Asian Journal of Microbiology and Biotechnology

Volume 9, Issue 2, Page 29-37, 2024; Article no.AJMAB.12160
ISSN: 2456-8341

Micropropagation of Two Varieties of *Vitis vinifera* Cabernet Franc and Pinot Noir, Comparison of Physiological and Production Parameters

Yanina S. García [a], Evelyn A. González [a], Mariano H. Gutiérrez [b], Eliana S. Fernández [b], A. Mariela Quiroga [b] and Hilda E. Pedranzani [a*]

[a] Facultad de Química Bioquímica y Farmacia, Departamento de Biología, Proyecto de Investigación N° 02-2523, Secretaría de Ciencia y Tecnología, Universidad Nacional de San Luis, Argentina.
[b] Facultad de Ingeniería y Ciencias Agropecuarias, Departamento de Ciencias Agropecuarias, Proyecto de Investigación N° 02-2523, Secretaria de Ciencia y Tecnología, Universidad Nacional de San Luis, Argentina.

Authors' contributions

This work was carried out in collaboration among all authors. Author HEP designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors YSG and EAG made all the experiments. Author MHG managed and controlled the chemical units used. Author ESF managed the literature searches, author AMQ made the introduction of two varieties of wine. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/ajmab/2024/v9i28755

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.ikprress.org/review-history/12160

Original Research Article

Received: 11/04/2024
Accepted: 15/06/2024
Published: 01/07/2024

**Corresponding author: E-mail: hepedra@unsl.edu.ar, hildaelizz@gmail.com;*

Cite as: *García, Yanina S., Evelyn A. González, Mariano H. Gutiérrez, Eliana S. Fernández, A. Mariela Quiroga, and Hilda E. Pedranzani. 2024. "Micropropagation of Two Varieties of Vitis Vinifera Cabernet Franc and Pinot Noir, Comparison of Physiological and Production Parameters". Asian Journal of Microbiology and Biotechnology 9 (2):29-37. https://doi.org/10.56557/ajmab/2024/v9i28755.*

ABSTRACT

Grapevine (*Vitis vinifera*) is considered one of the most important fruit crops in the world, and the Cabernet Franc and Pinot Noir varieties have high commercial value. The aim of this study was to compare the *in vitro* performance of both varieties from explant establishment to *in vitro* rooting, testing different hormone concentrations. It started from mother plants obtained *in vitro*, from which uninodal cuttings were taken for micropropagation. Disinfection consisted of washing with 70% alcohol and 20% sodium hypochlorite, with sterile water rinses. Explants were placed in a laminar flow chamber under aseptic conditions in a sterile growth medium. They were grown in a culture chamber with 16 hours of light: 8 hours of darkness and a temperature of 25°C. Establishment, growth, rooting and contamination data were recorded for eight weeks. The culture media used were Experiment 1: 50% Murashige and Skoog (MS) [1] medium; Experiment 2: MS at 50% plus indoleacetic acid (IAA) at 0.01%. The data were statistically analyzed; Cabernet Franc and Pinot Noir had similar production standards, high vigor, good *in vitro* growth and direct rooting. Both varieties performed better on the auxin enriched medium.

Keywords: In vitro culture; indoleacetic acid; growth; rooting.

1. INTRODUCTION

The Cabernet Franc grape is one of the most famous red grapes in the world and is combined with Cabernet Sauvignon and Merlot grapes in Bordeaux wine. It is lighter in color than Cabernet Sauvignon, with very coloured musts, high sugar content, very perfumed and with an aroma reminiscent of raspberries. Its wines are very colorful, pleasant, with characteristic aromas of raspberry and violet, with a very delicate bouquet, slightly lower acidity and polyphenols than Cabernet Sauvignon wines and not very astringent. They produce very light, aromatic and pleasant young wines. These wines age very well and are well suited to barrel aging [2]. The Pinot Noir variety originates from French Burgundy and is also known as Burgundy, Pineau, Klevner, Plant Fin, Noirien, Pinoz, Dorada. With regard to berries and pulp, Pinot Noir is a very delicate variety in its cultivation, as it is very sensitive to wood fungal diseases, sensitive to mildew and powdery mildew, very sensitive to botrytis, grape moth, cicadellidae and mites. It adapts well to temperate climates and accepts all types of well-drained soils, although it prefers calcareous soils. As far as its oenological potential is concerned, it produces a high-quality wine suitable for aging, fine, intense and complex. It produces musts rich in sugar when the grapes are properly ripened, with medium acidity. Suitable for cavas, champagnes and crianza wines with body and strength, with high aromatic complexity. Base for bouquet wines, especially when the grapes come from calcareous soils [3]. The use of micropropagation offers an important alternative to conventional plant propagation methods [4,5] and is an important tool for initiating breeding programmes [6]. The use of efficient micropropagation protocols will result in the production of numerous plants that can be maintained under controlled conditions in a reduced space until their transfer to soil for growth or grafting [7]. For this reason, information is needed on the viability of these *in vitro* grapevine varieties in their propagation. Among its advantages, it allows mass production of plants at any time of the year in a short time and *in vitro* propagated plants often show more vigorous growth than in vivo propagated plants, mainly because they are free of viruses and pests [8]. *In vitro* culture is valuable for the application of techniques such as mutation and induced selection and germplasm exchange [9].

When grapevine cultivars are propagated *in vitro* under standard growing conditions [10], their response is often related to the genotype and culture medium used, as well as the proportion of plant growth regulators used [11].

The aim of this study was to evaluate the performance of the two varieties Cabernet Franc and Pinot Noir in *in vitro* propagation, with different hormone levels in all the steps: establishment, multiplication, rooting and percentage of contamination at each stage.

2. MATERIALS AND METHODS

2.1 Vegetable Materials

Mother plants obtained *in vitro* were segmented into uninodal cuttings and, after disinfection, sown in a laminar flow chamber under aseptic conditions in sterile growth medium.

2.2 Growing Media

The culture medium used were:

Medium 1: Murashige Skoog (MS) 50% salts.
Medium 2: Murashige Skoog (MS) 50% + indoleacetic acid (IAA).

In both experiments, the culture medium MS with 50% salts was used and vitamins (thiamine 40 mg/l, nicotinic acid 40 mg/l and pyridoxine 50 mg/l) 10 ml and myo-inositol (1000 mg/l) 10 ml and 30 g/1 of sucrose were added. 30000 mg/l.

No hormones were added to the medium of experiment 1 and 10 ml 100 mg/l IAA (indoleacetic acid) was added to the medium of experiment 2. Once the nutrient solution was dissolved, it was mixed with the 8000 mg/l agar, previously diluted in 500 ml water. The pH was adjusted to 5.6 - 5.8 and distributed into flasks.

2.3 Sterilization

The elements required for disinfection and seeding were sterilized in an autoclave at 1 atm for 15 min at 120ºC.

2.4 Disinfection and *In-vitro* Seeding

Uninode stakes were rinsed with water and detergent for 5 min, then with 70% alcohol for 3 sec and then with 20% sodium hypochlorite for 20 min, with three rinses with sterile water between each step.

In a laminar flow chamber under aseptic conditions, 1-2 cuttings were sown per flask containing sterilized culture medium, then the flasks were wrapped in nylon film and kept in the culture chamber with a photoperiod of 16 h light and 8 h dark, and a temperature range of 22-25°C. Data on establishment, rooting, number of leaves and contamination were recorded for 8 weeks.

3. RESULTS AND DISCUSSION

The inductions of explants were of 60% in medium 1 (M1) and 100% in medium 2 (M2) for Cabernet Franc. In Pinot Noir the induction was 90 % in M1 1 and 100% in M2. Both varieties were able to grow better with the growing medium of experiment 2 achieving 100% establishment (Fig. 1).

3.1 Number of Leaves of Mother Plants

Weekly leaf count data for each variety were used to determine whether the cuttings were growing over a period of eight weeks. The non-parametric Mann-Whitney U test was used for statistical analysis [12].

In Cabernet Franc, leaf emergence was observed with 7 leaves per plant in the M1 and a maximum of 66 leaves in M2 (Fig. 2). This variety showed a highly significant difference ($p \leq 0.05$) between both growth mediums. In Pinot Noir, leaf emergence was observed, reaching a maximum of 18 leaves in M1 and a maximum of 92 leaves in M2 (Fig. 3). This variety showed a highly significant difference between M1 and M2, with $p \leq 0.05$ in relation to the number of leaves.

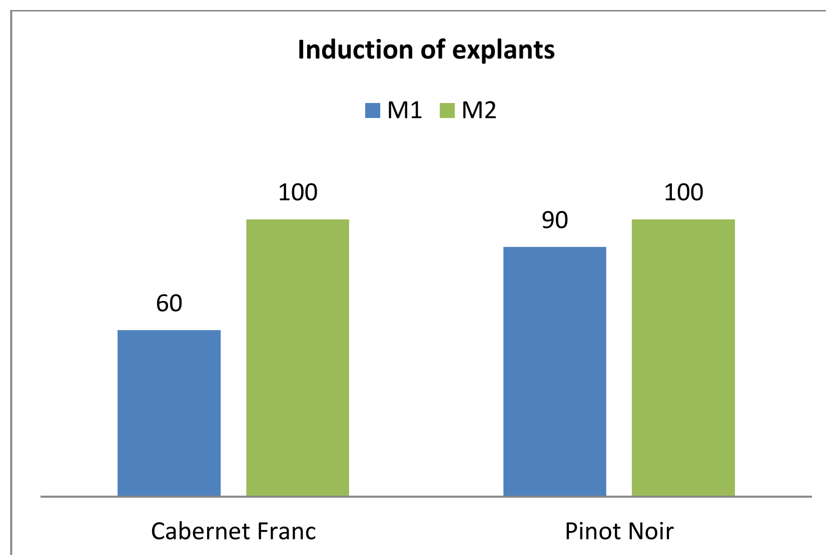

Fig. 1. Induction of explants of cabernet franc and pinot noir varieties in M1 and M2 at 8th week

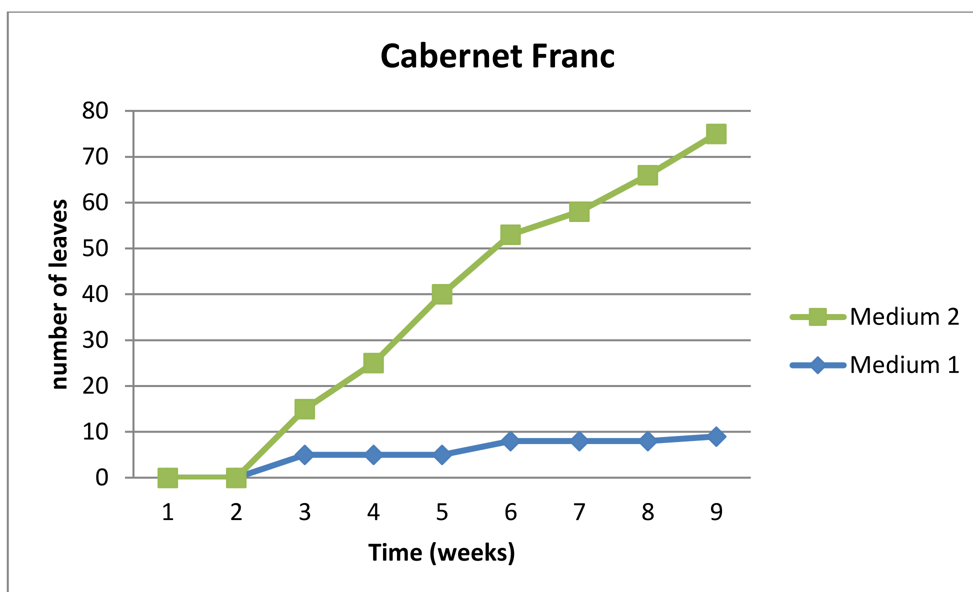

Fig. 2. Number of leaves in Cabernet Franc: M1: without AIA and M2: with AIA, over 8 weeks

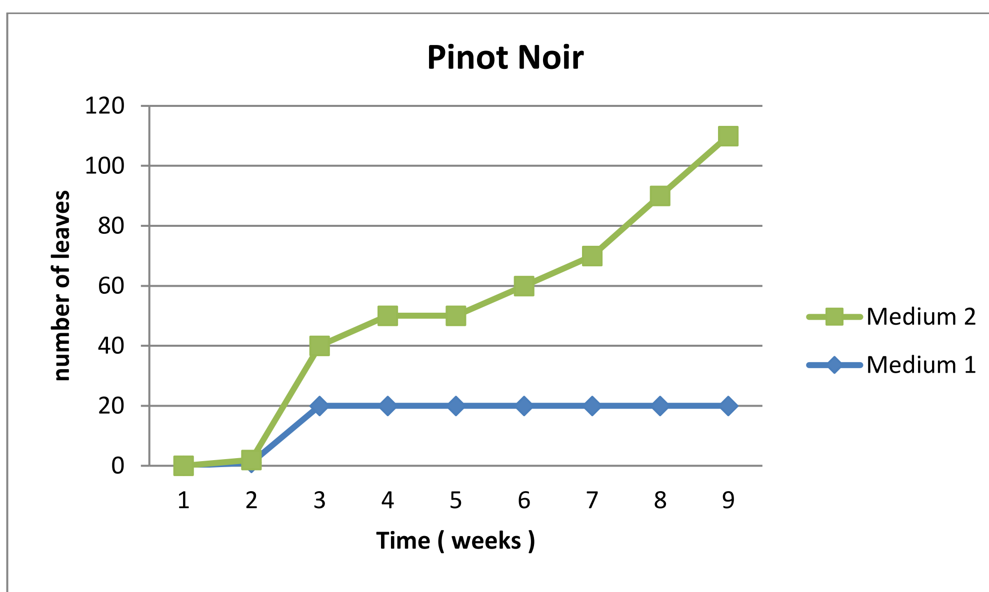

Fig. 3. Number of leaves in Pinot Noir: M1: without AIA and M2: with AIA, over 8 weeks

3.2 Rooting

Rooting was recorded once a week for eight weeks on the established shoots. Cabernet Franc in M1 no root development, in M2 80% of rooting was observed (eight weeks). This variety showed a highly significant difference ($p \leq 0.05$) between both growing mediums (Fig. 3). Cabernet Franc was a variety with a high root production, even in the first weeks. Pinot Noir in M1, 20% of the cuttings rooted in the fifth week, was maintained until the end of the experiment. In M2 cuttings rooted 70% and 90% in the second and third week respectively, and it was maintained until the end of the experiment. This variety showed a highly significant difference in rooting ($p \leq 0.05$) between medium (Fig. 4).

3.3 Contamination

The contamination data obtained in the eight weeks of the trial were analyzed using the non-parametric Mann-Whitney U test [12].

Cabernet Franc, in M1 70% of the cuttings were contaminated with bacteria and in M2 40% with fungal (Fig. 5). Statistical analysis showed that there was no significant difference between M1 and M2, with $p > 0.05$.

Pinot Noir in M1, 90% of the cuttings were contaminated and in M 2: 50% mainly by bacteria (Fig. 5). The results of the statistical analysis showed that there were significant differences in contamination between M1 and M2, with $p \leq 0.05$.

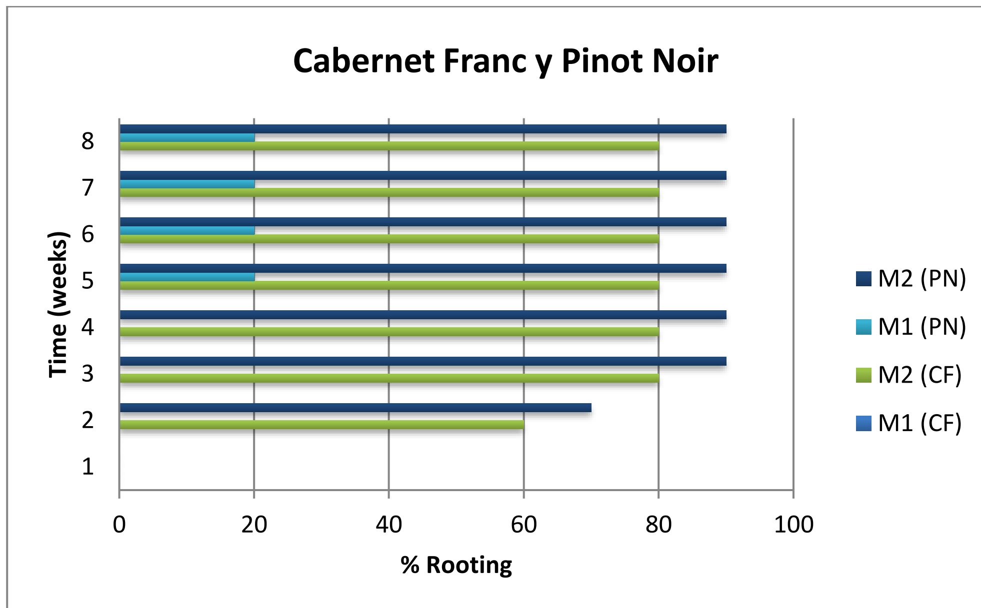

Fig. 4. Percentage of rooting *in vitro* culture of Cabernet Franc and Pinot Noir grapevines, during 8 weeks in different culture media 1: M1 and M2. ** Significant difference ($p \leq 0.05$)

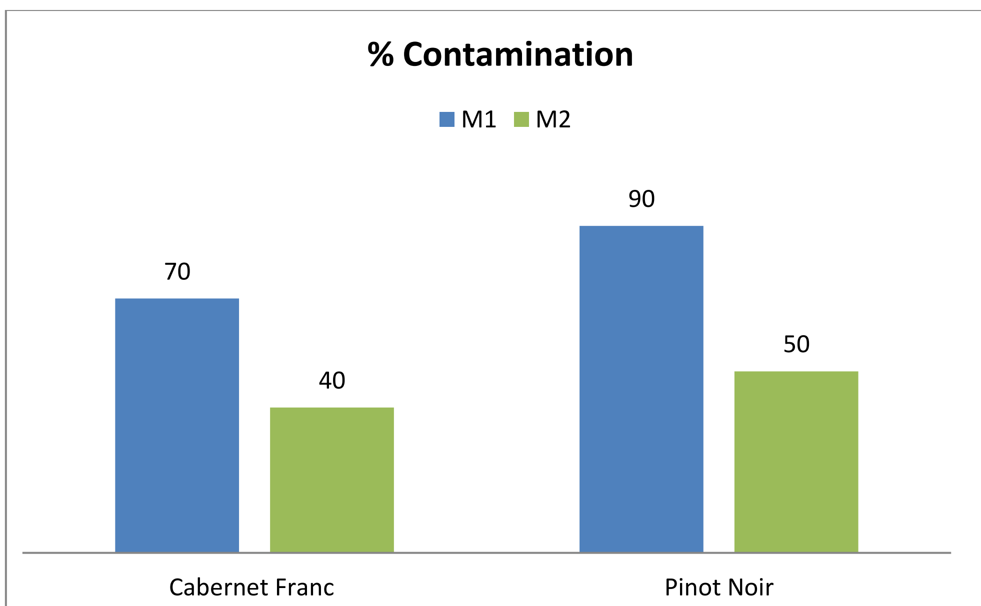

Fig. 5. Percentage of contamination in the *in vitro* culture of Cabernet Franc and Pinot Noir grapes during 8 weeks in two different culture media M1 and M2 ** Significant difference ($p \leq 0.05$)

Cabernet Franc

M1

M2

PinotNoir

M1

M2

Fig. 6. Comparative images of different steps: induction, growth, rooting of Cabernet Franc y Pinot Noir varieties in Medium 1 and 2

Table 1. Comparative parameters of Cabernet Franc and Pinot Noir in medium 1(M1) and medium 2 (M2)

Variety	Parameter	M1 MS al 50%	M2 MS al 50% + AIA
Cabernet Franc	Induction	↔	↑
	Aerial Growth	↓	↑
	Rooting	✕	↑
	Contamination	↑	↔
Pinot Noir	Induction	↑	↑
	Aerial Growth	↓	↑
	Rooting	↓	↑
	Contamination	↑	↔

4. DISCUSSION AND CONCLUSION

The conventional method of grapevine propagation is time consuming and allows for disease transmission, and the use of tissue culture allows for the mass production of genetically homogeneous populations and healthy plants. It is therefore a very important technique for grapevine breeding programmes [13].

Micropropagation of selected Vitis genotypes can be achieved by growing whole or fragmented apical meristems, axillary microsections of shoots, or development of adventitious or sparse shoots, among others [14,15].

The Cabernet Franc and Pinot Noir lines showed different results in the use of growth regulators from those reported by most authors reviewed in the literature [16,17,18,19], which indicate benzyl amino purine (BAP) as an indispensable cytokinin for Vitis micropropagation. Diab et al. [20] propose the cytokinins Benzyl Adenine (BA), Thidiazuron (TZ) or Kinetin (KIN) as essential hormones in different concentrations for grapevine micropropagation and regeneration. In our experience, no cytokinins other than those included in the M medium have been used and a high growth rate has been achieved.

Rooting occurred only with the addition of Indole Acetic Acid (IAA), which is considered a major finding as we tend to look for inexpensive culture media to advise on large-scale micropropagation. Plant growth regulators that are effective for one species may not be equally effective for another variety, cultivar or species [16].

Rooting, once the *in vitro* grown plant has developed aerially, it is usually transferred to a rooting medium for rooting proliferation [17,16]. The rooting medium usually has similar proportions of ANA, IAA, Indole Butyric Acid (IBA) or at least two of them to promote adventitious root development and proliferation in a short time, in our study we have managed to accelerate the production of both varieties, with the use of MS supplemented with IAA, for explant establishment, proliferation and rooting, without changing the medium or repotting.

Diab et al. [20] transferred vine cuttings of the hybrid 'Remaily Seedless' to Vitis Chée and Pool (C2D) medium supplemented with IAA, ANA or IBA (1 mg/l each) for root induction. The media with 1 mg/l IBA proved to be the best for rooting cuttings, followed by IAA. The rooting percentage was 80% using C2D medium supplemented with 1 mg/l IBA, with 16 out of 20 cuttings rooting. ANA was not suitable for rooting as the rooting percentage was only 35%.

Similarly, in a previous study on rooting of *in vitro* cuttings of cv. 'Pinot Noir', Jaskani et al. [21] reported that media containing 10 μM IBA were the best for rooting grapevine cuttings, while in the absence of IBA, the cuttings showed complete failure to root. In our work, 80% of Cabernet Franc and 90% of Pinot Noir cuttings develop roots in 50% MS medium and 100 mg/l IBA.

Cabernet Franc and Pinot Noir, were found to be susceptible to *in vitro* micropropagation, with very good yield an ability to acclimatize and tolerance by ex vitro transplantation.

Wue concluded that 50 % salt MS medium plus addition of 10 ml of IAA (indole acetic acid mg/l agar-agar 8000 mg/l and 30000 mg/l sucrose) is an economical culture medium for large-scale micropropagation of Cabernet Franc and Pinot Noir cutting.

ACKNOWLEDGEMENT

Thanks to the Secretary of Science and Technology, Consolidated Project No. 02-25223 of the National University of San Luis. San Luis, Argentina.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Murashige T, Skoog FM. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 1962;15:473-479.
2. PinotNoir: la más delicada. VitiViniCultura. Viveros Barber. Available:http://www.vitivinicultura.net/variedades-de-uvas-pinot-noir.html.
3. Cabernet Franc. Variedades de vinos tintos. VitiViniCultura. Viveros Barber. Available:http://www.vitivinicultura.net/cabernet-franc-variedades-vid.html.
4. Cruz-Cruz CA, González-Arnao MT, Engelmann F. Biotechnology and conservation of plant biodiversity. Resources. 2013;2(2):73-95.
5. Kumar PA, Tarun K. Efficient Micropropagation and Evaluation of Genetic Fidelity Of *In Vitro* Raised Plants of Commiphora Wightii Arn. (Bhandari)-A Medicinally Important Red-Listed Species of Arid Regions. Journal of Plant Development. 2012;19.
6. Alderete LM, Mori M, Kato A, Salvio Escandón A. Establishment of an *in vitro* micropropagation protocol for Mecardoniatenella. Electronic Journal of Biotechnology. 2006;9(3):0-0.
7. Cavazos-Galindo JM, Alvarado-Gómez O. G, Santos-Haliscak, J. A, Moreno-Degollado, G, Rodríguez Fuentes, H, Ojeda-Zacarías M, Carmen D. Clonal propagation of two adult grapevines cultivars (*Vitis vinifera* L.) for *in vitro* conservation. Polibotánica. 2018;45:181-190.
8. George EF, Hall MA, De Klerk GJ. Micropropagation: uses and methods in Plant propagation by tissue culture (29-64): Ed. Springer; 2008.
9. Pedro TS, Peiró R, Villanova J, Olmos A, Gisbert C. *In vitro* propagation of *Vitis vinifera* L. cv. 'Monastrell'. Electronic Journal of Biotechnology. 2017;27(1):80-83.
10. Melyan G, Sahakyan A, Harutyunyan A. Micropropagation of grapevine (*Vitis vinifera* L.) seedless cultivar Parvana through lateral bud development. Vitis. 2015;54:253-255.
11. Nookaraju A, Barreto SM, Agrawal DC. Rapid *in vitro* propagation of grapevine cv. Crimson Seedless-Influence of basal media and plant growth regulators. Journal of Applied Horticulture. 2008;10(1):44-49.
12. Ruxton GD. The unequal variance t-test is an underused alternative to Student's t-test and the Mann–Whitney U test. Behavioral Ecology. 2006;17(4):688-690.
13. Kinfe B. Multiple shoots regeneration of grape vine (*Vitis vinifera* L.): Optimum BAP concentration for shoot initiation, survival and multiplication optimum IAA concentration for rooting. LAP Lampert Acad. Publ. 2010;64.
14. Heloir MC, Fournioux JC, OziolL, Bessis R. An improved procedure for the propagation *in vitro* of grapevine (*Vitis vinifera* cv. Pinot noir) using axillary bud micro cuttings Plant Cell Tiss. Org. Cult. 1997;49:223-225
15. Monette PI. Grapevine (*Vitis vinifera* L.), Biotechnology in Agriculture and forestry, Crops Bajaj Y.P.S. (ed.) Springer Verlag Berlin Heidelberg, Germany). 1988;6(6):3-37.
16. Poudel PR, KataokaI, Mochioka R. Effect of plant growth regulators on *in vitro* propagation of Vitis ficifolia var. ganebu and its interspecific hybrid grape. Asian Journal of Plant Sciences. 2005;4:466-71.

17. Alizadeh M, Singh SK, Patel VB. Comparative performance of *in vitro* multiplication in four grape (*Vitis spp.)* rootstock genotypes. International Journal of Plant Production. 2010;4(1):41-50
18. Křižan B, Ondrušiková E, Moudrá J. The effect of media composition on multiplication of grape rootstocks *in vitro*. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis. 2012;60(8):141-144.
19. Khan N, Ahmed M, Hafiz I, Abbasi N, Ejaz S, Anjum M. Optimizing the concentrations of plant growth regulators for *In vitro* shoot cultures, callus induction and shoot regeneration from calluses of grapes. OENO One. 2015;49(1):37-45.
20. Diab AA, Khalil SM, Ismail RM. Regeneration and micropropagation of grapevine (*Vitis vinifera* L.) through shoot tips and axillary buds. IJABR. 2011; 2(4):484-491.
21. Jaskani MJ, Haider A, Sultana R, Khan MM, Qasim M, Iqrar AK. Effect ofgrowth hormones on micropropagation. of *Vitis vinifera* L. CV. Perlette. Pak. J. Bot. 2008;40(1): 105-109.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of the publisher and/or the editor(s). This publisher and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.ikprress.org/review-history/12160