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# Micropropagation of grapevine (*Vitis vinifera* L.) seedless cultivar 'Parvana' through lateral bud development

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## Summary

**Factors affecting successful establishment *in vitro*, rapid proliferation, rooting and acclimatization of grapevine seedless cultivation 'Parvana' were studied. A treatment combination of 2.0 % calcium hypochlorite for 7 minutes plus 70 % ethanol for 3 minutes was found to be effective with 75.0 % of aseptic culture establishment *in vitro*. The highest number of new microshoots (4.5) was obtained in a culture medium (Gambourg's B5 modification) supplemented with 0.6 mg·L$^{-1}$ BAP + 0.2 mg·L$^{-1}$ KIN + 0.5 mg·L$^{-1}$ GA3. Presence of GA3 in combination with BAP and KIN promoted elongation of shoots. A concentration of 0.4 mg/l IAA in half strength of MS (Murashige and Skoog) proved to be the best for rooting (84.4 % root induction, 4.66 roots with 7.9 cm length). In vitro rooted plantlets were successfully acclimatized, with 82.2 % survival rate in plastic pots containing garden soil, sand and peat moss(1:1:1). In this study an efficient micro propagation technique of grapevine seedless cultivar 'Parvana' was successfully achieved through lateral bud culture. Such a technique would be useful for large scale plant production and in vitro conservation.**

K e y w o r d s : *in vitro*; micropropagation; explants; grapevine; plant regeneration; lateral bud.

## Introduction

Grapevine (*Vitis vinifera* L.) is one of the most important fruit crop grown in Armenia. *In vitro* propagation is an alternative method to propagate grapevines and has several advantages over traditional propagation methods. It allows the large-scale production of plantlets of high genetic and phytosanitary quality in a short time, small area and no matter of the season. The numerous methods for grapevine *in vitro* propagation have been described (Fanizza *et al.*1984, Gray and Fisher 1985; Guta *et al.* 2008). After the independence of the Republic of Armenia in 1991, the vineyards area significantly decreased and a lot of valuable grape cultivars were endangered among of which was 'Parvana'. Now there are only a few individual plants of 'Parvana' cultivated in some farmer vineyards, whereas this cultivar due to the large, uniform fruits would be of great interest for the domestic market. *In vitro* propagation offers another method for increasing availability of plant material of this cultivar. The purpose of this study was to develop an efficient micropropagation technique *i.e.* explants sterilization, shoot regeneration, rooting, acclimatization for grapevine seedless cultivar 'Parvana' through lateral bud both for large scale plant production and *in vitro* conservation.

## Material and Methods

'Parvana', a seedless table grapevine cultivar for eating as fresh fruit or raisins, was constituted in 1966 by the Armenian Scientific Research Institute of Viticulture, Wine-making, Fruit growing crossing 'Katta Kurgan' and 'Kishmish Khishrau' cultivars. The research was conducted in the tissue culture laboratory of the Scientific Center of Agrobiotechnology, ANAU.

Explants preparation and sterilization: Nodal explants bearing a single lateral bud were used to initiate shoot culture. Explants were sterilized in a 1-3 % solution (5, 7, 15 min) of calcium hypochlorite containing one drop of Tween 20, followed by immersion in 70 % ethanol at various time scale (3, 7 min).

C u l t u r e e s t a b l i s h m e n t : Modified Gamborg's B5 medium with different levels and combinations of PGRs (Plant Growth Regulators): 6-benzilaminopurine - BAP (0.5; 0.6; 1.0 mg·L$^{-1}$); N6-furfuryladenine - KIN (0.2; 0.5 mg·L$^{-1}$) and gibberellic acid - $GA_3$ (0.5; 1.0 mg·L$^{-1}$) was used for regeneration. Fifteen explants were used for each treatment and experiment was repeated three times. Observations for shoot proliferation were evaluated 5 weeks after the beginning of the experiment, and the number of shoots per explant and length of shoots were recorded.

R o o t f o r m a t i o n : Various concentrations (0; 0.2; 0.3; 0.4; 0.5; 1.0 mg·L$^{-1}$) of 3-indolacetic acid (IAA) were supplemented to the half strength of Murashige and Skoog (MS/2) agar-solidified medium for root formation. The rooting percentage, average number and length (cm) of roots for each rooted shoot were evaluated after 21 d of culture on rooting medium.

A c c l i m a t i z a t i o n : The rooted plantlets were transferred to plastic pots containing garden soil, sand and peat moss (1:1:1), covered with transparent polythene bags and placed for acclimatization at 25 ± 2 °C with 80-90 % relative humidity. After 10 d, transparent bags were gradually removed from pots for proper hardening. The plant survival was evaluated after 30 d of their transfer to *ex vitro* condition. Correspondingly data were pooled from three independent experiments and expressed as the mean.

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Treatment means were compared with the standard error (SE) of the mean, the student's *t*-test from GraphPad software was used to find significant differences between the means.

## Results and Discussion

The efficiency and successful rate of plant tissue culture relies on several factors such as a selection of sterilizing agents, exposure period, types and source of explants (Garg *et al*., 2014). The resulting effects of different concentrations of sterilants and exposure time on surface sterilization of the explants are displayed in Tab. 1. The results of this study showed, that with the increasing of the concentration and exposure time of sterilants the infection was decreased, but the number of non growing cultures increased. The high number (20 or 55.5 %) of non growing culture was obtained when explants were surface sterilized with 3.0 % $Ca(OCl)_2$ (15 min) + 70 % ethanol (7 min) and the lowest mean (1 or 2.3 %) was obtained by treating the explants with 1.0 % $Ca(OCl)_2$ (5 min) + 70 % ethanol for 3 min. A treatment combination of 2.0 % calcium hypochlorite for 7 min plus 70 % ethanol for 3 min was found to be effective with 75.0 % of aseptic culture establishment *in vitro*. KALADHAR (2010) found almost the same combination and concentration of sterilants was effective for sterilization of explants in the other plant species *Tridenteta* L. From the data presented in the Tab. 2 it is clear, that the number of shoots newly formed from lateral buds varied with the concentration of different PGRs and their concentration. In this respect, YERBOLOVA *et al.* (2013) also reported that the *in vitro* regeneration efficiency of the grape cultivars is not identical and depends on hormonal composition of the medium. Received data showed that studied cultivar survived and formed shoots (90-100 %) on all the tested media, but on medium without PGRs explants did not form shoots. The highest number of microshoots/explant (4.5) was obtained in a culture medium supplemented with 0.6 $mg \cdot L^{-1}$ BAP + 0.2 $mg \cdot L^{-1}$ KIN + 0.5 $mg \cdot L^{-1}$ $GA_3$. Presence of $GA_3$ in combination with BAP and KIN promoted elongation of shoots. ZHOU and LIU (2009) indicated that the medium supplemented with 0.5 $mg \cdot L^{-1}$ $GA_3$ can significantly increase the elongation of shoots in Chinese Jujube.

In our study the maximum length of shoots was 3.1, 3.4 and 3.8 cm in combinations of 0.6 $mg \cdot L^{-1}$ BAP + 0.5 $mg \cdot L^{-1}$ $GA_3$ + 0.2 $mg \cdot L^{-1}$ KIN; 0.6 $mg \cdot L^{-1}$ BAP + 0.5 $mg \cdot L^{-1}$ $GA_3$ and 0.5 $mg \cdot L^{-1}$ BAP + 1.0 $mg \cdot L^{-1}$ $GA_3$ respectively. Earlier MARTINEZ and TIZIO (1989) reported that combination of 1.0 $mg \cdot L^{-1}$ BAP + 0.5 $mg \cdot L^{-1}$ $GA_3$ was the most useful for *in vitro* culture ability of seven different grape varieties.

The success of micropropagation of woody and shrub plants depends on the rooting ability of the plants. Data presented in the Tab. 2 indicated that culture media with and without IAA induced roots of cultivar 'Parvana'. SAJID *et al.* (2006) observed that root induction of grape cultivars possible on hormone free media, however they indicated also that the presence of NAA or IAA in the media had a profound effect on the root induction. The maximum average number (33.81; 4.66) of roots per shoot was recorded for 0.3 and 0.4 $mg \cdot L^{-1}$ IAA respectively. The maximum average root length (6.9; 7.9, 6.3 cm) was obtained in a culture medium supplemented with 0.3; 0.4 and 0.5 $mg \cdot L^{-1}$

Table 1

Effect of sterilization of explants on culture The experiment was repeated 3 times with 12 explants in each replication for a total of 36 explants. The observations were recorded regularly for 30 d for the non growing cultures, infected cultures and healthy cultures

| No. | Sterilants / Strength | | | Number of contaminated explants | Number of survived and growing explants | Number of non growing cultures |
|---|---|---|---|---|---|---|
| | $Ca(OCl)_2$ | | Ethanol | | | |
| | (%) | (min) | (min) | | | |
| 1 | 1 | 5 | 3 | 28 (77.8 %) | 7 (19.4 %) | 1 (2.3 %) |
| 2 | 1 | 7 | 3 | 29 (80.5 %) | 5 (13.9 %) | 2 (5.5 %) |
| 3 | 1 | 15 | 3 | 24 (66.7 %) | 9 (25 %) | 3 (8.3 %) |
| 4 | 1 | 5 | 7 | 22 (61.1 %) | 10 (27.8 %) | 4 (11.1 %) |
| 5 | 1 | 7 | 7 | 20 (55.5 %) | 12 (33.3 %) | 4 (11.1 %) |
| 6 | 1 | 15 | 7 | 22 (61.1 %) | 9 (25 %) | 5 (13.8 %) |
| 7 | 2 | 5 | 3 | 13 (36.1 %) | 20 (55.5 %) | 3 (8.3 %) |
| 8 | 2 | 7 | 3 | 7 (19.4 %) | 27 (75.0 %) | 2 (5.5 %) |
| 9 | 2 | 15 | 3 | 7 (19.4 %) | 19 (52.8 %) | 10 (27.8 %) |
| 10 | 2 | 5 | 7 | 11 (30.5 %) | 20 (55.5 %) | 5 (8.3 %) |
| 11 | 2 | 7 | 7 | 8 (22.2 %) | 18 (50 %) | 10 (27.8 %) |
| 12 | 2 | 15 | 7 | 9 (25.0 %) | 16 (44.4 %) | 11 (30.5 %) |
| 13 | 3 | 5 | 3 | 10 (27.8 %) | 19 (52.8 %) | 7 (19.4 %) |
| 14 | 3 | 7 | 3 | 8 (22.2 %) | 18 (50.0 %) | 10 (27.8 %) |
| 15 | 3 | 15 | 3 | 3 (8.3 %) | 18 (50.0 %) | 15 (41.7 %) |
| 16 | 3 | 5 | 7 | 8 (22.8 %) | 18 (50.0 %) | 10 (27.8 %) |
| 17 | 3 | 7 | 7 | 5 (13.9 %) | 17 (47.2 %) | 14 (38.9 %) |
| 18 | 3 | 15 | 7 | 4 (11.1 %) | 12 (33.3 %) | 20 (55.5%) |

Table 2

Effect of plant growth regulators on in vitro shoot regeneration and rooting of microshoots: 15 explants and 15 microshoots were used for each treatment respectively for regeneration and rooting. The experiment was repeated three times. The values followed by the same letter are not significantly different at $p < 0.05$

| Concentration of Plant Growth Regulators (PRGs) | Regeneration (%) | Number of shoots per explant (mean ± SE) | Length of shoots (cm) (mean ± SE) |
|---|---|---|---|
| Medium without PGRs | - | - | - |
| BAP 0.5 mg·L$^{-1}$ + $GA_3$ 0.5 mg·L$^{-1}$ | 95.5 | 1.2 ± 0.1$^{a}$ | 2.8 ± 0.1$^{a}$ |
| BAP 0.6 mg·L$^{-1}$ + $GA_3$ 0.5 mg·L$^{-1}$ | 93.3 | 1.2 ± 0.03$^{a}$ | 3.4 ± 0.1$^{b}$ |
| BA P 1.0 mg·L$^{-1}$ + $GA_3$ 0.5 mg·L$^{-1}$ | 100.0 | 1.5 ± 0.03$^{b}$ | 3.2 ± 0.2$^{ab}$ |
| BAP 0.5 mg·L$^{-1}$ + $GA_3$ 1.0 mg·L$^{-1}$ | 91.1 | 1.13 ± 0.1$^{a}$ | 3.8 ± 0.2$^{b}$ |
| BAP 0.6 mg·L$^{-1}$ + $GA_3$ 1.0 mg·L$^{-1}$ | 100.0 | 1.3 ± 0.1$^{a}$ | 3.3 ± 0.1$^{b}$ |
| BAP 1.0 mg·L$^{-1}$ + $GA_3$ 1.0 mg·L$^{-1}$ | 93.3 | 1.3 ± 0.04$^{a}$ | 3.0 ± 0.2$^{a}$ |
| BAP 0.5 mg·L$^{-1}$ + KIN 0.2 mg·L$^{-1}$ | 100.0 | 2.4 ± 0.1$^{c}$ | 1.9 ± 0.1$^{c}$ |
| BAP 0.6 mg·L$^{-1}$ + KIN 0.2 mg·L$^{-1}$ | 100.0 | 3.0 ± 0.1$^{d}$ | 2.2 ± 0.2$^{c}$ |
| BAP 1.0 mg·L$^{-1}$ + KIN 0.2 mg·L$^{-1}$ | 88.9 | 1.6 ± 0.04$^{eb}$ | 2.0 ± 0.1$^{c}$ |
| BAP 0.5 mg·L$^{-1}$ + KIN 0.2 mg·L$^{-1}$ + $GA_3$ 0.5 mg·L$^{-1}$ | 93.3 | 3.6 ± 0.1$^{f}$ | 2.6 ± 0.2$^{ab}$ |
| BAP 0.6 mg·L$^{-1}$ + KIN 0.2 mg·L$^{-1}$ + $GA_3$ 0.5 mg·L$^{-1}$ | 100.0 | 4.5 ± 0.1$^{g}$ | 3.1 ± 0.1$^{b}$ |
| BAP 1.0 mg·L$^{-1}$ + KIN 0.2 mg·L$^{-1}$ + $GA_3$ 0.5 mg·L$^{-1}$ | 100.0 | 1.5 ± 0.1$^{be}$ | 2.5 ± 0.1$^{a}$ |
| | Rooting (%) | Number of roots per shoot (mean ± SE) | Length of roots (cm) (mean ± SE) |
| Medium without IAA | 44.4 | 1.7 ± 0.1$^{a}$ | 3.6 ± 0.2$^{a}$ |
| IAA 0.1 mg·L$^{-1}$ | 51.1 | 2.1 ± 0.1$^{a}$ | 3.5 ± 0.3$^{a}$ |
| IAA 0.2 mg·L$^{-1}$ | 48.9 | 3.5 ± 0.1$^{b}$ | 3.9 ± 0.1$^{a}$ |
| IAA 0.3 mg·L$^{-1}$ | 55.0 | 3.8 ± 0.2$^{b}$ | 6.9 ± 0.3$^{b}$ |
| IAA 0.4 mg·L$^{-1}$ | 84.4 | 4.7 ± 0.1$^{d}$ | 7.9 ± 0.4$^{d}$ |
| IAA 0.5 mg·L$^{-1}$ | 60.0 | 3.6 ± 0.2$^{b}$ | 6.3 ± 0.2$^{b}$ |
| IAA 1.0 mg·L$^{-1}$ | 46.7 | 3.0 ± 0.1$^{c}$ | 2.6 ± 0.2$^{c}$ |

IAA respectively. A lowest root length (2.6 cm) was observed in concentration of 1.0 mg·L$^{-1}$ IAA, with callus formation. IAA at 0.4 mg·L$^{-1}$ was found to be optimum for root induction (84.4 % root induction, 4.66 roots/explant with 7.9 cm length).

In fact, success of the most hazardous step of propagation, *i.e.* transfer and acclimatization of *in vitro* rooted plants to an *ex vitro* environment, depends on the quality of the root system. *In vitro* rooted plantlets were successfully acclimatized, with 82 % survival rate in plastic pots containing garden soil, sand and peat moss (1:1:1).

## Conclusions

The results obtained in the present work demonstrated the efficient micropropagation of grapevine (*Vitis vini-fera* L.) cultivar 'Parvana' through lateral bud development. These techniques can be widely applied, not only to increase propagation rates but also for the conservation of germplasm.

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