Choise of Explant Material and Media for *in vitro* Callus Regeneration in Sultana Grape Cultivar *(Vitis vinifera* L.*)*

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The study was carried out to choise of explant material and culture media for callus regeneration in 'Sultana' (*Vitis vinifera* L.). Leaf disc and node explants were the main explant materials which were taken from *in vitro* shoots which were obtained from macroshoot tip explants. The initial macroshoot tip were cultured on MS (Murashige and Skoog) medium including BAP (6-benzylaminopurine) (1 mg L^{-1}) and *in vitro* shoots were subcultured on shoot multiplication medium with BAP (1 mg L^{-1}) + IBA (Indole-3-butyric acid) (0.1 mg L^{-1}) In order to investigate callus regeneration potential of Sultana grape cultivar, leaf disc explants were cultured on two different MS medium including BAP in combination with 2,4-D (2,4-dichlorophenoxyacetic acid) whereas node explants cultured on four different MS medium including BAP in combination with 2,4-D and NAA (naphthalene acetic acid). The intensity of callus proliferation was greater in leaf disc culture than in node culture. In all media combinations, MS medium including BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}) were found to be the most effective on callus regeneration. In this medium, callus regeneration rate was found to be 100% and the average diameter of callus was found to be 6.3 mm.

Keywords: 'Sultana' (*Vitis vinifera* L.), tissue culture, callus, leaf disc, node

Sultani Çekirdeksiz Üzüm Çeşidinde (*Vitis vinifera* L.) *in vitro* Kallus Rejenerasyonunda Eksplant ve Ortam Seçimi Üzerinde Araştırmalar

Bu çalışmada Sultani Çekirdeksiz üzüm çeşidinde başarılı bir kallus rejenerasyonu sağlamak için eksplant materyali ve kültür ortamı seçimi üzerinde çalışılmıştır. Ana eksplant materyalleri olarak, *in vitro* makro sürgün ucu materyallerinin *in vitro* sürgünlerinden elde edilen, yaprak diskleri ve boğum parçaları kullanılmıştır. Başlangıçta, makro sürgün ucu 1 mg L^{-1} BAP (6-benzylaminopurine) içeren MS (Murashige ve Skoog) besin ortamına kültüre alınmıştır. Bunun ardından *in vitro* sürgünler BAP (1 mg L^{-1}) + IBA (Indole-3-butyric acid) (0.1 mg L^{-1}) içeren sürgün çoğaltma besin ortamına alt kültüre aktarılmıştır. Sultani Çekirdeksiz üzüm çeşidinde kallus rejenerasyon potansiyelini araştırmak için; yaprak diskleri 2,4-D (2,4-dichlorophenoxyacetic acid) ile BAP kombinasyonlarını içeren iki farklı MS ortamında, boğum parçaları ise 2,4-D ve NAA (naphthalene acetic acid) ile kombinasyon halinde BAP içeren dört farklı MS ortamında kültüre alınmıştır. Kallus çoğaltım oranı, yaprak disk eksplantında boğum eksplantına göre daha yüksek oranda gerçekleşmiştir. Tüm uygulama kombinasyonlarının içinde kallus rejenerasyonunda en etkili sonuç BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}) içeren MS ortamından elde edilmiştir. Bu ortamda ortalama kallus çapı 6.3 mm ve kallus rejenerasyon oranı ise 100% oranında bulunmuştur.

Anahtar Kelimeler: 'Sultani Çekirdeksiz' **(***Vitis vinifera* L.), doku kültürü, kallus, yaprak disk, boğum

Introduction

Grapevine has been the subject of callus culture studies aimed at obtaining the best callus production for somatic embryogenesis, genetic transformation and cell suspension culture. Callus culture based procedures also represent opportunities for breeding studies. In the literature, different organs such as anther (Perl *et al*. 1995; Nakajima *et al*. 2000; Martinelli *et al*. 2001; Lopez-Perez et al. 2005; Cutanda *et al*. 2008; Zhang et al. 2009), immature ovule (Xu et al. 2005), immature inflorescences (Lopez-Perez *et al*. 2005; Cutanda *et al*. 2008; Acanda *et al*. 2013), ovary (Lopez-Perez *et al*. 2005), leaf (Passos *et al*. 1999; Keskin and Kunter 2007-2008), leaf petiole (Tassoni *et al*. 2005), nodal and stem segments (Jaskani *et al*. 2008; Chao *et al*. 2015), tendrils (Salunkhe *et al*. 1997) were used for callus regeneration. However differences in the rate of callus initiation and proliferation intensity have been reported depending on the genotype, explant type and culture media used. (Lopez-Perez *et al*. 2005, Jaskani *et al*. 2008; Diab *et al*., 2011; Khan *et*

al. 2015). Because there is not a general protocol for grapevine callus culture, improvements are needed in cultivar specific base.

'Sultana' (*Vitis vinifera* L.) is the well-known stenospermocarpic grapevine genotype. It is used as either seedless table or dried grape. Also 'Sultana' is the progenitor of seedless hybrids. Therefore there is a particular interest in the area of *in vitro* researches in 'Sultana'. On the other little is known about callus proliferation in local research area. The aim of this study was to choice of explant material and culture media led to *in vitro* regeneration of callus in *Vitis vinifera* L. cv. Sultana.

Materials and Methods

Plant material

The study was carried on *Vitis vinifera* L. cv. Sultana, the well-known ancestral genotype of seedless table grapes. The initial materials were one year old dormant cuttings of *Vitis vinifera* L. cv. Sultana which were obtained from Kalecik Viticultural Research Station of Faculty of Agriculture, Ankara University. Two or three budded dormant canes were cultured to initiate shoot proliferation. Buds were forced to burst in controlled growth cabinet at 24 °C. After shoots appeared macroshoot tips were used as explant material. Explants (macroshoot tip) were washed in tap water during 120 min, followed by immersion in 5 and 10% (v/v) actijen supplemented with a few drops of Tween 20 for 5 and 10 min, and rinsed three times with sterile distilled water for 5 min each.

Shoot regeneration and multiplication

After sterilization macroshoot tip were excised from shoot tip then placed onto shoot regeneration medium individually in 32 x 150 mm culture tubes with 20 ml shoot regeneration medium containing of MS medium (Murashige and Skoog, 1962) with 1 mg L^{-1} BAP and 30 g L^{-1} sucrose. pH was adjusted to (5.6-5.8) before sterilization at 121 °C and 102.97 kPa for 20 min. Plant growth regulators (BAP, IBA, NAA, 2,4-D) added to the nutrient medium were sterilized using a filter syringe, 0.2 µm in size, in a laminar flow hood. For regeneration, the culture tubes were incubated in culture room at 24±1 ºC with 16h photoperiod for 4 weeks. After 3-4 weeks of establishment of *in vitro* shoots regeneration, the healthy shoots were transferred on shoot multiplication MS medium supplemented with BAP (1 mg L^{-1}) + IBA (0.1 mg L^{-1}). Cultures were incubated at 24±1 °C with a 16-h photoperiod under 2.000-2.500 lux light intensity provided by cool-white fluorescent. Cultures were evaluated after four weeks of culture and observed on a weekly basis for necrosis, bacterial and fungal contamination, and explant survival rate.

Callus regeneration

After subculture, callus cultures were formed with leaf disc and node explants from the 'Sultana' (*Vitis vinifera* L.). Callus was initiated in 15 x 90 mm petri dishes containing 20 ml of MS medium supplemented with different concentrations and combinations of plant growth regulators. Callus incubated in dark at 24±1 °C were subcultured twice with 4-6 weeks. In order to investigate callus regeneration potential of 'Sultana', leaf disc explants were cultured on two different MS medium including BAP in combination with 2,4-D, whereas node explants cultured on four different MS medium including BAP in combination with 2,4-D and NAA.

Statistical analysis

All analyses were performed in 3 replications. All data were subjected to analysis by two-way analysis of variance (ANOVA) of SPSS Version16.0 (Snedecor and Cochran, 1980).

Results and Discussion

Callus regeneration was attempted by used various initial explants on different basal nutrient media and PGR's *in vitro*. In the present study, the optimum conditions for callus culture was determined by applying two different explant type (leaf disc and node) and three different callus culture medias. In first application, leaf disc explants were cultured in MS medium including 5, 10 and 15 µM NAA. After 30 days, no callus formation was found in either medium type (Table 1). The findings of Jaskani *et al*. (2008) have shown percentage of callus induction rate differs to explant type and the media used for culture. In their study, callus induction rate derived from node explant was found to be 70% and 80% in media containing 5 and 10 µM NAA respectively whereas 40% and 50% for leaf disc explants.

In the second application, leaf disc explants were planted in MS medium modified by Babalık and Baydar (2008) containing BAP (0,5 mg L^{-1}) + 2,4-D (1 mg L^{-1}) and BAP (1 mg L^{-1}) + 2,4-D (2 mg L^{-1}). Callus culture was incubated under dark conditions at 24 °C and no callus formation was observed 15

days later. Callus regeneration rate (57%) was observed on MS medium supplemented with 2,4-D (2 mg L^{-1}) + BAP (0.3 mg L^{-1}) + NAA (0.2 mg L^{-1}) derived from with node explant under dark conditions (Table 2). In contrast to our results Babalık and Baydar (2008), were found higher callus regeneration rate from stem and leaf stalk explants (62.50% and 100%, respectively) under dark conditions on this medium 2,4-D (2 mg L^{-1}) + BAP (0.3 mg L^{-1}) + NAA (0.2 mg L^{-1}). Babalık and Baydar (2008) also reported to the best callus regeneration (97.56%) was obtained in the MS medium supplemented with BAP (1 mg L^{-1}) + 2,4-D (2 mg L^{-1}) + casein hydrolysate (1 g L^{-1}) derived from leaf stalk explants for 'Kalecik Karası' (*Vitis vinifera* L.) under dark conditions. Passos *et al*. (1999) described to found to rate of callus regeneration (100%) derived from leaf disc explants in the MS medium supplemented with NAA (4 mg L^{-1}) and TDZ (0.9 mg L^{-1}) for 'Seyve Villard 5276' (*Vitis* sp.).

In the third application, callus culture was established using leaf and node explants in two different MS medium including BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}) modified by Keskin and Kunter (2007) and 2,4-D (2 mg L^{-1})+ BAP (0.3 mg L^{-1}) + NAA (0.2 mg L^{-1}) (Khan *et al*., 2015) and 2,4-D (4 mg L^{-1}) + BAP (0.6 mg L^{-1}) + NAA (0.4 mg L^{-1}). In the third application, callus regeneration rates were %100, %70 and %93, respectively in 'Sultana' (*Vitis vinifera* L.) (Table 2). Callus regeneration rate (70%) was found in the MS medium including 2,4-D (2 mg L^{-1}) + BAP (0.3 mg L^{-1}) + NAA (0.2 mg L^{-1}) derived from with node explant (Table 2). Results of this study were similar with the findings of Khan *et al*. (2015), whose also observed callus regeneration rate at 73% on MS medium including 2,4-D (2 mg L^{-1}) + BAP (0.3 mg L^{-1}) + NAA (0.2 mg L^{-1}).

In our study, among media combinations, the most effective callus texture was found in the MS medium including BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}) from node explant. Also callus size was larger than the other MS medium. Callus induction was initiated in the 4^{th}-6^{th}. weeks of culture and average callus size was 6.3 mm in this medium. Calluses derived from node explant type were green or white and friable whereas those derived from leaf disc explant type were became brown and necrotic after subcultures. Best callus regeneration response (93%) derived from with leaf disc explant was observed callus cultured on MS medium supplemented with BAP (0.5 mg L^{-1}) + 2,4-D (1 mg L^{-1}) (Table 1). Therefore the quality of regenerated callus materials could be used for cell culture.

Table 1. Effect of different concentrations of various PGRs on leaf disc explant on callus regeneration

Growth regulator combinations	**Number of explants cultured****	**Number of calluses induced****	**Frequency (%) of callus regeneration****
5 mg L^{-1} NAA	20	no callus formation	0.00
10 mg L^{-1} NAA	20	no callus formation	0.00
15 mg L^{-1} NAA	20	no callus formation	0.00
0,5 mg L^{-1} BAP + 1 mg L^{-1} 2,4-D	14	13 a	93.00 a
1 mg L^{-1} BAP + 2 mg L^{-1} 2,4-D	14	8 b	57.00 b
F			no results (*p*=0,000)

** p<0.01, Data represent mean of 3 repeats.

Table 2. Effect of different concentrations of various PGRs on node explant on callus regeneration

Growth regulator combinations	**Number of explants** cultured**	**Number of calluses** induced**	**Frequency (%) of callus** regeneration**
1 mg L^{-1} BAP + 2 mg L^{-1} 2,4-D	15	14	93.00 b
1 mg L^{-1} BAP + 0,1 mg L^{-1} 2,4-D	33	33	100.00 b
2 mg L^{-1} 2,4-D + 0,3 mg L^{-1} BAP + 0,2 mg L^{-1} NAA	33	23	70.00 a
4 mg L^{-1} 2,4-D + 0,6 mg L^{-1} BAP + 0,4 mg L^{-1} NAA	98	93	97.00 b
F			69,790 (*p*=0,000)

**p<0.01, Data represent mean of 3 repeats.

Conclusions

The findings obtained from this study and discussions of these findings with the results of the previous literature were supported that callus regeneration ratio and quality depend on the explant material and media. In this study, node explant was found to be the best explant type for callus regeneration. Callus regeneration rate of node explant has been 100% on the MS medium including BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}). As the result node explant regenerated on MS medium including BAP (1 mg L^{-1}) + 2,4-D (0.1 mg L^{-1}) could be recommended for callus regeneration study in Sultana grape cv.

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