Erwerbs-Obstbau (2018) 60(Suppl 1):S55–S59
https://doi.org/10.1007/s10341-018-0393-7

ORIGINAL ARTICLE

Influence of BAP Concentrations and Nutrient Medium Composition on *In Vitro* Regeneration of 'Öküzgözü' and 'Boğazkere' (*Vitis vinifera* L.) Cultivars

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Received: 21 June 2017 / Accepted: 31 May 2018 / Published online: 27 June 2018

Abstract
This study has been conducted with the aim to determine the type of nutrient medium that can be used in micropropagation studies for 'Öküzgözü' and 'Boğazkere' and to specify BAP concentrations. In the study where ejectors with a length of 0.7–0.8 cm that are obtained with single-node culture are used, it was focused on four different nutrient media such as MS, DKW, QL and WPM and on six different concentrations such as 0.2–0.4–0.6–0.8–1.0–1.5 mg l^{-1} BAP. Single-node suspension explants which will be used in initiating the culture, are taken into culture in MS nutrient medium and the nutrient medium is supported with 30 g l^{-1} sucrose, 6 g l^{-1} agar and 1 mg l^{-1} BAP. In the trial environment, parameters such as number of shoots, shoot length (cm), number of nodes and callus ratio have been investigated. For both grape varieties, the best outcome was obtained with MS nutrient medium with respect to number of shoots, shoot length, and number of nodes. These values were found as 4.66, 1.24 and 6.39 for 'Öküzgözü' variety respectively, whereas they are determined as 6.28, 1.15 and 6.81 for 'Boğazkere' variety respectively. In both grape varieties in DKW nutrient medium, starting from the 2nd week of culture, obscuration began to appear on the shoots and after this stage no other development has taken place.

Keywords 'Öküzgözü' · 'Boğazkere' · In vitro · BAP · Nutrient medium

Einfluss von BAP-Konzentrationen und Nährmedienzusammensetzung auf die *in vitro* Revitalisierung bei den Weinreben-Sorten Öküzgözü' und 'Boğazkere' (*Vitis vinifera* L.)

Schlüsselwörter: 'Öküzgözü' · 'Boğazkere' · In vitro · BAP · Nährmedium

Introduction

In vitro regeneration, systems are generally shaped in the triangle of genotype, explant source, and culture conditions. In this respect, for *Vitis vinifera* L., there is no general protocol, whereas the required nutrition environment content, plant growth regulators, and environmental conditions show variations as per the varieties (San Pedro et al. 2017).

For in vitro multiplication of different *Vitis spp*. varieties, protocols have been developed aiming to determine the requirements of the necessary nutrition environments (Yildirim et al. 2016). In the study realized from the shoot sample pieces of grape type of 'Cabernet Savignon' for plant regeneration, in MS nutrient medium supported with 2 mg/l BA, thousands of plant production is realized in a very short time. In the in vitro multiplication study conducted to produce sapling being free from viruses, from 'Baco' grapes as being a French hybrid grape type, modified MS and modified B5 nutrient environments have been used and it is notified that ¾ MS nutrient medium being supported with 3 mg l^{-1} BAP gave the best outcome. Later on, this study is applied to 21 *vinifera* and their hybrids (Harris and Stevenson 1982). For example, in the study relating with in vitro shoot proliferation of *V. rotundifolia*, MS (Murashige and Skoog 1962) and ½ MS nutrient

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Table 1 Effect of basal culture medium on in vitro shoot proliferation of the grapevine cultivars

Type of nutrient medium	Shoot number	Shoot Length (cm)	Node number	Explant ratio (%) forming callus
Öküzgözü Grape Cultivar				
MS	MS	4.66 ± 0.57 a	6.39 ± 0.28 a	60.00 ± 11.23 a
DKW	0.00 ± 0.00 c	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 b
QL	2.50 ± 0.45 b	1.05 ± 0.04 b	4.00 ± 0.23 c	60.00 ± 11.23 a
WPM	3.75 ± 0.29 a	1.09 ± 0.04 ab	5.03 ± 0.20 b	86.67 ± 9.08 a
Boğazkere Grape Cultivar				
MS	6.28 ± 0.50 a	1.15 ± 0.04 a	6.81 ± 0.20 a	80.00 ± 10.69 ab
DKW	0.00 ± 0.00 d	0.00 ± 0.00 c	0.00 ± 0.00 d	0.00 ± 0.00 c
QL	1.93 ± 0.33 c	1.12 ± 0.05 a	4.72 ± 0.25 b	73.33 ± 11.81
WPM	4.00 ± 0.39 b	0.93 ± 0.03 b	3.50 ± 0.12 c	100.00 ± 0.00 a

For each parameter the same letter represents values with no significant difference according to the Duncan's range test ($P \leq 0.05$). Means in a column followed by different letters are significantly different (Duncan Multiple Comparison, $P < 0.05$)

medium showed the same impact, whereas it is stated that scrub shoots were obtained from WPM (Llyod and McCown 1980) nutrient medium (Gray and Benton 1991). In the study realized with the aim to determine the optimum environment requests in the in vitro multiplication with grape varieties of Kober 5BB rootstock, 'Magarach', 'Zhemchug Magaracha' and 'Sverkhrannii Magaracha', MS (1, ½, ¼) and liquid and solid phase situations were analysed and it was stated that for the development of shoot and root development, macro-micro elements and vitamin ratio in the nutrient medium were influential (Zlenko et al. 1995). Furthermore, in the shoot multiplication of *Vitis thunbergii*, it was observed that WPM nutrient medium was better than MS ve NN (Nitsch and Nitsch 1969) nutrient medium. It was reported that in the study conducted by Mhatre et al. (2000), in the different stages of in vitro multiplication of grape varieties 'Thompson Seedless', 'Sonaka', 'Tas-e Ganesh', different modified forms of MS, NN and WPM have been used. In the study realized for the regeneration of grape varieties 'Öküzgözü' ve 'Boğazkere' from the leaf, it is observed that NN nutrient medium is used and that good results were obtained (Ozden et al. 2008). In the study conducted as relating with the micropropagation of grape variety 'Perlette', it was reported that regeneration ratio was 80% in MS nutrient medium which was supported with 1 mg l^{-1} BA (Jaskani et al. 2008). In the study realized for determining nutrient medium composition and plant growth regulators for the in vitro multiplication of deGrasset (*Vitis champinii* Planch.) which is used as rootstock, among the MS, MS-1, B5 (Gamborg et al. 1968) ve WPM nutrient medium, it was stated that the best shoot proliferation was obtained from MS-1 nutrient medium (Mukherjee et al. 2010). In the study conducted to develop the micropropagation of 'Malagouzia' and 'Xinomavro' being among the grape varieties of Greece, MS, WPM, GAL, MS-GAL, QL-MS, QL-WPM nutrient media were used. It was stated that the best result for 'Malagouzia' variety was obtained with GAL nutrient medium and that the best regeneration was obtained with ZL/QL-MS nutrient medium for 'Xinomavro' variety (Skiada et al. 2010). In the micropropagation study that was realized with the aim to improve the multiplication of grape variety 'Muscat Alexandria' which is under danger, it was stated that it was appropriate to use MS nutrient medium being supported with 3 mg l^{-1} BAP +0.2 mg l^{-1} NAA for proliferation (Abido et al. 2013). In the micropropagation study for grape varieties of 'Khoshnav', 'Bidaneh Sefid' and 'Farkhi' in BS nutrient medium being supported with BA and in WPM nutrient medium, while the longest shoots were obtained from MS nutrient medium being supported with 0.5 mg l^{-1} BA, it was stated that with regards to the number of shoots, the best results were obtained from MS nutrient medium being supported with BA (Mozafari et al. 2016). Similarly, while in vitro protocols for *Vitis* spp types were defined, studies were also realized for the optimization of cytokinins. Although types and concentrations of cytokinins show variations as per different types of Vitis, it was revealed with studies that they showed variations even for two different varieties of the same type. For example in the in vitro studies of varieties of Muscat grape (*V. rotundifolia*), it was found out that TDZ was more influential than BAP (Sudarsono and Goldy 1988). However, Gray and Benton (1991) have reported that BAP gave a better result for the same type. Furthermore, Mhatre et al. (2000) have stated that for the proliferation of *V. vinifera*, BAP was the most suitable cytokinin. While 1.13 mg l^{-1} BAP was used for 'Carlos' grape, being among Muscat grape varieties, it was found out that for Fry grape variety usage of 4.5 mg l^{-1} BAP, which was almost 4 times more than 1.13 mg l^{-1} BAP, was more appropriate. In a study realized as relating with the multiplication of deGrasset, being one of the grape-vine rootstocks, it was stated that the application of 1 mg l^{-1} BAP gave better results when compared with TZD, Zeatin and the other concentrations of BAP (Mukherjee et al. 2010).

Table 2 Effect of different concentrations of BAP on in vitro shoot proliferation of the grapevine cultivars

BAP Concentration mg l^{-1}	Shoot Number	Shoot Length (cm)	Node Number	≤5 mm Shoot Number
'Öküzgözü' Grape Cultivar				
0.2	1.86 ± 0.25 c	1.25 ± 0.10 ab	5.14 ± 0.30 b	3
0.4	3.13 ± 0.41 b	1.28 ± 0.07 ab	6.31 ± 0.30 a	9
0.6	3.35 ± 0.16 ab	1.41 ± 0.06 a	6.53 ± 0.19 a	–
0.8	3.70 ± 0.30 ab	1.20 ± 0.07 ab	5.97 ± 0.26 a	2
1.0	4.44 ± 0.44 a	1.24 ± 0.05 ab	6.25 ± 0.24 a	7
1.5	4.42 ± 0.46 a	1.14 ± 0.04 b	4.43 ± 0.14 c	28
'Boğazkere' Grape Cultivar				
0.2	2.13 ± 0.36 c	1.20 ± 0.07 ab	5.68 ± 0.30 bc	4
0.4	2.80 ± 0.51 c	1.15 ± 0.05 ab	5.46 ± 0.26 bc	4
0.6	4.93 ± 0.24 b	1.31 ± 0.09 a	6.21 ± 0.24 ab	13
0.8	4.26 ± 0.33 b	1.23 ± 0.05 ab	6.00 ± 0.22 b	5
1.0	6.28 ± 0.50 a	1.15 ± 0.04 ab	6.81 ± 0.20 a	19
1.5	4.60 ± 0.32 b	1.11 ± 0.04 b	5.20 ± 0.16 c	12

For each parameter the same letter represents values with no significant difference according to the Duncan's range test ($P \leq 0.05$). Means in a column followed by different letters are significantly different (Duncan Multiple Comparison, $P < 0.05$)

In this study it is aimed to determine the impact of nutrient media composition and plant growth regulators with respect to the development of in vitro regeneration in 'Öküzgözü' and 'Boğazkere' grape varieties.

Materials and Methods

Plant Material

Within the scope of work, wood steels belonging to 'Öküzgözü' and 'Boğazkere' grape varieties were rooted in greenhouse conditions. Shoots with the length of 5–10 cm, being obtained from the rooted steels formed the beginning material. Shoots with the length of 7–8 mm being formed of explants which are stitched to the nutrient media in single node were used in sub-culture studies.

Nutrient Medium and Sterilization

As culture starting environment, Modified MS (Murashige and Skoog 1962) nutrient medium is used, while culture nutrient medium was supported with 30 g l^{-1} sucrose, 6 g l^{-1} agar and 1 mg l^{-1} BAP. Within the scope of the study, WPM (Llyod and McCown 1980), QL (Quoirin and Lepoivre 1977) and DKW (Driver and Kuniyuki 1984) nutrient medium and 0.2–0.4–0.6–0.8–1.0–1.5 mg l^{-1} concentrations of BAP (Benzylaminopurine) were used. After explants with single nodes were prepared and washed in tap water, they were made subject to pre-sterilization for 45 s with ethanol with 70% concentration and right after that, they were rinsed off with sterile distilled water. Explants, to which surface sterilization was applied for 15 min with NaOCl (Sodium Hypochlorite 53%-Axion) with a concentration of 10%, were rinsed off for three times for 5 min each with sterile distilled water. Distilled water, blotting paper, and glass materials that were used in the study were sterilized for 2 h at 180 °C in the stove and pliers and bistouries were wrapped in aluminium foil in groups of 10 and they were sterilized at 300 °C in dry air sterilizer. pH value of nutrient medium was adjusted at 5.7 before autoclaving and sterilization was done at 121 °C for 15 min. Explants were transplanted in Magenta GA7 culture cases containing 50 ml nutrient medium.

Growth Room Conditions and Evaluation of Data

Lighting conditions of growth room are provided with white fluorescence lamps so that the lighting density would be 40 µmol m^{-2} s^{-1}. Environment temperature was adjusted as 25 ± 2 °C by controlling with air-conditioner. At the end of 4th week following the transplantation of explants at the nutrient medium, measurements of number of shoots, length of shoots (cm), number of nodes and callus ratio (%) were made.

Research was planned so that coincidence parcels would have 3 repetitions per trial design and that each repetition would be comprised of 10 explants. Results were made subject to ANOVA with SPSS 13th Statistical package program. For determining the differences, DUNCAN multi-comparison test was used.

Results and Discussion

Results obtained from the study realized with the aim of determining nutrient medium and BAP concentrations regarding in vitro regeneration of 'Öküzgözü' and 'Boğazkere' grape varieties, are given in Table 1 and 2.

In the study conducted for determining the appropriate in vitro nutrient medium for grape varieties of 'Öküzgözü' and 'Boğazkere', with respect to tbe parameters measured, the results were found to be significantly important (Table 1). For both varieties, with respect to MS nutrient medium shoot number, shoot length, node number, and callus formation ratios, for 'Öküzgözü' variety, best results obtained were 4.66–1.24–6.39 and 6%0 respectively, whereas the best results obtained for 'Boğazkere' variety were 6.28–1.15–6.81–80% respectively. In DKW nutrient medium, starting from the 2nd week of culture, obscuration began to occur in the shoots and no development could be observed. According to the studies conducted after the 1970s as relating with the micropropagation of *Vitis vinifera* L. varieties, it was determined that MS nutrient medium was the nutrient medium that was mostly used as giving the best outcome (Barlass and Skene 1978; Harris and Stevenson 1982; Gray and Benton 1991; Zlenko et al. 1995; Jaskani et al. 2008; Mukherjee et al. 2010; Abido et al. 2013; Mozafari et al. 2016). WPM and QL, which are the other nutrient media used in our study, were ranked as second and third ones with regards to the parameters which were measured. Mhatre et al. (2000), have reported that in different stages of in vitro multiplication of grape varieties of 'Thompson Seedless', 'Sonaka', 'Tas-e Ganesh', different modified forms of MS, NN and WPM were used. In the micromultiplication of 'Malagouzia' and 'Xinomavro', being the grape varieties of Greece, nutrient media combinations (MS, WPM, GAL, MS-GAL, QL-MS, QL-WPM) were focused on. It was stated that 'Malagouzia' variety regenerated better in GAL nutrient medium and that 'Xinomavro' variety regenerated better in the combined nutrient media being composed of ZL/QL-MS (Skiada et al. 2010) Furthermore, in a study conducted for the regeneration of grape varieties of 'Öküzgözü' and 'Boğazkere' from the leaf, it was stated that NN nutrient medium gave good results (Ozden et al. 2008).

In the study realized for determining the appropriate BAP concentration for grape varieties of 'Öküzgözü' and 'Boğazkere', the results obtained were found to be statistically important with regards to the parameters measured. (Table 2) For the variety of 'Öküzgözü', maximum shoot number was found out to be 4.44 pieces from 1 mg l^{-1} BAP. While shoot length of 1.41 cm and node number of 6.53 wre obtained from 0.6 mg l^{-1} BAP concentration, it was determined that breaking point was this ratio. It was observed that as concentration ratio increased, number of shoots that were smaller than 5 mm increased and that they turned into scrub shoots and that this situation had a negative impact on the number of shoots that were quality and which could be taken to sub-culture. For the variety of 'Boğazkere', the highest number of shoots was found as 6.28 and the number of nodes was found as 6.81 in 1 mg l^{-1} BAP. With regards to the shoot length, best result was obtained as 1.31 cm in 0.6 mg l^{-1} BAP concentration. In fact for both varieties it was determined that for proliferation usage of 1 mg l^{-1} BAP was appropriate and that for the elongation of shoot, usage of 0.6 mg l^{-1} BAP was appropriate. In the micropropagation studies realized as relating with different types and varieties of *Vitis*, BA and different concentrations of BAP were used. While in vitro protocols were defined for different V*itis* types and varieties, studies were also made for the optimization of cytokinins. While types and concentrations of cytokinin showed variations according to *Vitis* types, it was also revealed with studies that variations occured even for two different varieties of the same type. It was stated that for the proliferation of *V. vinifera*, BAP was the most suitable cytokinin (Mhatre et al. 2000); 2 mg l^{-1} BA was the most suitable option for grape variety of 'Cabernet Savignon' (Barlass and Skene 1978); 3 mg l^{-1} BAP was the most suitable option for in vitro multiplication of 'Baco' grape (Harris and Stevenson 1982); 1 mg l^{-1} BAP was the most suitable option for regeneration of varieties of 'Öküzgözü' ve 'Boğazkere' from the leaf (Ozden et al. 2008); 1 mg l^{-1} BA was the most suitable option for the microprolangation of grape variety of 'Perlette' (Jaskani et al. 2008); that deGrasset (*Vitis champinii* Planch.) which was used as rootstock was better than TDZ and Zeatine of 1 mg l^{-1} BAP in the in vitro multiplication (Mukherjee et al. 2010); 3 mg l^{-1} BAP was most suitable option for the multiplication of grape variety of 'Muscat Alexandria' (Abido et al. 2013); that 1 mg l^{-1} BA was the most suitable option for the proliferation of grape varieties of 'Khoshnav', 'Bidaneh Sefid' and 'Farkhi'; 0.5 mg l^{-1} BA was the most suitable option for obtaining maximum shoot length (Mozafari et al. 2016); that while 1.13 mg l^{-1} BAP was used for 'Carlos' grape being among Muscat grape varieties, it was appropriate to use 4.5 mg l^{-1} BAP, being 4 times more than the previously mentioned one, for Fry grape variety (Gray and Benton 1991); and that TDZ was more effective than BAP in the in vitro studies of Muscat grape (*V. rotundifolia*) varieties. (Sudarsono and Goldy 1988).

Conclusion

With regards to supporting the improvement studies carried out as relating with 'Öküzgözü' and 'Boğazkere', being among the important wine grape varieties of our country, with tissue cuılture which is among the important compo-

nents of plant biotechnology, in vitro regeneration studies have a significant importance. When in vitro studies carried out in viticulture are investigated, it is seen that different nutrient media and cytokinins are used. In this study which was conducted to determine the impacts of nutrient media and cytokinin concentrations on the in vitro regeneration of the two grape varieties being mentioned, among the nutrient media that were used, MS nutrient medium gave the best outcome. Among the particulars determining the success of in vitro regeneration, it was reached to the conclusion that usage of 1 mg l^{-1} BAP was appropriate in the shoot multiplication environments and that usage of 0.6 mg l^{-1} BAP was appropriate in the shoot prolongation environments.

Conflict of interest H. Yildirim and G. Ozdemir declare that they have no competing interests.

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