Combined Proceedings IPPS 74:144-151. 2024.

Micropropagation and Applications of In Vitro Systems for Grapevine (*Vitis* spp.)

Puthiyaparambil Josekutty

Research Manager, Skybury Farms, 136 Ivicevic Rd, Paddy's Green, QLD 4879 Australia

josekutty964@gmail.com

Keywords: tissue culture, organogenesis, shoot proliferation, rooting, acclimation, high-health plants

Summary

Grapevines (*Vitis vinifera* L.) are of great significance to the Australian economy as they comprise vine grapes and table grapes. Grapevine rootstocks belong to several *Vitis* species such as *Vitis rupestris*, *V. riparia*, *V. berlandieri* and *V. champini* etc. from America. In vitro propagation systems for grapevine like micropropagation, organogenesis, somatic embryogenesis, protoplast culture and cryopreservation are important for various reasons such as rapid and reliable cloning, international transfer of germplasm, virus elimination, rapid crop improvement through cell-level selection, genetic engineering, gene editing as well as in vitro conservation of valuable germplasm. Micropropagation of three open varieties of table grapes undertaken for rapid reliable cloning of virus-free stock material for orchard establishment is described. Also, various in vitro methods applied to grapes and their applications are discussed.

INTRODUCTION

Skybury farms, 136 Ivicevic Rd, QLD 4880 in Australia (www.skybury.com.au) is a diversified agriculture business commercially producing Papaya, Coffee and a variety of value-added products from our farm produce, for example, the papaya vodka that won international award in the London spirit show. Papaya cream is another example of its premier products. Skybury Farms is also a proud supporter of horticulture development in Australia offering cost-effective, results oriented research and development services.

Grapevine industry is a major player in the Australian horticulture sector valued at about 50 billion AUD in 2022. Wine grape industry is the major player with a cultivated area of 146,224 million ha., annual production of 1.48 billion litres wine of which 48% is exported, and employing 136,790 persons. Although much smaller (AUD 1.5 Billion), the table grape industry is significant as 70% of the Australian table grapes are exported according to the Australian Grape and Wine website (https://www.agw.org.au/).

Although there are several reports on micropropagation of grapes as recently reviewed, our objective was to develop a rapid and reliable cloning technology that works at the Skybury lab which is specialising in horticulture research and development. This technology could be used to assist wine growers and wine variety importers in Australia to rapidly clone new varieties of grapes they introduce to Australia. A mid to longer term goal of Skybury lab is to provide crop improvement research service (improved variety development) to wine growers using the in vitro breeding technique we successfully applied to the rapid development of the world's best papaya, the Skybury, Sweet, Red variety (Puthiyaparambil et al. 2023).

MATERIALS AND METHODS

We used healthy (disease-free) grafted plants of three most popular table grape varieties with distinct fruit characteristics {'Menindee seedless' an erly, seedless, green grape; 'Çrimson seedless' a late season seedless, red variety, and 'Autumn royal' also a late variety but with seedless, black berries} obtained from a reputed plant nursery. We maintained the mother stocks in the insect- proof greenhouse in Skybury nursery for 60 days and regularly observed for visual symptoms of common diseases of grapevine. We applied insecticides and fungicides as normally applied to our papaya production nursery which maintains over 50,000 tissue culture papayas for our farm use.

Shoot tips and tender nodal segment were collected and used as explants for micropropagation. The explants were surface sterilised with a 10-15 min wash with Johnson's$^{(T)}$ baby shampoo followed by a one-minute rinse with 70% (v/v) ethanol, then rinsed once with sterile (autoclaved) RO water. Further, treated the explants for 3-5 min with 5% (v/v) household bleach diluted with sterilised RO water and finally rinsed four times with sterile RO water. Surface sterilised explants were trimmed to 2.0 – 3.0 cm shoot apieces and nodal segments with single nodes. The cleaned explants were inoculated on to autoclaved media in a laminar flow hood. All the cultures were incubated in a growth room maintained at 25± 2°C, lighted with red + blue LED lights at approximately 80 µmol/s/m^2.

RESULTS AND DISCUSSION

Culture Initiation

Initiation was easy from shoot tip and nodal explants in MS medium (Murashige and Skoog, 1962) modified with benzyl adenine (1.0-2.0 mg/l) or kinetin (1.0-2.0 mg/l) + naphthaleneacetic acid (NAA; 0.1-0.5 mg/l) + 25 g/l sucrose, with / without 500 mg/l charcoal, pH adjusted to 6.25 before adding 2.8 g/l Gelzan and sterilisation (**Fig. 1**). On average, shoot culture initiation required 25-30 days from the day of culture establishment. Fungal contamination (10-12%) was more than bacterial contamination (3-5%) observed at initiation stage. The tropical climate in which the plants were maintained explains the high rate of contamination at the initiation stage.

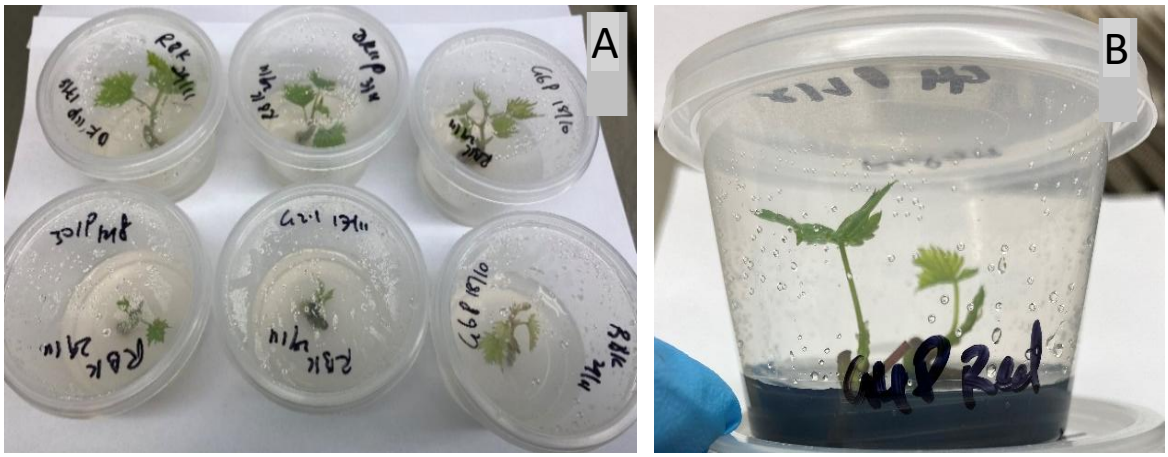

Figure 1. A) Culture initiation in charcoal-free medium and **B**) in charcoal supplemented medium.

Shoot Proliferation

According to recent reviews, (Yancheva et al. 2018; Carimi et al. 2019; XiuMing et al. 2021) there is a significant genotype effect on micropropagation of grapevine varieties. However, in this experiment, we didn't notice much differences between the varieties at the initiation and proliferation stages.

Best medium for shoot proliferation was MS medium supplemented with 25 g/l sucrose, 100 mg/l Polyvinylpyrrolidone (PVP 40), 2.0 mg/L Kinetin + 0.2 mg/L NAA, 2.8 g/l Gelzan and pH adjusted to 6.25 before autoclaving. Shoot proliferation started from day 10-15 of culture and 3-5 shoots developed in 30 days on average from cultured shoots. The shoot multiplication rate was also similar (6-8 shoots / culture) between the three varieties. From the third monthly subculture, a 600 ml jar with 10 shoots easily generated 50-60 shoots and filled up the jar in 3 weeks (**Fig. 2**). Due to the high rate of proliferation and growth, I found that transferring the cultures on to hormone free MS medium containing 20 g/l sucrose for alternate monthly culture cycle kept the shoots with little or no vitrification. Specific media requirements for obtaining high frequency shoot proliferation from different grape varieties is on record as per the recent reviews (Yancheva et al. 2018; Carimi et al. 2019; XiuMing etal. 2021). However, all three varieties I studied responded very well in the same medium.

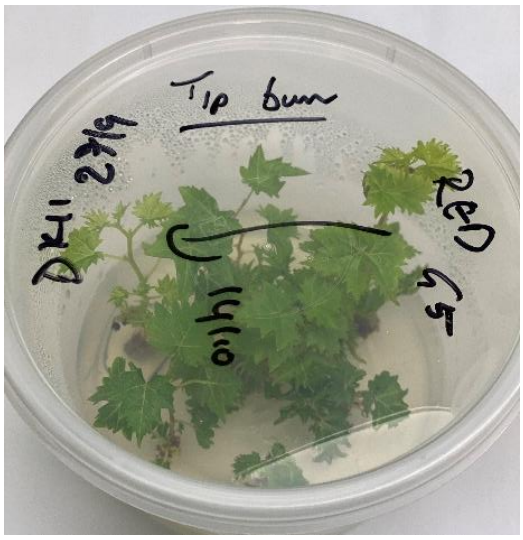

Figure 2. Shoot multiplication

Rooting

All three varieties of grapevine rooted easily on the same i. e. ½ strength MS medium supplemented with 15 g/l sucrose, 0.5 mg/l indole-3-butyric acid, 3 mg/l Thiamine and 6.0 g/l agar, pH adjusted to 6.0 before autoclaving (Fig. 3A & B). It has been reported that different grapevine varieties require their own unique basal media supplemented with different plant growth regulators (Yancheva et al. 2018, Carimi et al. 2019; Xiu Ming et al. 2021). However, all the three grapevine varieties in our experiment rooted efficiently, and roots proliferated in the same medium.

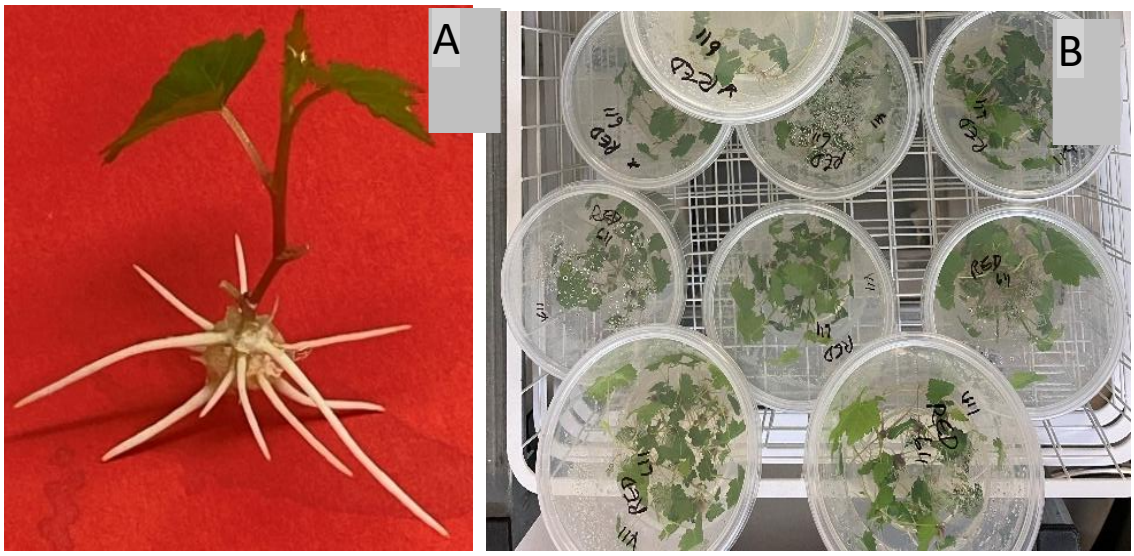

Figure 3. Rooting of microshoots of grapevine. **A**) after 2 weeks, **B**) after 4 weeks.

Acclimation

Well rooted grapevine plantlets when transplanted to porous potting mix (Searles) and maintained in a climate-controlled greenhouse acclimated at high frequency (90-95% survival) and the primary hardening only required 3 weeks (**Fig. 4**). The acclimated plants established and developed further in the net house with 50% shade (**Fig. 5**).

Figure 4. Primary hardening in the climate-controlled greenhouse

Figure 5. Secondary hardening of tissue cultured grapevine in the net house with 50% shade.

They sun-hardened efficiently in a fully open net house and survived field planting. Once planted in the farm with a supporting shade, 100% of them established and produced fruit as normal grafted grapevine (**Fig. 6**). Acclimation of tissue culture plants is complicated by the change in environment, poor development of root-shoot juncture, delicate leaves with poorly developed cuticle and stomata. Therefore, ambient high humidity and moderate temperature are required for acclimation of micropropagated plants. The high humidity and low temperature provided at primary hardening in the climate-controlled greenhouse may have helped to achieve high rates of acclimation of tissue cultured grapes.

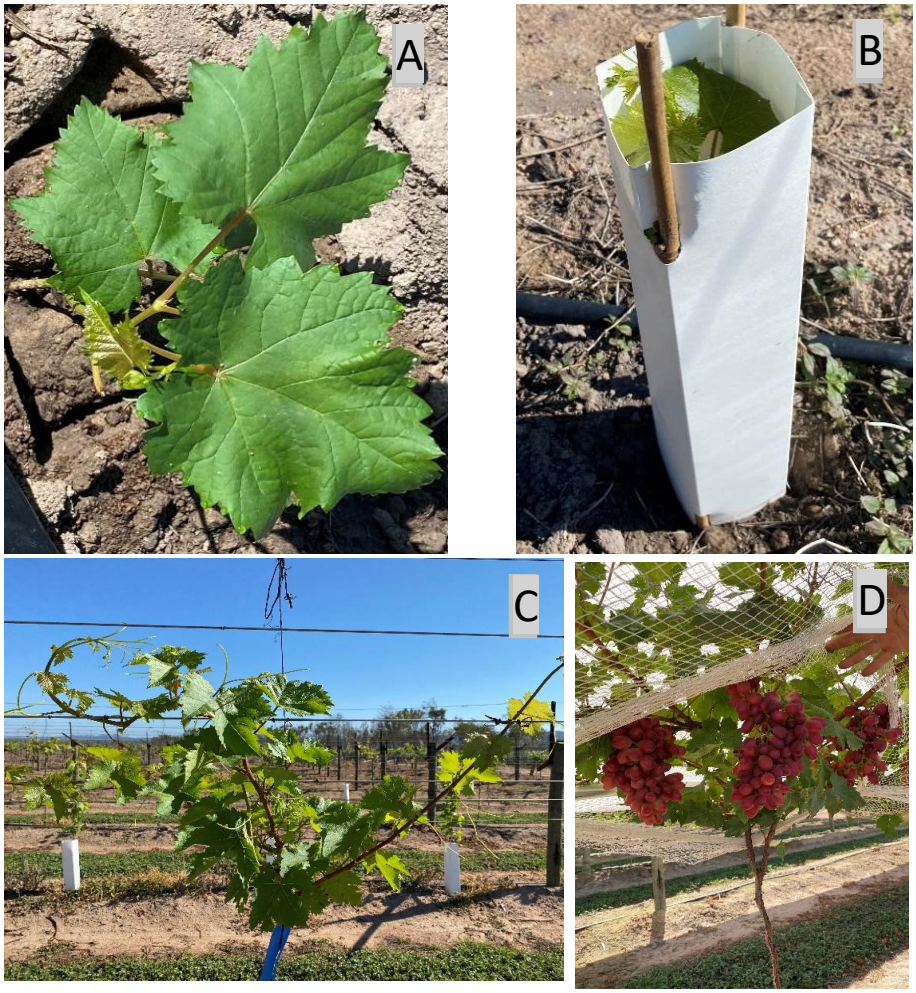

Figure 6. Field testing of micropropagated grapevine. **A**) on planting day, **B**) after 30 days of planting, **C**) three months after planting and **D**) maturing red grapes.

Applications of tissue culture techniques for grapes

Somatic embryogenesis can be used for rapid cloning of grapevine varieties and, for selecting non-genetically modified grapevine with resistance to fungal pathogens (Li et al. 2014). Somatic embryogenesis was also used for regenerating genetically modified grapevine with improved characteristics (Dhekney et al. 2016). Cryotherapy and thermotherapy were applied to in vitro shoots of infected plants for virus elimination and rapid cloning of virus-free plant material (Pathirana et al. 2015). Long term storage of several accessions of grapevine germplasm in vitro has been achieved and maintained under cryopreservation (Bettoni et al. 2021). Accelerated mutation breeding of grapevine was also achieved using tissue culture technology (Pathirana and Carimi, 2023). Gene editing was successfully used to make disease resistant grapevine (Wang et al. 2018) and grapevine with Muscat flavour (Yang et al. 2024).

CONCLUSION

Micropropagation of the three grapevine varieties studied was easy and behaved similarly in this experiment. All the three varieties initiated, multiplied and rooted in the same culture medium and with similar efficiency.

Acknowledgements

I acknowledge the support (facilities and finance) for the research by Skybury Farms, 136 Ivicevic Rd, QLD 4880 and the able technical assistance of Mrs Jay Pui Lam.

LITERATURE CITED

Bettoni, J.C. et al. (2021). Grapevine shoot tip cryopreservation and cryotherapy: Secure storage of disease-free plants. Plants *10*:2190. https://doi.org/10.3390/plants10102190

Dhekney, S.A. Li, Z.T., Grant, T.N.L. and Gray, D.J. (2016). Somatic embryogenesis and genetic modification of *Vitis*. In: Germana, M., Lambardi, M. (eds) In Vitro Embryogenesis in Higher Plants. Methods in Molecular Biology *1359*. Humana Press, New York. https://doi.org/10.1007/978-1-4939-3061-6_11

Li, Z.T., Kim, K., Dhekney, S.A., Jasinski, J.R. Creech, M.R. and Gray, D.J.. (2014). An optimized procedure for plant recovery from somatic embryos significantly facilitates the genetic improvement of *Vitis*. Horticulture Research. *24*:1. https://doi.org/10.1038/hortres.2014.27

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. *15*:473-497. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x

Pathirana, McLachlan, A., Hedderley, D., Carra, A., Carimi, F. and Panis, B. (2015). Removal of leafroll viruses from infected grapevine plants by droplet vitrification. Acta Hort. *1083*, ISHS 2015. https://doi.org/10.17660/ActaHortic.2015.1083.64

Pathirana, R and Carimi, F. (2023). Studies on improving the efficiency of somatic embryogenesis in grapevine (*Vitis vinifera* L.) and optimising ethyl methanesulfonate treatment for mutation induction. Plants *12*: 4126. DOI: 10.3390/plants12244126

Puthiyaparambi, J.C., Pagie, M., Teressita, S., Jay, P.M., Bongani, J.N., Paul, F., Candy, M., Mark, M., Farion, M., Ian, M, Sanskruti, L., and Nitin, M. (2023) Rapid papaya crop improvement through accelerated in vitro breeding and molecular diagnostics. Acta Hort. *1362*:499-506. https://doi.org/10.17660/ActaHortic.2023.1362.67

Xiu-ming, Z, Wu, Y., Li, Z., Song, C., and Wang, X. (2021). Advancements in plant regeneration and genetic transformation of grapevine (*Vitis* spp.). Journal of Integrative Agriculture *20*:1407–1434.

Wang, X., Tu, Ml, Wang, D., Liu, J., Li, Z., Wang, Y., and Wang, X. (2018). CRISPR/Cas9-mediated efficient targeted mutagenesis in grape in the first generation. Plant Biotechnol J. *16*: 844-85524. https://doi.org/10.111/pbi.12832

Yancheva, S., Marchev, P., Yaneva, V., Roichev, V., and Tsvetkov, I. (2018). In vitro propagation of grape cultivars and rootstocks for production of pre-basic planting material. Bulgarian Journal of Agricultural Science *24*:801–806.

Yang, Y., Wheatley, M., Meakem, V., Galarneau, E., Gutierrez, B., and Zhong, G. (2024). Editing VvDXS1 for the creation of muscat flavour in *Vitis vinifera* cv. Scarlet Royal. Plant Biotechnol J. 22(6):1610-1621. DOI: 10.1111/pbi.14290