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ORIGINAL RESEARCH

Direct organogenesis *in vitro* for mass propagation and conservation of grapevine cv. 'Merdzavani Vaghahas'

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Abstract. An efficient *in vitro* mass propagation and conservation protocol was developed for grapevine (*Vitis vinifera* L.) cultivar 'Merdzavani Vaghahas' through direct organogenesis. Maximum quantity of aseptic cultures was obtained when nodal segments were treated with 70% ethanol for 30 s + 2.0% $Ca(ClO)_2$ for 15 min Explants were cultured on Murashige and Skoog (MS) medium sup-plemented with different concentrations and combinations of Benzilaminopurin (BAP), Gibberellic acid (GA_3) and Kinetin (Kin) for multiplying axillary shoots. The maximum number (3.8) of shoot formation and the maximum shoot length with average of 2.7 cm appeared on medium supplemented with 0.5 mg/l BAP + 0.5 mg/l Kin + 0.5 mg/l GA_3. Best rooting responses were obtained on ½ MS medium + 0.8 mg/l IBA (Indole-3-butyric acid) resulting mean value of 8.6 ± 0.5 root number and 14.4 ± 0.6 cm root length. For *in vitro* conservation and storage, two temperature regimes (15°C and 24°C) were used. The plants maintained at 15°C were smaller than those at 24°C. The *in vitro* plants were maintained at 15°C for more than 8 months without subculture. *In vitro* rooted plantlets were hardened successfully using perlite, forest soil and bio humus in 2:1:0 proportions with 88.0% survival. The described method can be successfully used for large-scale propagation and *in vitro* conservation of grapevine cv. 'Merdzavani Vaghahas'.

Key words: grapes; direct organogenesis; tissue culture; micropropagation; acclimatization; *in vitro*.

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Прямой органогенез *in vitro* сорта винограда Мердзавани Вагаас для массового размножения и сохранения

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Аннотация. Разработан эффективный протокол для массового размножения методом прямого органогенеза и дальнейшего сохранения *in vitro* виноградной лозы (*Vitis vinifera* L.) сорта Мердзавани Вагаас. Максимальное количество асептических культур было получено при обработке узловых сегментов 70%-ным этанолом в течение 30 с + 2,0%-ным $Ca(ClO)_2$ в течение 15 мин. Для размножения пазушных побегов экспланты культивировали на среде Мурасиге-Скуга (МС) с добавлением различных концентраций и комбинаций бензиламинопурина (БАП), гиббереллиновой кислоты ($ГК_3$) и кинетина (Кин). Наибольшее количество (3,8) побегообразования и максимальная длина побегов, в среднем 2,7 см, наблюдались на среде с добавлением 0,5 мг/л БАП + 0,5 мг/л Кин + 0,5 мг/л $ГК_3$. Максимальный ризогенез был получен на среде ½ МС + 0,8 мг/л ИМК (Индолил-3-масляная кислота). Среднее значение количества корней составило 8,6 ± 0,5 шт., а длина корней 14,4 ± 0,6 см. Для консервации, сохранения *in vitro* использовали два режима температуры, 15°C и 24°C. Высота растений, выдерживаемых при 15°C, была ниже, чем хранящихся в условиях 24°C. Растения *in vitro* поддерживались при температуре 15°C более 8 мес., без пересадок. Укоренившиеся *in vitro* проростки были успешно адаптированы *in vivo* с использованием перлита, лесной почвы и биогумуса в пропорциях 2:1:0 с приживаемостью 88,0%. Разработанный метод может быть успешно использован для крупномасштабного размножения и хранения *in vitro* винограда сорта Мердзавани Вагаас.

Ключевые слова: виноград; прямой органогенез; культура тканей; микроразмножение; акклиматизация; *in vitro*.

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Introduction

Grapevine (*Vitis vinifera* L.) is one of the main important fruit crops cultivated worldwide.

Presently, grapevines are highly susceptible to diseases in the general method of propagation (by hardwood cuttings).

Micropropagation of grapes has played a very significant role in multiplication of cultivars with desirable traits and production of healthy plants. Propagation of

uniform, disease free plants is produced in a relatively short period and space because this process is independent of the time of year and weather conditions.

In grapevine, there are various reports which indicate rapid *in vitro* regeneration and multiplication via tissue culture [1–3].

Plant germplasm conservation is the most successful method to conserve the genetic traits of endangered and commercially valuable species and plant varieties for future proliferation, development and it is also the basis of agricultural production [4].

This investigation is aimed at developing an efficient method for mass micropropagation of true-to-type grapevine plants of Armenian grapevine cultivar 'Merdzavani Vaghahas' through direct organogenesis and determining the optimal methods for *in vitro* conservation.

Objects and methods of research

The studies were performed at the Scientific Center of Agrobiotechnology of Armeniam National Agrarian University. Grapevine cultivar 'Merdzavani Vaghahas' was selected for *in vitro* studies.

'Merdzavani Vaghahas' is an Armenian grapevine table variety with muscat flavor, was created in 1965 by the Armenian Scientific Research Institute of Viticulture, Wine-making, Fruit Growing by crossing 'C 484' ('Madeline Angevine' x 'Chasselas Musque') x 'Kishmish Khishrau' ('Nimerang' x 'Kishmish Noir').

The plant materials of this research were obtained from the Armenian National Field Collection of Grapevine.

Explants sterilization: The nodal segments were treated to 1) 70% ethanol, 2) 2.0% calcium hypochlorite and their combinations for varying time durations: T1) ethanol (70%) for 5 min, T2) ethanol (70%) for 10 min, T3) calcium hypochlorite (2.0%) for 10 min, T4) calcium hypochlorite (2.0%) for 15 min, T5) calcium hypochlorite (2.0%) for 20 min, T6) ethanol (70%) for 30 s + 2.0% $Ca(OCl)_2$ for 15 min, T7) ethanol 70% for 30 s + 2.0% $Ca(OCl)_2$ for 20 min.

Culture establishment: Murashige and Skoog (MS) basal medium was used through *in vitro* culture of plantlets. Nodal segments, approximately 0.3 cm were cultured on full–strength modified MS medium supplemented with different combinations and concentrations (0.0; 0.5; 1.0 mg/l) of benzilaminopurin (BAP), (Kin) kinetin (0.0; 0.5; 1.0 mg/l) and Gibberelin acid (GA_3) (0.0; 0.5; 1.0 mg/l) for multiplying axillary shoots. Observations for shoot proliferation were evaluated 35 days after the beginning of the experiment, and the number of shoots per explant and length of shoots were recorded.

Root formation: Half-strength MS basal medium supplemented with different concentrations (0.3; 0.5; 0.8 and 1.0 mg/l) of IBA were used for rooting stage. Medium without auxin was used as a control. Percentage of rooting; mean number and length (cm) of roots for each rooted shoot were evaluated after 30 days of cultivation on the rooting medium.

The pH of the media was adjusted to 5.8 before adding agar. Media were gelled with 0.5% agar. The cultures were incubated at 26 ± 1°C under cool white fluorescent light for a daily 16 h photoperiod. Twenty explants were used for each treatment, experiment was repeated three times.

For *in vitro* conservation the effects of temperature on growth of *in vitro* plants were investigated using MS medium supplemented with 20 g/l sucrose, 50 mg/l ascorbic acid, 0.1 mg/l IBA. The plants were kept in two growth chambers with the temperature adjusted to 15°C and to 24°C, light intensity of 2000 lux. After 6 month, 12 plants, from each temperature culture condition, were assigned to biometric analyses: length of the longest root, numbers of roots and length of the shoots.

Ex vitro acclimatization: For ex vitro acclimatization the rooted plantlets were taken out from the test tubes and gently washed to free agar from surface. Well-developed plantlets (>10 cm of shoots and >8-10 cm of roots) were transferred to plastic pots containing mixture of perlite, forest soil and bio humus in different proportion

1)1:1:0; 2)1:1:1; 3)1:2:1; 4)2:1:0; 5)1:1:2; 6)1:2:0.

The plantlets were maintained for 3 weeks at about 25°C to 27°C under high humidity (>75%) by covering the pots with transparent polyethylene sheets. After two weeks the covers were removed from the pots.

Statistical analysis: Experiments were repeated three times. The results are expressed as mean ± standard error. The differences among averages of the recorded parameters for all treatments were tested for significance at 5% level.

Results and discussion

The study results showed that on increasing the exposure time of ethanol (70%) and calcium hypochlorite (2.0%) the infection was decreasing in both chemicals, but in the same time the number of non-growing cultures was increasing. Higher (10 min) duration of 70% ethanol (T2) showed lower infection (12.3%) and maximum non-growing cultures (36.7%). In case of 5 min duration (T1), the percentage of infected and non-growing cultures was correspondingly 47.7% and 9.0%. Explants treated with 2.0% calcium hypochlorite for 10 min (T3) showed 47.7% contamination and 6.7% non-growing cultures, while 2.0% calcium hypochlorite for 15 min (T4) and 20 min (T5) were correspondently 22.3%, 20.0% and 10.3%, 26.7%. The minimum survival (43.3%) was observed with 70% ethanol and 5 min exposure time. Maximum healthy cultures (80.0%) were recorded when nodal segments were treated with 70% ethanol for 30 s + 2.0% $Ca(ClO)_2$ for 15 min (T6) followed by ethanol 70% for 30 sec + 2.0% $Ca(OCl)_2$ for 20 min (T7) with 68.3% survival. Improper choice of sterilant exposure time has lethal effect on plant cells and limits the development of explants.

Regeneration of grapevine is possible through both organogenesis and embryogenesis. Explants including shoot tips, buds, leaves and tendrils can regenerate into somatic embryos directly or indirectly through the callus phase [5].

In the present study regeneration was processed through direct organogenesis from bud explants. Somatic embryogenesis was strongly influenced by Plant Growth Regulators (PGR). The effects of various concentrations and combinations of PGR in full strength modified MS medium on shoot regeneration were evaluated (Table).

Shoot proliferation (shoots/explants) and shoot length (cm) was recorded after 35 days.

Nutrient medium MS without PGRs did not show any shoot induction. Shoot formation was noted in all concentrations and combinations of growth regulators, but the number and length of shoots per explant varied. Medium containing 0.5 mg/l BAP combined with 0.5 mg/l Kin and 0.5 mg/l GA_3 showed the maximum frequency of shoot regeneration, with an average shoot length of 2.7 cm and an average of 3.8 shoots per explant. The regenerated plants did not show any obvious phenotypic variation.

Presence of GA_3 in combination with BAP and Kin promoted elongation of shoots. This is in conformity with the observation by D.H. Tejavathi and B.L. Manjula (2010) in *Ruta graveolens* L. [6].

Auxin is a key regulator of plant growth and development. Because of its effective impact on cell division, cell growth and differentiation, auxin is generally used for artificially controlling plant growth [7, 8].

As shown in Fig. 1, the differences in rooting percentage, root number and length were significant among different treatments. Highest rooting percentage (85.5%) was observed in ½ MS medium fortified with 0.8 mg/l IBA followed by ½ MS + 0.5 mg/l IBA (80.6%). Root induction was not observed in the control (medium without IBA). An IBA concentration of 0.8 mg/l gave a greater number of roots (8.6 ± 0.5) and maximum root length (14.4 ± 0.6 cm) (Fig. 3, a) than other concentrations (0.3; 0.5; 1.0 mg/l). Beura (2003) also reported that ½ MS supplied with 0.5–1.0 mg/l IBA was the best for *in vitro* rooting for the most of horticulture crops [9].

Temperature is a critical environmental factor regulating *in vitro* plant growth and development. Temperature influences plant tissue culture and micropropagation. Plants kept at 15°C showed significantly smaller length and less dry mass of both roots and shoots, in relation to those kept at 24°C, thus demonstrating the influence of low temperature on growth. An average of 90% of *in vitro* plants of cv. 'Merdzavani Vaghahas' were efficiently stored for 8 months at 15°C without subculture on half strength MS medium containing 30 g/l sucrose in culture tubes, while at 24°C it was required every 1.5–2.0 months.

An important, and often limiting, part of any micropropagation system is the transition from *in vitro* to *ex vitro* growth or acclimatization [10].

Statistical analysis of variance showed that the effect of substrate mixtures was significant on survival rate of ex vitro acclimatized plantlets. Among the different types and combinations of substrate used for adaptation, a combination of perlite, forest soil and bio humus (2:1:0) was found superior, with higher survival rate of plantlets (88.0%). The lowest (67.0%) survival was recorded in substrate mixture with 1:2:1 ratio (Fig. 2).

Plantlets grown on T4 substrate mixture exhibited vigorous growth and deep green leaves (Fig. 3).

Conclusions

This study presents a rapid and efficient method for micropropagation of grapevine cv. 'Merdzavani Vaghahas' from nodal explants, which carries a high potential for rapid multiple shoot regeneration and subsequent *in vitro* propagation. It was revealed that the productivity of plant regeneration and root formation depends on the applied phytohormones and their concentration. A low incubation temperature of 15°C reduces culture growth and surv ival up to 8 months of time. The described protocol can be used not only as an alternative means for mass propagation of this cultivar, but also for *in vitro* conservation.

Table. The effect of PGRs treatment on shoot regeneration from nodal explants of grapevine (*Vitis vinifera* L.) cv. 'Merdzavani Vaghahas'

Таблица. Влияние обработки PGR на регенерацию побегов узловых эксплантов винограда (*Vitis vinifera* L.) сорта Мердзавани Вагаас

PGRs (mg/L)			Shoot proliferation (shoots/explant) (Mean ±SE)	Shoot length (cm) (Mean ±SE)
Kin	BAP	GA_3		
-	-	-	-	-
-	0.5	-	1.4±0.1	2.0±0.2
-	1.0	-	1.9±0.2	2.3±0.2
-	0.5	0.5	1.3±0.1	2.5±0. 2
-	1.0	1.0	2.1±0.1	1.9 ±0.4
0.5	-	-	1.0±0.2	1.8 ±0.2
1.0	-	-	1.5±0.1	1.6±0.4
0.5	-	0.5	1.1±0.3	2.4±0.2
1.0	-	1.0	1.7±0.2	2.9±0.1
0.5	0.5	0.5	3.8±0.3	2.7 ±0.2
0.5	0.5	-	3.4±0.4	1.3±0.1

- No growth regulators added

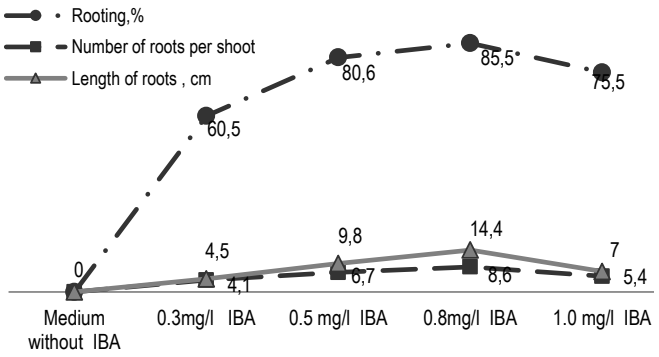

Fig. 1. Influence of IBA on rooting of grapevine shoots produced *in vitro*

Рис. 1. Влияние IBA на укоренение побегов винограда, полученных *in vitro*

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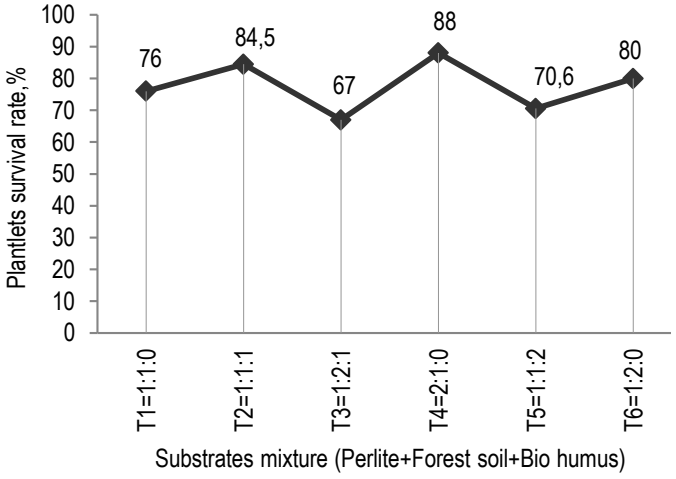
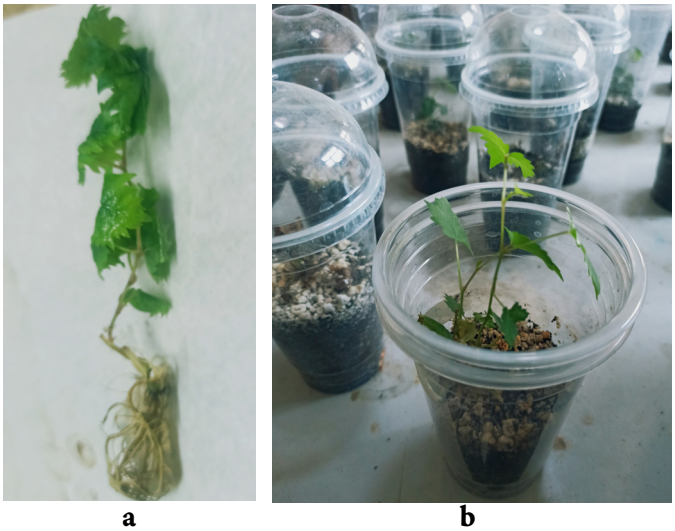


Fig. 2. The effect of different substrate mixture combinations (perlite: forest soil: bio humus) on survival rate of *ex vitro* acclimatized grapevine plantlets

Рис. 2. Влияние различных комбинаций субстратных смесей (перлит: лесная почва: биогумус) на приживаемость саженцев винограда, акклиматизируемых *ex vitro*



a **b**

Fig. 3. Rooted *in vitro* (a) and *ex vitro* acclimatized grapevine plantlets of cv. 'Merdzavani Vaghahas' on T4 substrate mixture (b)

Рис. 3. Укорененные *in vitro* (a) и акклиматизированные *ex vitro* саженцы винограда сорта Мердзавани Вагаас на субстратной смеси Т4 (b)

Conflict of interests

Not declared.

Конфликт интересов

Не заявлен.

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