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# Factors affecting *in vitro* cultivation of grape (*Vitis vinifera* L.): a review

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**A B S T R A C T**

Grape (*Vitis vinifera* L.) is globally cultivated as commercial fruit crop usually used for fruit purpose or industrial product. The objective of the current review is to review and identify the research gap on the effect of different growth media and vitrification on shooting and rooting performance of grape. Factors affecting rooting of grape cuttings can be internal or external factors. Currently, grapevines are very sensitive to disease in the conventional method of propagation. Even if tissue culture is recommended for healthy propagation of the grape varieties, still factors affecting the growth of the plant verifications were reported. This, review paper progressively revised for the existing factors and possible solutions during *in vitro* propagation of grapevines.

**Keywords**: Growth regulators, Grapevine, Shoot, Vitrification, Factors.

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## Introduction

Grape (*Vitis vinifera* L.) is globally cultivated as commercial fruit crop usually used for fruit purpose or industrial product (Orhan *et al.*, 2009). It also used in folk medicine for its biological activities since ancient times. From commercial viticulture perspective, nearly all grape varieties are propagated through stem cutting, layering and grafting in most parts of the world. However, this increases the susceptibility of cultivated varieties to disease causing agents (microbes, mites, insects, nematodes, fungi, bacteria, viruses and more importantly *Phylloxera)* (Alizadeh *et al.*, 2010). Factors like slow and seasonal multiplication and infection with pathogens have constrained the use of conventional plant propagation methods, thus lead to development of new and novel methods of propagation like *in vitro* multiplication, which ensures the production of virus and disease free elite planting material in large numbers. Even, *in vitro* micro propagation of grape cultivars challenged by different factors: media types, concentration of hormones, vitrification and growing mechanisms. Study conducted on shoot multiplication in grape cv. perlette clearly described, the role of cultivation media (Jamwal *et al.*, 2013). Thus, the combination of various growth regulators and their concentrations significantly influences shoot length due to their effect on cell division and cell expansion (Khan *et al.*, 2015). Virtification is another factor (bottleneck) for the establishment of grape tissue culture (Bi *et al.*, 2017). Though it is known that, grapevine (*Vitis vinifera* L.) is one of the most widely distributed fruit crop of the world, today the need for grapevine fruit is increasing (Richard *et al.*, 2010). This happened because of increase in the number of wine industries and more demand for fresh and dried fruits (Fayek *et al.*, 2009). Therefore, to fit the demand for grape a healthy micro-propagation is too much needed (Patrice *et al.*, 2006). The aim of this work was to assess the factors affecting tissue culture of grapes.

### Objectives

- To review the factors of different growth regulators on *in vitro* growth performance of grape
- To identify research gap on the investigated results and forwarding recommendations.

### Literature reviews on factors affecting *in vitro* cultivation of grape

*Review on hyperhydricity (vitrification) and controlling mechanism*

Hyperhydricity of micro propagated shoots also known as Vitrification is unconditionally results from growth and culture condition, which affects

 

the survival and quality of micro-propagated grape cultivars (Gemechu and Feyissa, 2016). Vitrification affects the survival and quality of micro propagated plants. It highly affects the shoots and leaf parts of the plants. To get healthy propagules, it is necessary to look the cause and *in vitro* controlling of Vitrification (Rasco and Pateña, 1997).

Liquid and low agar media is also one causing agent of Vitrification as it induced cellulose formation along with induced and disoriented cellulose biosynthesis, which is manifested in non-functional guard cells. Mal-functioning stomata in addition affect the cuticle contributed to increased transpiration and desiccation of *in vitro* formed leaves. Thus, agar should not be considered simply as a means of solidifying culture media: In general, the concentrations of agar affect the chemical and physical characteristics of a culture medium (Ziv, 1991). The problem of Vitrification on micro propagation of the grapevine has been reported (Alizadeh *et al*., 2010; Kinfe, 2010) but there are few reports which mention decreases of Vitrification.

As, Vitrification of shoots appear during the multiplication stages, reductions of Vitrification *in vitro* result in increment of shoot numbers (Kumsa, 2016). There are a number of mechanisms used to reduce Vitrification: can be reduced by aeration of culture volume and changing of the concentration of growth regulators (Sharma and Mohan, 2006). In another way, an effective procedure for obtaining healthy shoots from *in vitro* culture of propagates was ventilating the culture vessels (Laia *et al*., 2005).

*Adjusted Agar and BAP concentrations to produce non-vitrified shoots of grapevine*

Study conducted on two grape varieties (Canonannon and Chenin blanc) confirmed that 0.5 mg $L^{-1}$ BAP and 7.5 g $L^{-1}$ agar concentrations were contributed in production of maximum number of normal shoots/explant (Table 1).

Table 1. Effect of agar and BAP on normal and vitrified shoots of grapevine at 3 weeks after culturing.

| Agar (g $L^{-1}$) | BAP (mg $L^{-1}$) | 'Canonannon' | | 'Chenin blanc' | |
|---|---|---|---|---|---|
| | | Mean no of normal shoots/explant | Mean no of vitrified shoots/explant | Mean no of normal shoots/explant | Mean no of vitrified shoots/explant |
| 0.0 | 0.0 | 0.0 ± 0.0$^{d}$ | 0.0 ± 0.0$^{d}$ | 0.0 ± 0.0$^{d}$ | 0.0 ± 0.0$^{d}$ |
| 6.0 | 0.5 | 2.5± 0.8$^{b}$ | 2.5± 0.8$^{b}$ | 2.0± 0.1$^{c}$ | 2.0± 0.1$^{c}$ |
| 6.0 | 1.0 | 1.0± 0.6$^{c}$ | 1.0± 0.6$^{c}$ | 0.7± 0.2$^{c}$ | 0.7± 0.2$^{c}$ |
| 6.0 | 1.5 | 2.2± 0.3$^{c}$ | 2.2± 0.3$^{c}$ | 2.1± 0.8$^{c}$ | 2.1± 0.8$^{c}$ |
| 6.0 | 2.0 | 1.5± 0.2$^{c}$ | 1.5± 0.2$^{c}$ | 1.3± 0.6$^{c}$ | 1.3± 0.6$^{c}$ |
| 6.0 | 2.5 | 1.0± 0.1$^{c}$ | 1.0± 0.1$^{c}$ | 0.8± 0.5$^{c}$ | 0.8± 0.5$^{c}$ |
| 6.5 | 0.5 | 2.9± 0.5$^{b}$ | 2.5± 0.5$^{b}$ | 2.3± 0.4$^{c}$ | 2.3± 0.4$^{c}$ |
| 6.5 | 1.0 | 2.0± 0.8$^{c}$ | 2.0± 0.8$^{c}$ | 1.6± 0.3$^{c}$ | 1.6± 0.3$^{c}$ |
| 6.5 | 1.5 | 2.2± 0.2$^{c}$ | 2.2± 0.2$^{c}$ | 2.1± 0.1$^{c}$ | 2.1± 0.1$^{c}$ |
| 6.5 | 2.0 | 1.2± 0.1$^{c}$ | 1.2± 0.1$^{c}$ | 1.2± 0.8$^{c}$ | 1.2± 0.8$^{c}$ |
| 6.5 | 2.5 | 2.8± 0.7$^{b}$ | 2.8± 0.7$^{b}$ | 2.4± 0.9$^{c}$ | 2.5± 0.9$^{b}$ |
| 7.0 | 0.5 | 3.0± 0.9$^{ab}$ | 0.5 ± 0.9$^{c}$ | 2.5.± 0.5$^{b}$ | 0.0 ± 0.0$^{d}$ |
| 7.0 | 1.0 | 2.7± 0.5$^{b}$ | 1.5± 0.5$^{c}$ | 2.2± 0.8$^{c}$ | 1.0 ± 0.8$^{c}$ |
| 7.0 | 1.5 | 2.1± 0.3$^{c}$ | 1.1± 0.3$^{c}$ | 1.1± 0.4$^{c}$ | 0.0 ± 0.0$^{d}$ |
| 7.0 | 2.0 | 2.3± 0.5$^{c}$ | 0.3± 0.5$^{c}$ | 2.0± 0.3$^{c}$ | 1.0± 0.3$^{c}$ |
| 7.0 | 2.5 | 1.9± 0.2$^{c}$ | 0.9± 0.2$^{c}$ | 2.5± 0.4$^{b}$ | 0.0± 0.0$^{d}$ |
| 7.5 | 0.5 | 6.0± 0.1$^{a}$ | 0.0 ± 0.0$^{d}$ | 5.0± 0.2$^{a}$ | 0.0± 0.0$^{d}$ |
| 7.5 | 1.0 | 2.8±0.1$^{b}$ | 0.8± 0.1$^{c}$ | 2.2± 0.8$^{c}$ | 0.0 ± 0.0$^{d}$ |
| 7.5 | 1.5 | 3.0 ± 0.3$^{ab}$ | 0.0 ± 0.0$^{d}$ | 2.5± 0.1$^{b}$ | 1.0 ± 0.1$^{c}$ |
| 7.5 | 2.0 | 3.0 ± 0.3$^{ab}$ | 1.0 ± 0.3$^{c}$ | 2.8± 0.2$^{b}$ | 1.0 ± 0.2$^{c}$ |
| 7.5 | 2.5 | 2.9± 0.3$^{b}$ | 0.9± 0.3$^{c}$ | 2.5± 0.1$^{b}$ | 0.8 ± 0.1$^{c}$ |
| 8.0 | 0.5 | 2.0± 0.2$^{c}$ | 0.0± 0.0$^{d}$ | 1.8± 0.6$^{c}$ | 1.4± 0.6$^{c}$ |
| 8.0 | 1.0 | 2.2± 0.2$^{c}$ | 0.2± 0.2$^{c}$ | 2.0± 0.3$^{c}$ | 1.0± 0.3$^{c}$ |
| 8.0 | 1.5 | 1.2± 0.8$^{c}$ | 0.2± 0.8$^{c}$ | 1.1± 0.1$^{c}$ | 0.1± 0.1$^{c}$ |
| 8.0 | 2.0 | 1.8± 0.6$^{c}$ | 0.8± 0.6$^{c}$ | 1.0± 0.7$^{c}$ | 0.2± 0.1$^{c}$ |
| 8.0 | 2.5 | 1.0± 0.8$^{c}$ | 0.0± 0.0$^{d}$ | 0.9± 0.2$^{c}$ | 0.1± 0.2$^{c}$ |

*Column means with the same letter are not significantly different at P<0.05 probability level.*

*Source: (Gemechu and Feyissa, 2016).*

***Effect of time intervals on number of normal and vitrified shoots/explant***

At the day, intervals after culture were 0 to 20; numbers of vitrified shoots were low. But, when the days after culture were increased to 20 to 40, normally produced shoots were changed to vitrified shoots in both varieties (Fig. 1).

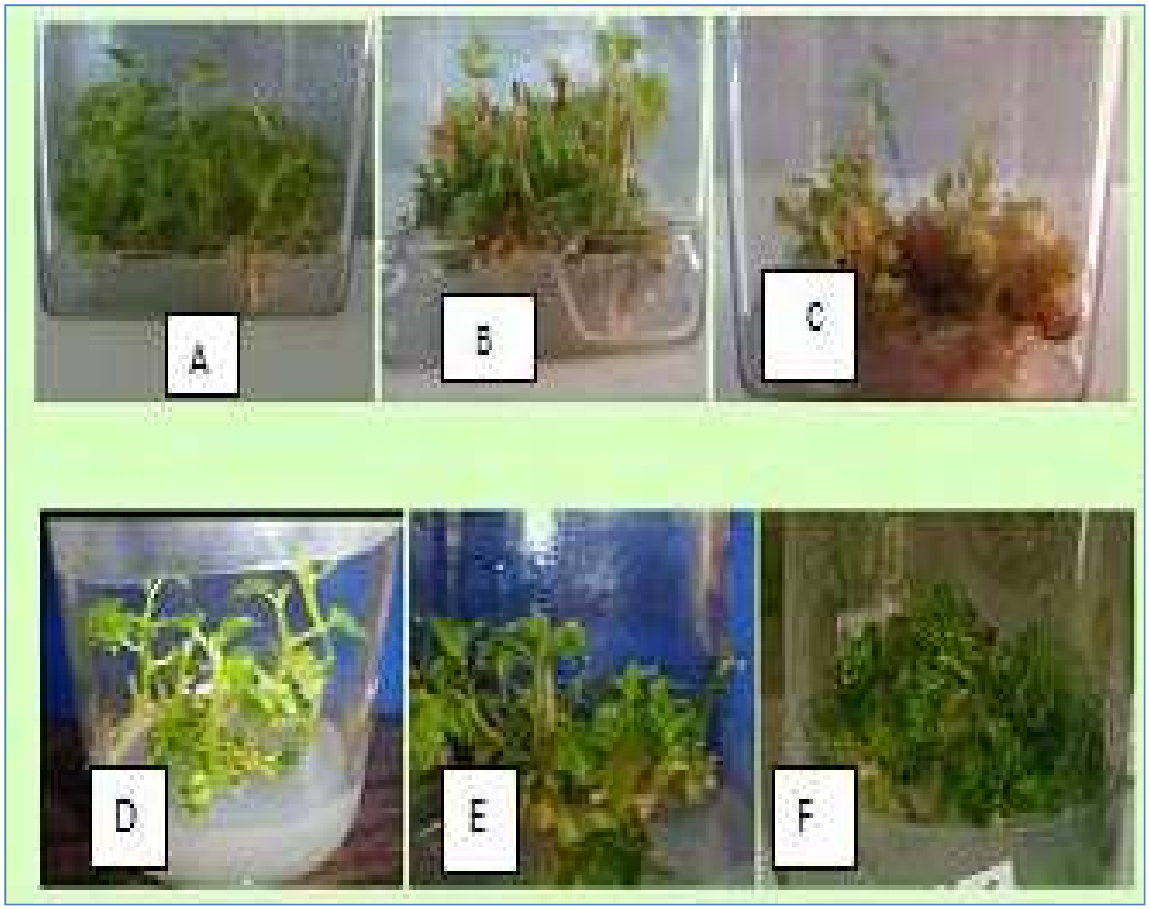


Fig. 1. Effect of Vitrification on two grape varieties at different intervals of time on MS medium supplemented with 0.5 mg $L^{-1}$ BAP and 7.5 g $L^{-1}$ agar. (A= Chenin blanc at 21 days, B= Chenin blanc after 30 days, C= Chenin blanc after 4 weeks, D= Canonannon at 21 days, E= Canonannon after 30 days, F= Canonannon after 4 weeks).

*Source: (Kumsa, 2011).*

*Adjusted Agar and IAA on Roots /Explant*

The maximum mean numbers of normal shoot roots/explant (5.8 ± 0.3) were obtained when gelling agent was 7.5 g $L^{-1}$ in 4.0 mg $L^{-1}$ IAA for both varieties. In contrast maximum vitrified shoot roots of canonannon variety were occurred in medium gelled at 7.5 g $L^{-1}$ in 2.0 mg $L^{-1}$ IAA (Kumsa, 2017; Jaleta and Sulaiman, 2019).

Table 2. Effect of agar and IAA concentrations on differentiation of roots/explant at 3 weeks after culturing.

| | | 'Canonannon' | | | | 'Chenin blanc' | | | |
|---|---|---|---|---|---|---|---|---|---|
| Agar (g $L^{-1}$) | IAA (mg $L^{-1}$) | Mean no of normal roots /explant | Mean length of normal roots /explant | Mean no of vitrified roots /explant | Mean Length of vitrified roots /explant | Mean no of normal /explant | Mean length of normal roots /explant | Mean no of vitrified roots /explant | Mean Length of vitrified roots /explant |
| | | 1.0± 0.6$^{c}$ | 1.0± 0.6$^{c}$ | 0.7± 0.2$^{c}$ | 0.7± 0.2$^{c}$ | 1.0± 0.6$^{c}$ | 1.0± 0.6$^{c}$ | 0.7± 0.2$^{c}$ | 0.7± 0.2$^{c}$ |
| 6.0 | 3.0 | 1.2± 0.3$^{c}$ | 2.2± 0.3$^{c}$ | 1.0± 0.8$^{c}$ | 1.1± 0.3$^{c}$ | 2.0± 0.3$^{c}$ | 2.8± 0.3$^{b}$ | 1.0± 0.8$^{c}$ | 1.8 ± 0.8$^{c}$ |
| 6.0 | 4.0 | 2.5± 0.2$^{b}$ | 2.5± 0.2$^{b}$ | 2.1± 0.6$^{c}$ | 2.3± 0.3$^{c}$ | 1.5± 0.2$^{c}$ | 3.5± 0.2$^{ab}$ | 1.0± 0.6$^{c}$ | 2.5± 0.6$^{b}$ |
| 6.5 | 1.0 | 2.8± 0.5$^{b}$ | 2.5± 0.5$^{b}$ | 2.3± 0.4$^{c}$ | 2.3± 0.1$^{c}$ | 2.9± 0.5$^{b}$ | 2.5± 0.5$^{b}$ | 2.3± 0.4$^{c}$ | 2.3± 0.4$^{c}$ |
| 6.5 | 2.0 | 2.9± 0.8$^{b}$ | 2.0± 0.8$^{c}$ | 2.4± 0.3$^{c}$ | 1.6± 0.3$^{c}$ | 2.0± 0.8$^{c}$ | 2.0± 0.8$^{c}$ | 1.6± 0.3$^{c}$ | 1.5± 0.2$^{c}$ |
| 6.5 | 3.0 | 2. 8± 0.2$^{b}$ | 2.3± 0.2$^{c}$ | 2.5± 0.1$^{b}$ | 2.1± 0.1$^{c}$ | 2.2± 0.2$^{c}$ | 2.2± 0.2$^{c}$ | 2.1± 0.1$^{c}$ | 1.1± 0.1$^{c}$ |
| 6.5 | 4.0 | 3.0 ± 0.1$^{ab}$ | 2.5± 0.1$^{b}$ | 2.8± 0.8$^{b}$ | 1.2± 0.8$^{c}$ | 2.8 ± 0.1$^{b}$ | 1.2± 0.1$^{c}$ | 2.2± 0.8$^{c}$ | 1.2± 0.8$^{c}$ |
| 7.0 | 1.0 | 3. 0± 0.9$^{ab}$ | 2.8 ± 0.9$^{b}$ | 2.9.± 0.5$^{b}$ | 2.0 ± 0.5$^{c}$ | 3.0± 0.9$^{ab}$ | 1.0 ± 0.9$^{c}$ | 2.5.± 0.5$^{b}$ | 0.5 ± 0.5$^{c}$ |
| 7.0 | 2.0 | 3.1± 0.5$^{ab}$ | 2.9± 0.5$^{b}$ | 2.5± 0.8$^{b}$ | 2.0 ± 0.8$^{c}$ | 2.7± 0.5$^{b}$ | 1.5± 0.5$^{c}$ | 2.2± 0.8$^{c}$ | 0.5 ± 0.8$^{c}$ |
| 7.0 | 30 | 3.1± 0.3$^{ab}$ | 3.1± 0.3$^{ab}$ | 2.9± 0.4$^{b}$ | 2.0 ± 0.4$^{c}$ | 2.1± 0.3$^{c}$ | 1.1± 0.3$^{c}$ | 2.1± 0.4$^{c}$ | 0.1 ± 0.4$^{c}$ |
| 7.0 | 4.0 | 3.3± 0.5$^{ab}$ | 3.3± 0.5$^{ab}$ | 2.8± 0.3$^{b}$ | 2.1± 0.3$^{c}$ | 2.3± 0.5$^{c}$ | 0.3± 0.5$^{c}$ | 2.0± 0.3$^{c}$ | 1.0± 0.3$^{c}$ |
| 7.5 | 1.0 | 3.2 ± 0.2$^{ab}$ | 6.6 ± 3.0$^{a}$ | 2.6± 0.1$^{b}$ | 2.8 ± 0.1$^{c}$ | 3.0 ± 0.2$^{ab}$ | 6.1 ± 4.1$^{a}$ | 2.1± 0.1$^{c}$ | 2.8 ± 0.1$^{b}$ |
| 7.5 | 2.0 | 4.1 ± 0.1$^{ab}$ | 8.0 ± 4.2$^{a}$ | 3.0± 0.8$^{ab}$ | 3.2± 0.8$^{ab}$ | 3.2 ± 0.2$^{ab}$ | 7.0 ± 3.2$^{a}$ | 2.2± 0.8$^{c}$ | 3.2± 0.8$^{ab}$ |
| 7.5 | 3.0 | 4.5 ± 0.3$^{ab}$ | 7.0 ± 0.3$^{a}$ | 2.8.± 0.5$^{b}$ | 3.5.± 0.5$^{ab}$ | 3.8 ± 0.1$^{ab}$ | 7.0 .± 0.2$^{a}$ | 2.5.± 0.5$^{b}$ | 3.5.± 0.5$^{ab}$ |
| 7.5 | 4.0 | 5.8 ± 0.3$^{a}$ | 8.5 ± 1.4$^{a}$ | 2.1± 0.8$^{c}$ | 3.2± 0.8$^{ab}$ | 4.0 ± 0.5$^{ab}$ | 7.2 ± 0.3$^{a}$ | 2.2± 0.8$^{c}$ | 3.0± 0.8$^{ab}$ |
| 8.0 | 1.0 | 5.0± 0.2$^{a}$ | 5.2± 0.2$^{a}$ | 2.2± 0.4$^{c}$ | 2.1± 0.4$^{c}$ | 2.5.± 0.5$^{b}$ | 2.5.± 0.5$^{b}$ | 2.1± 0.4$^{c}$ | 1.1± 0.4$^{c}$ |
| 8.0 | 2.0 | 3.2± 0.2$^{ab}$ | 4.2± 0.2$^{ab}$ | 2.0± 0.3$^{c}$ | 2.0± 0.3$^{c}$ | 2.2± 0.8$^{c}$ | 2.2± 0.8$^{c}$ | 2.0± 0.3$^{c}$ | 2.0± 0.3$^{c}$ |
| 8.0 | 3.0 | 2.2± 0.8$^{c}$ | 3.2± 0.8$^{ab}$ | 1.1± 0.1$^{c}$ | 2.1± 0.1$^{c}$ | 1.1± 0.4$^{c}$ | 1.1± 0.4$^{c}$ | 1.1± 0.1$^{c}$ | 1.1± 0.4$^{c}$ |
| 8.0 | 4.0 | 1.8± 0.6$^{c}$ | 2.8± 0.6$^{b}$ | 1.0± 0.7$^{c}$ | 2.0± 0.1$^{c}$ | 2.0± 0.3$^{c}$ | 2.0± 0.3$^{c}$ | 1.1± 0.2$^{c}$ | 1.1± 0.4$^{c}$ |

*Means followed by the same letters in the same column are not significantly different at 5 % level of probability.*

*Sources: (Gemechu and Feyissa, 2016; Kumsa, 2011)*

When the time of culture increased, there is an increment of vitrified shoots/explants, which resulted in mal-growth of plant roots/explant in both cultivars (Table 2).

## Summary and Conclusion

Currently, grapevines are very sensitive to disease in the conventional method of propagation. Even if tissue culture is recommended for healthy propagation of the grape varieties, still factors affecting the growth of the plant verifications were reported. This, review paper progressively revised for the existing factors and possible solutions during *in vitro* propagation of grapevines.

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