1. Effect of MS[z] basal medium supplemented with different concentrations of benzyl adenine (BA) on shoot length (cm) of three grape cultivars

BA Concentration (µM)	Cultivar		
	'Khoshnav'	'Farkhi'	'Bidaneh Sefid'
0.0	2.62±0.14c[‡]	2.7±0.12cd	2.31±0.19c
2.2	4.29±0.04a	4.46±0.05a	3.4±0.2a
4.4	3.07±0.04b	3.05±0.08b	2.71±0.15b
6.6	2.38±0.04cd	2.46±0.04dc	2.11±0.1c
8.8	1.89±0.06e	1.67±0.06e	1.44±0.12d

[z]: Morashige and Skoog medium (Morashige and Skoog, 1962); [‡]Mean value followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05). Mean ± SE

Table 2. Effect of WPM[z] basal medium supplemented with different concentrations of benzyl adenine (BA) on shoot length (cm) of three grape cultivars

BA Concentration (µM)	Cultivar		
	'Khoshnav'	'Farkhi'	'Bidaneh Sefid'
0.0	0.51±0.04e[‡]	0.45±0.02d	0.43±0.02e
2.2	1.6±0.11a	1.19±0.06ab	1.18±0.1a
4.4	0.83±0.07ab	1.3±0.1a	0.8±0.04b
6.6	0.72±0.03c	0.66±0.05lb	0.63±0.02lc
8.8	0.61±0.12cd	0.51±0.04c	0.48±0.04de

[z]: Woody Plant Medium (Lloyd and McCown, 1981); [‡]Mean values followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05). Mean ± SE

Table 3. Effect of MS basal medium and benzyl adenine (BA) on shoot number in three grape cultivars

Concentration (µM)	Cultivars		
	'Khoshnav'	'Farkhi'	'Bidaneh Sefid'
0.0	0.0±0.0e[‡]	0.0±0.0e	0.0±0.0e
2.2	0.79±0.08d	0.88±0.09d	0.54±0.09d
4.4	1.54±0.09c	1.62±0.08c	1.29±0.08c
6.6	1.96±0.08b	2.04±0.13b	1.62±0.08b
8.8	2.71±0.13a	3.13±0.08a	2.13±0.08a

[‡]Mean values followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05). Mean ± SE

Table 4. Effect of WPM basal medium and benzyl adenine (BA) on shoot number in three grape cultivars

Concentration (µM)	Cultivars		
	'Khoshnav'	'Farkhi'	'Bidaneh Sefid'
0.0	0.0±0.0d[‡]	0.0±0.0d	0.0±0.0e
2.2	0.29±0.08c	0.21±0.09b	0.21±0.09c
4.4	0.54±0.09b	0.46±0.08ab	0.38±0.0b
6.6	0.88±0.09a	0.54±0.09a	0.46±0.08a
8.8	0.21±0.09c	0.13±0.08c	0.13±0.08d

[‡]Mean values followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05). Mean ± SE

Table 5. Effect of MS basal medium and benzyl adenine (BA) on leaf number in three grape cultivars

Concentration (μM)	Cultivars		
	'Khoshnav'	'Farkhi'	'Bidaneh Sefid'
0.0	5.83±0.34e‡	5.91±0.41d	3.74±0.28e
2.2	6.41±0.34d	6.24±0.28cd	4.74±0.36d
4.4	7.83±0.34c	7.49±0.29cb	6.24±0.28c
6.6	8.83±0.48b	9.08±0.45b	8.24±0.36b
8.8	9.99±0.43a	10.16±0.21a	9.24±0.37a

‡Mean values followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05). Mean ± SE

Table 6. Effect of WPM basal medium and benzyl adenine (BA) on leaf number in three grape cultivars

Concentration (μM)	Cultivars		
	'Khoshnav'	'Farkhi'	'Bidaneh Sefid'
0.0	2.99±0.13c‡	2.74±0.16b	2.66±0.13b
2.2	4.16±0.67fab	3.72±0.17ab	3.12±0.31ab
4.4	4.66±0.52a	4.24±0.28a	3.33±0.23a
6.6	3.75±0.14fb	2.33±0.27bc	1.53±0.07c
8.8	3.25±0.47b	1.49±0.21c	1.24±0.15d

‡Mean values followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05). Mean ± SE

(Tables 5 and 6). Results showed that the number of leaves per adventitious shoot increased by increasing the concentration of BA. On the MS medium, the highest number of leaves was observed at 8.8 μM BA and the lowest number of leaves at control (Table 5). The highest number of leaves in all studied cultivars was produced on the MS basal medium supplemented with 4.4 μM BA (Table 5).

In the present study, medium culture type and growth regulator concentration had significant effect on leaves number in three cultivars. It is well known fact that *in vitro* regeneration of an explant is influenced by several internal and external factors such as nature and hormonal composition of culture medium, species, genotype, explants and various other culture conditions (Kurmi et al., 2011). The difference in the leaves number production in three cultivars may be related to genotype. In general, cultivar 'Khoshnav' showed appropriate responses and better adaptation in comparison to cultivars 'Farkhi' and 'Bidaneh Sefid'. Present study revealed that MS medium was more effective compared to WPM for leaves production, therefore these results indicated that WPM medium is inappropriate medium for leaves production in three cultivars. The amount of nutrient depletion after culture may be greater for low-salt medium, such as WPM, than for more concentrated media such as MS (Bosela and Michler, 2008). Therefore, MS medium had higher effect on leaf production than WPM (Bosela and Michler, 2008).

Status of the leaf

'Khoshnav' and 'Farkhi' showed similar status. Worse leaves in the regenerated shoots were observed in cultivar 'Bidaneh Sefid' (Figure 1). MS medium produced leaves with better status in comparison to WPM medium (Figure 2). The effect of different

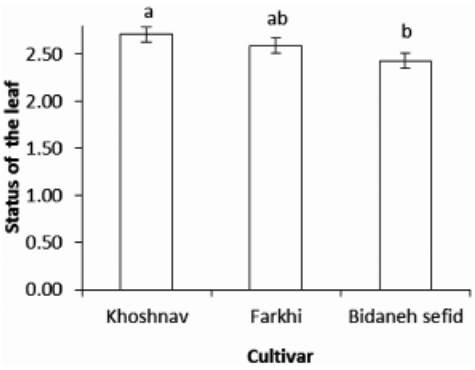

Figure 1. Leaf status mean in three grape cultivars *in vitro* conditions. Mean values followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05).

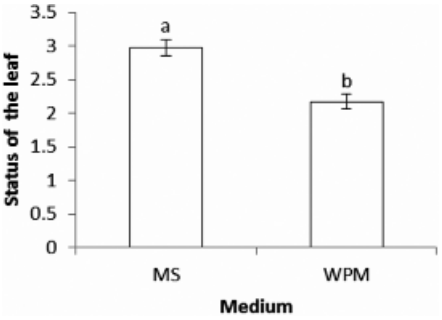

Figure 2. Leaf status mean of the three grape cultivars under different basal media. Mean values followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05).

BA concentrations on the status and the size of the leaves showed that best leaves (size and appearance of the leaves) obtained in 2.2 and 4.4 μM BA concentrations (Figure 5). With increasing BA concentration, the leaves showed vitrification and deformation and inappropriate leaves were obtained at 8.8 μM BA concentration (data not showed).

In both media leaf vitrification and abnormality were produced by high BA concentration (6.6 and 8.8 μM). High cytokinin concentration, high relative humidity in containers of medium, ethylene synthesis and specific ions such as nitrate in the medium caused vitrification and abnormal leaves production (Ibáñez et al., 2003). Differences in vitrification frequency were related to differences in ionic strength; 44 meq l^{-1} for WPM versus 96.2 meq l^{-1} for MS, respectively (George et al., 1987). Bosela and Michler (2008) reported that during culture initiation, vitrification was observed at frequencies of 60–70% on WPM compared to frequencies of <5% on MS medium. The shoots that regenerated on WPM also shared a 'small leaf' phenotype, compared with normal leaf sizes on MS media (Bosela and Michler, 2008).

This problem is the consequence of culture conditions, and leads to losses of plantlets. According to the results obtained in present study, it would seem advisable for optimal proliferation of shoots to use lower concentrations of BA (4.4 μM) since

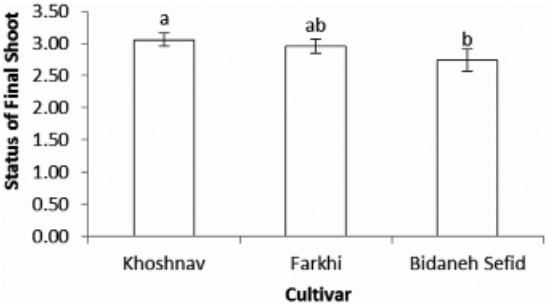

Figure 3. Shoot status mean in three grape cultivars under different levels of BA (Benzyl adenine). Mean values followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05).

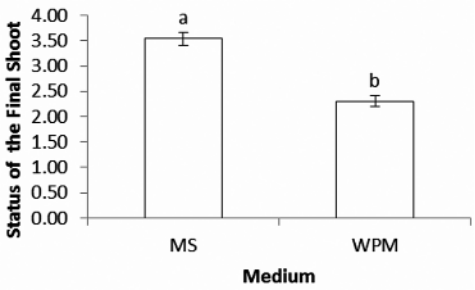

Figure 4. Shoot status mean in three grape cultivars under different media. Mean values followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05).

it lead to normal growth of the branches of appropriat size. For vitrification elimination Morini et al. (1985) suggested reduction of, BA concentration to 2.2 µM. Reduction in BA concentration leads to low shoot production rate without complete vitrification removal.

Shoot status

'Khoshnav' and 'Farkhi' showed better shoots status in comparison to 'Bidaneh Sefid' (Figure 3), although the difference between 'Farkhi' and 'Bidaneh Sefid' was not significant. MS produced more appropriate shoots than WPM medium (Figure 4). The most appropriate shoots were produced by 4.4 µM BA concentration (Figure 5).

Result showed that 6.6 and 8.8 µM BA concentrations were detected as inappropriate factor for nodal cutting regeneration on both meda and for researched cultivars. Most appropriate shoots were formed in MS medium containing 4.4 µM BA in three cultivars. In this respect, Jaskani et al. (2008) obtained the best shoots on MS medium containing 5 and 10 µM BA for *Vitis vinifera* L. cv. Perlette. Bigger (2010) found that more inappropriate shoots were produced by 8 µM BA in comparison to 2 and 4 µM BA, and that is similar to the results of present experiment.

Rooting stage

Root induction

The primary roots became visible after 12 days of culture. Shoots were rooted in all hormonal treatments during four week after subculture. Lu (2005) observed root initiation in

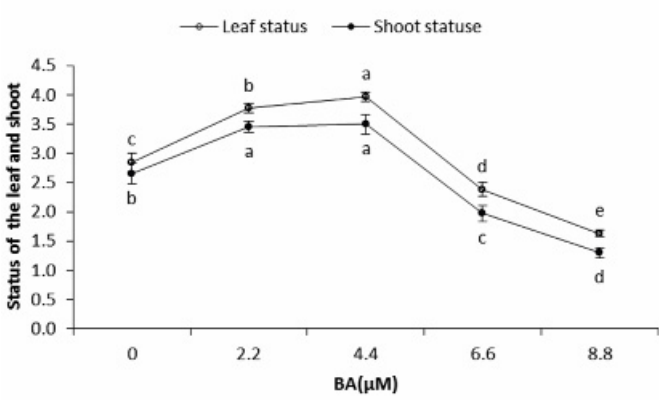

Figure 5. Shoot and leaf status mean in three grape cultivars under different levels of Benzyl adenine (BA). Mean values followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤0.05). (Mean value is comparable for shoot and leaf statues separately)

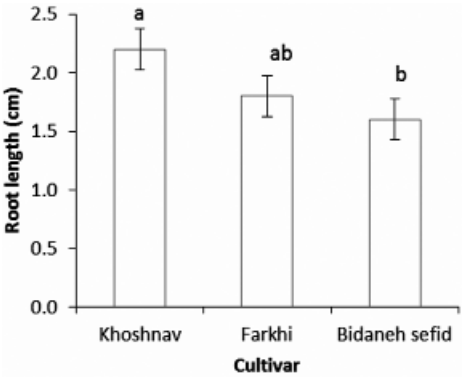

Figure 6. Root length mean in three grape cultivars under different levels of IBA (Indol-3-butiric acid). Mean values followed by different letters indicate significant differences according to Duncan's multiple range test (P ≤ 0.05).

grape species of *Vitis thunbergii* 11 days after plantlets cultivation. Probably time of root initiation start in grape is different depending on genotype and hormonal treatments. A high endogenous auxin concentration at the beginning of the rooting process is normally associated with a high rooting rate (Caboni et al., 1997). An alternative explanation for loss of rooting capacity in adult material may be associated with the differential expression of genes that affect one or all of the phases in the process (Vidal et al., 2009).

Number of rooted shoots

With increasing time of establishment of explants in rooting medium, rooted shoots percentage increased in three cultivars. However, higher rooted shoots were observed in 'Khoshnav' in initial days of cultivation in compare to other cultivars. The rooting percentage was almost 100% in all treatments four weeks after culture. These results indicated that root initiation and rooting rate is affected by genotype. Present results is comparable to other results (Barreto and Nookaraju, 2007) that indicated that the MS medium supplemented with IBA and NAA gave 95% rooting shoot in grape cultivar Perlette.

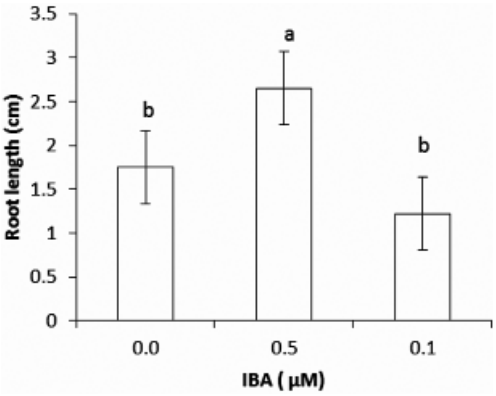

Figure 7. Root length mean in grape cultivars at 0.0, 0.5 and 0.1 µM of IBA (Indol-3-butiric acid) added to the culture medium. Mean values followed by different letters indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$)

Root length

'Khoshnav' produced significantly ($p<0.01$) longer roots than 'Bidaneh Sefid'. Roots length was similar in 'Farkhi' and and 'Bidaneh Sefid' (Figure 6). Longer roots were obtained at 0.5 µM IBA in comparison to 0.0 and 1.0 µM IBA. The shortest root length was observed in 1 µM IBA treatment (Figure 7). Higher root proliferation ability (89.94%) and root length (7.75 cm) was exhibited by MS rooting medium supplemented with 1 mg l^{-1} IBA (Kurmi et al., 2011). Skiada et al. (2010) suggested that 0.5 µM IBA application improved root formation in 'Malagouzia' and 'Xinomavro' grape cultivars that is similar to the results that were obtained in present study.

Table 7. Effect of culture medium and indole-3-butyric acid (IBA) on root number in three grapes cultivars

Concentration (µM)	Cultivars		
	'Khoshnav'	'Farkhi'	'Bidaneh Sefid'
0.0	7.62±0.23a‡	3.33±0.23de	3.10±0.31e
0.5	6.00±0.4b	4.50±0.28c	4.12±0.31cd
1.0	4.62±0.37c	6.00±0.4b	5.00±0.4bv

‡Mean values followed by different letters indicate significant differences according to Duncan's multiple range test ($P \leq 0.05$). Mean ± SE

Number of root

Higher root number was produced by cultivar 'Khoshnav' in comparison to 'Farkhi' and 'Bidaneh Sefid' in all treatments except for 1 µM IBA (Table 7). Whereas, the number of roots produced using 0.5 and 1 µM IBA was higher than control (Figure 7). These results showed that rooting was affected by genotype and IBA concentration. Thicker roots were obtained with increasing of IBA concentration. Root number in 'Khoshnav' was reduced by increased of IBA concentration. This result may be related to higher internal auxin in 'Khoshnav' compared to other cultivars. The results clearly indicated that lower to moderate concentrations of IBA is required for rooting of grape shoot under *in vitro* conditions. Padoul et al. (2005) obtained thicker root by concentration higher than 2 mg l^{-1} IBA in two grape cultivars. Banilas and Korkas (2007) reported that the best root number and percentage of rooting were produced in medium supplemented by 5 µM IBA.

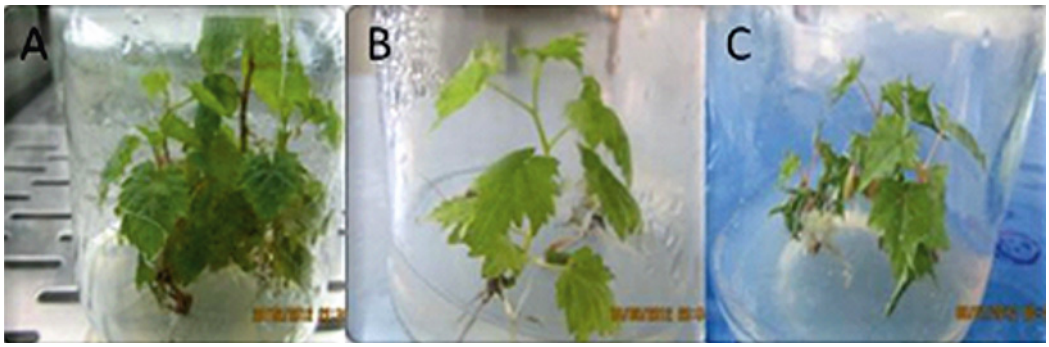

Figure 8. The shoot regeneration of the three grape cultivars on MS medium after two months of *in vitro* culture on multiplication medium supplemented with 8.8 µM BA; (A) 'Khoshnav', (B) 'Bideneh Sefid, (C) 'Farkhi'.

Figure 9. Adapted plantlet with environmental conditions two monts after transplant (A) 'Farkhi', (B) 'Bideneh Sefid', (C) 'Khoshnav'

Acclimatization

For acclimatization, rooted plantlets were transplanted to pots containing perlite, peat and soil (in 1:1:1 ratio) (Figure 9). Then incubated in 20±1°C, 55 µmol $m^{-2}s^{-1}$ light intensity and 80% relative humidity for 18 days. To prevent the loss of moisture, seedlings grown in pots were covered by transparent polyethylene covering that was removed after 18 days. Plants were adapted to environmental condition during 4 days after removal of polyethylene cover. About 98% of plants survived and grew normally.

Conclusion

Tissue culture is rapid and efficient method for grape propagation. *In vitro* grape regeneration potential depends on genotype, the basal medium and concentration of growth regulator. These factors strongly affect survival rate of explants, shoot regeneration, number of produced shoots and shoots morphology.

MS medium was more suitable than WPM medium for the growth and regeneration of explants of three grape cultivars 'Khoshnav', 'Farkhi' and 'Bidaneh Sefid'. WPM culture medium was inappropriate for micropropagation of these cultivars, because callus production was high. To achieve suitable shoot proliferation, the initial explants were cultured on MS medium containing 4.4 µM BA. To increase the length of plantlets, the culture on the MS medium containing 2.2 µM BA was shown to be effective. For rooting of, 2.2 µM IBA can be recommended. Rooting of grape plantlets *in vitro* required to lower the levels of IBA in rooting basal medium. These results showed that the efficiency of *in vitro* propagation method is highly dependent on cultivar and nodal explants can be used for commercial propagation of cultivars 'Khoshnav', 'Farkhi' and 'Bidaneh Sefid'.

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