Advances in Bioresearch
Adv. Biores., Vol 5 (4) December 2014: 73-79
©2014 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV 7.20 [Poland]

Advances in Bioresearch

ORIGINAL ARTICLE

A New Commercial Protocol for Micropropagation of Myrtus tree

Amir Rezaee[1*], Kazem Kamali[2]
[1]M.S. Graduated in Horticultural Science, Ardakan University, Iran
[2]Assistant Professor, Ardakan University, Iran
Corresponding author: kkamali@yazd.ac.ir

ABSTRACT
This research was conducted in 2013, in order to propagation of Myrtus communis tree via tissue culture technique. In this study, the rate of shoot proliferation and rooting were evaluated. The results indicated that between, MS (Murashige & Skooge 1962) and WPM (woody plant Medium), media ,WPM showed better result rather than MS. In Shoot proliferation stage were used benzyl adenine(BA) at levels of 0, 0.5, 1, 2, 3, 4, 5 and 6 mg /l and indole butyric acid(IBA)at levels of 0 and 0.1 mg /l, then three traits containing: leaf number, shoot length, and shoot proliferation were examined. The best result was obtained by using of modified WPM medium with BA in 4and IBA in 0.1mg/l. The mean of shoot in this treatment was 40 shoots in each tube. For rooting, MS medium was used which adjusts IBA in 0, 1, 2, 3, 4, 5 mg/l that the best result was obtain by using of 3 mg/l IBA and dark period of 8 days. For adaptation stage of this plant, two contexts were used; jiffy pots as well as combination of 60% washed sand with 40% of peat moss which was successful in both contexts.
Keywords: Adaptation, Rooting, proliferation, MS, Myrtus communis, WPM

Received 07/10/2014 Accepted 29/11/2014

How to cite this article:
Amir R, Kazem K.A New Commercial Protocol for Micropropagation of Myrtus tree. Adv. Biores., Vol 5 [4] December 2014: 73-79: DOI: 10.15515/abr.0976-4585.5.4.7379

INTRODUCTION

Myrtus (*Myrtus Communis*)is a member of *Myrtaceae* family which includes 100 genera and 3000 growing species existingin temperate, tropical and subtropical regions. It grows wildly throughout the Mediterranean and northern hemisphere zone [1].It has been considered and used by different nations such as Iranians, Christians, and Jewssince ancient time and nowadays by identifying its different combinations and properties such as astringent, reinforcing, antiseptic and anti-parasitic is used for curing many disease such as in healing wounds and also can be used orally in treatment of digestive system and the urinary tract disorders [2, 3].Using propagation new techniques such as micropropagation can induce integrate production of plants and additionally rapid production of this plant [4,5,6,7,8]. Miller showed that the different ratios of cytokinin over auxin in medium can control the formation of organs such as roots and stems [9]. Higher ratio of auxin rather than cytokinin lead to root production and higher ratios of cytokinin rather than auxin lead to stem production; therefore, today root and stem production is controlled in tissue culture by adjusting auxin and cytokinin ratio under in vitro condition10. Browning plant tissues is often the result of being damaged and generally results in dead of the explant. In general, browning in plants is by poly phenol oxidase (PPO) enzymeactivity11. However ascorbic acid is very unstable in culture medium, but some of its breakdown products act well as antioxidant12. Propagation of plant colonies can be associated by successful micropropagation and an available way for short-term maintenance culture medium [13]. Micropropagation is an appropriate way to obtain a large number of true to type plants [14-17] .In this research we produced many plants via tissue culture technique and then translated all of them to the field successfully.

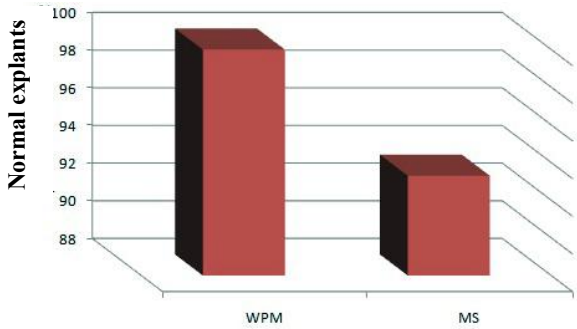
MATERIALS AND METHODS

This study was evaluated as a factorial experiment in acomplete randomized design in the stage of shoot proliferation and rooting of *Myrtus Communis*.Material plants with the size of 0.5 to 1 cm were provided from terminal and lateral buds. Culture medium were considered sucrose ,20 mg/l ,Agar6 mg/l and pH of 5.8 and conditions of growth chamber was: light intensity 5000 lux which was provided by florescent lamps, The light photo period was 8 hours of darkness with 25 degrees centigrade and 16 hours of light with 27degrees centigrade. Relative humidity was 45%. For disinfection of explants, sodium hypochlorite 20%and 0.05 ascorbic acid was used for 5 minutes for preventing of browning explants.

After 75explants of *myrtuscommunis* established in each of free-hormones MS and WPM media were evaluated for 4 weeks which were examined after locating explants in desired culture medium, shoot proliferation stage were done in a randomized design with 16 treatments and 4 replicates, with BA concentration in 0, 0.5, 1, 2, 3, 4, 5, and 6 mg/l and IBA in 0 and 0.1 mg/l . In this stage three traits,containing leaf number, shoot length, and proliferation were evaluated. For proliferation ,verified WPM, that amount of NH_4NO_3 and thiamine were doubled, agar was verified to 6g/l, sucrose to 20 g/l,$GA_3$to 2.5 mg/l, and TDZ to 0.1 mg/l, pH to5.7, and light changed from 5000 lux to 10,000 lux. After shoot proliferation stage, produced branches of 10 to 20 mm were entered to rooting stage which examines MS culture in a completely randomized design with 6 treatments and 4 replicates with growth regulators,IBA in 0, 1, 2, 3, 4 and 5 mg/l, in this stage ,two traits, number and length of root were evaluated. Plant adaptation stage was accomplished by two contexts of 60% washed sand, 40% peat and jiffy palette. All statistical data analyses were evaluated using SPSS and MSTAT-C software.

RESULTS AND DISCUSSION

In this study, two MS and WPM mediums free hormones were evaluated for a period of 4 weeks which can be observed in Graph 1-1.



Graph 1-1.The effect of different medium on establishment of Myrtus explants

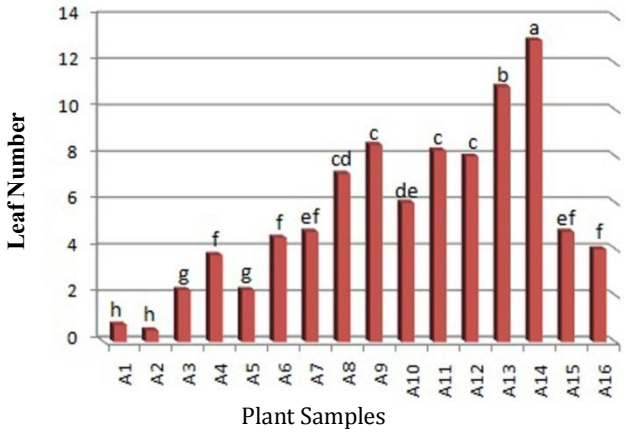
Of 75 studied explants in two cultures, 100% of explants were established in WPM, and 93.3% of explants remained normal in MS which is comparable. The results indicated which for shoot proliferation of this plant, using of WPM medium is better than MS. The studied results are shown in Table 1-2.

Table 1-2:Myrtus regeneration variance analysis

		Square average		
Change sources	Freedom degree	Number of leaves	Stem length	Shoot proliferation
Replicate	3	0.012^{ns}	0.125^{ns}	0.224^{ns}
BA	1	5.062^{**}	7.562^{**}	15.016^{**}
Error a	3	0.021	2.354	0.379
IBA	7	100.777^{**}	287.964^{**}	16.786^{*}
Effect of BA & IBA	7	6.277^{**}	2.205^{**}	1.266^{**}
Error b	21	0.807	2.140	0.266

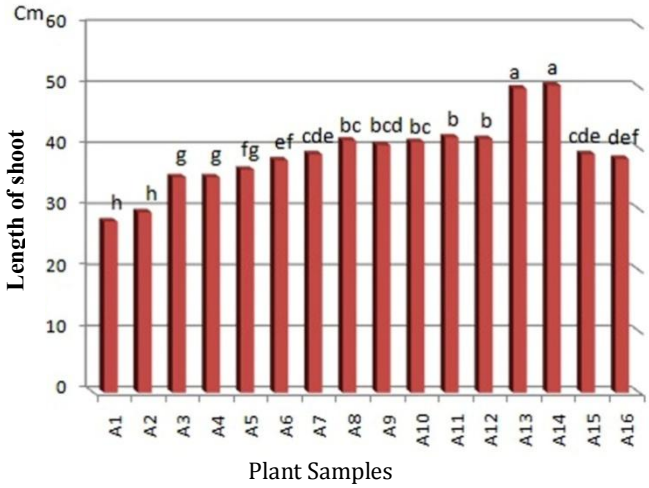
*, **, and ns are significant at the levels of 1% and 5% and no significant variance respectively.

The obtained results showed from variance analysis of BA and IBA effect on the number of leaf, stem length and shoot proliferation are shown in graph 1-2 respectively.



Graph1-2: number of leaf

In graph 1-2 the lowest number of leaf was for BA in 0.5 mg/l (A_2) and control treatment (A_1) and the highest number was for BA in 4 mg/l and IBA in 0.1 mg/l treatment (A_{14}) .
Increase in number of leaves led to increase in shoot length which is shown in graph 1-3.



Graph 1-3: the length of branch

According to graph 1-3 the highest branch length was for control (A_{13}) and BA in 0.5 mg/l (A_{14}) treatment and the lowest branch length was for BA in 4 and IBA in 0.1 mg/l (A_1) treatment as well as BAin 3 and IBA in 0.1 mg/l (A_2) treatment which is shown in the figures (figure 1-3 the highest and figure 1-4 the lowest branch length).

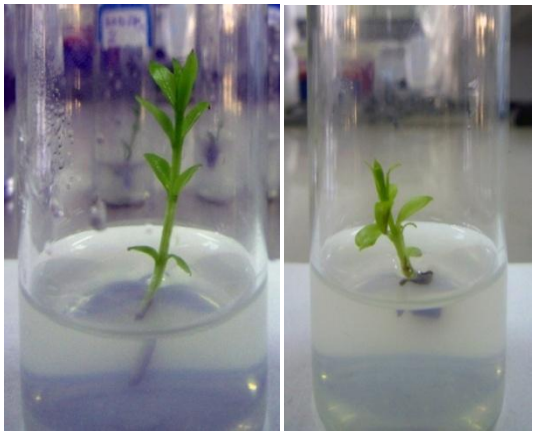
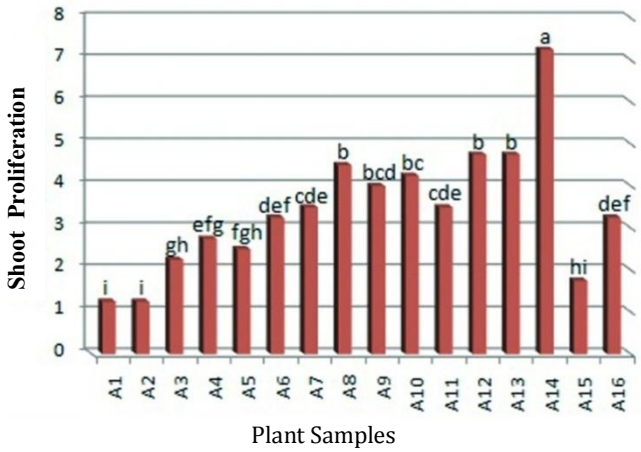


Figure 1-3: the highest branch length Figure1-4: the lowest branch length

One of the most aims of tissue culture is shoot proliferation explant. Although there is a reciprocal effect between the number of leaves and the length of the branch, increase in the number of the leaves led to increase in the length of the branch, is shown in graph 1-4.



Graph 1-4: Shoot proliferation

According to graph 1-4, the lowest number of shoot proliferation was contributed to control (A_1) and benzyl adenine of 0.5 mg/l (A_2) treatment and the highest number of shoot proliferation was for benzyl adenine of 4 and indole butyric acid of 0.1 mg/l (A_{14}) treatment which is shown in the figures (figure 1-5 the lowest number and figure 1-6 the highest number).

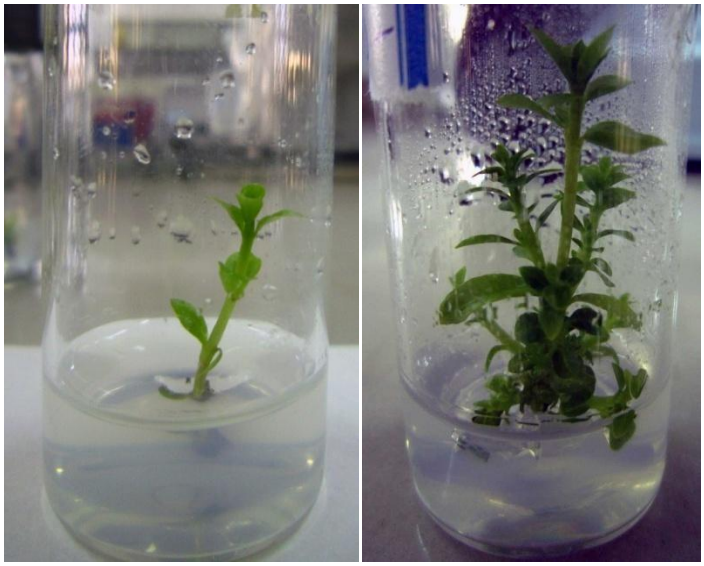


Figure 1-5: the lowest number of branchFigure1-6: the highest number of branch

The results showed that BA and IBA regulators had reciprocal effect on each other, low amount of indole butyric acid led to growth of explants; therefore, high concentration of BA is effective to IBA activity in buds and can be reduced its activities. Increase in the number of leaves and stem length resulted in the higher number of branches in the study. After putting produced plantlets in verified WPM, it was observed that the growth of the plantlet in this culture medium was very good and resulted plantlets are desirable in terms of stem and leaf formation and shape and size of the leaves. After a period of 28 days, it led to formation of around 40 branches in the culture which can be observed in figures 1-7 and 1-8.

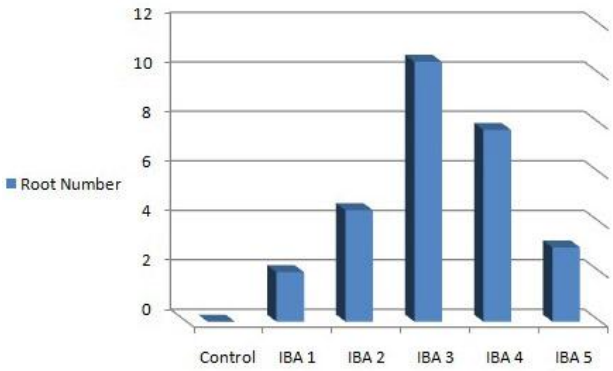

Figure 1-7: shoot proliferation


Figure 1-8: shoot proliferation

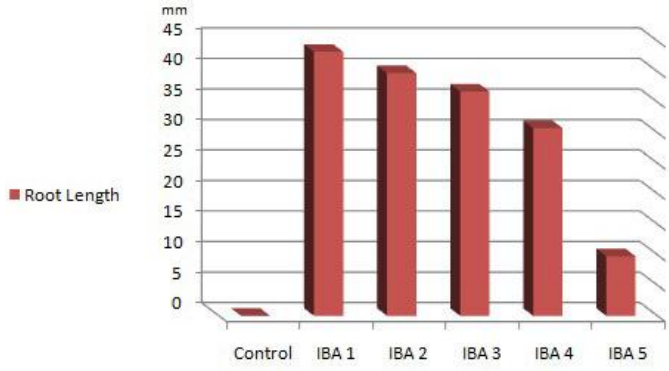
The grown- up branches in the culture free from hormones which were 4 - 5 cm high were transferred to root proliferation culture. Root proliferation in MS was evaluated by two factors of light and darkness. Root proliferation in light took long for 25 days. Increase in darkness period had a significant effect on root proliferation for 10 days which 8 day-period is optimum rather than periods of 5 and 10 days. However it is possible that some explants ruin due to senility in long darkness period; generally light of 2000 - 3500 lux was used for root proliferation. Root proliferation experiment was evaluated with 6 treatments, 4 replicates and two traits of number and length of roots. Variance analysis is observable in table 1-3 which was significant at the level of Duncan 1%.

Table 1-3: Mytrus root proliferation variance analysis

		Average of squares	
Change sources	Freedom degree	Length of root	Number of root
Replicates	3	1.153^{ns}	0.153^{ns}
Indole butyric acid	5	1249.44^{**}	60.175^{**}
Error	15	0.686	0.686



According to graph 1-5 rooting had reciprocal effect on the length and the number of roots, length of root in low concentration (indole butyric acid of 1 mg/l) led to increase in the length of the root (figure 1-9) and increase in the concentration (indole butyric acid of 5 mg/l) decreased the length of the root. However low concentration (indole butyric acid of 1 mg/l) included the lowest number of roots and increase in concentration by 3 mg/l had the highest number of root; increase more than 3 mg/l led to descending trend.



Graph 1-5.Rooting of Myrtus plant

Figure 1-9

Adaptation stage in both cultures: jiffy pot and 60% washed sand, 40% peat were studied. Observations showed that it was successful in both contexts. However leaf and stem formation growth efficiency and shape of leaf was more desirable in 60% washed sand, 40% peat (figure 1-10).

Figure 1-10 Adaptation and acclimatization of Myrtus plants

REFERENCES

1. Traveset, N. Riera and R.E.(2001). Mas -Ecology of Fruit-colour Polymorphism in Myrtuscommunis and Differential Effects of Birds and Mammals on Seed Germination and Seedling Growth, Journal of Ecology, 89, p. 749-760
2. Balfour, J.H. (1857). The Plants of Bible: Trees and Shrubs, Edinburgh, T. Nelson and Sons, Paternoster Row.

3. Philpot J.H.(2004).The Sacred Tree in Religion and Myth, New York, Courier Dover Publications.
4. Capuana, M.& Ponti,F.(2008). In Vitro: Medium Term Conservation of Myrtus communis L. Propagation of Ornamental Plants 8(2): 111-113.
5. Pierik, R.L.M.(1997). In Vitro Culture of Higher Plants: Kluwer Academic Publishers Netherlands, Fourth Edition, pp. 213-220.
6. Kongjika, E., Zekaj, ZH.,Caushi, E &Stamo, I.(2002). Bioteknologjia e Bimeve- Kulturat" in vitro: Academia e shkencava,Tirane, pp. 97-105.
7. Damiano, C., Padro, M.D.A. & Frattarelli, A.(2008). Propagation and Establishment in vitro of Myrtle (*Myrtuscommunis* L.), Pomegranate (*Punicagranatum* L.) and Mulberry (Morus alba L.). Propagation of Ornamental Plants, 8 (1): 3-8.
8. NOBRE, J. (1997).Micropropagation of *Myrtus communis* L. (Mediterranean myrtle).In: Bajaj P.S.(Ed). Biotechnology in Agriculture and Forestry. Vol. 39, Hight-Tech and Micropropagation V, - Springer-VeriagPBerlin Heidelberg, pp. 127-134.
9. Miller, C.O.(1956).Similarity of Some Kinetin and Red Light Effects". Plant physiol.31:318.
10. Krikorian, A.D. (1995). Hormones in Tissue Culture and Micropropagation. In Davies, P.J.(ed). Plant Hormones: Physiology, Biochemistry and Molecular Biology. Kluwer, Dordrecht, pp. 774-796.
11. Marks, T., and Simpson, S. (1990). Reduced Phenolic Oxidation at Culture Initiation in Vitro Following the Exposure of Field-grown Stockplants to Darkness or Low Levels of Irradiance. J. Hortic. Sci. 65: 103-111.
12. Elmore, H., Samples, B., Sharma, S., and Harrison, M. (1990).Influence of Cultural and Physiochemical Factors on AscorbateStability in Plant Tissue Culture Media.Plant Cell Tissue Organ Cult. 20: 131-135.
13. Capuana, M. &ponti, F. (2008). In vitro:Medium Term Conservation of *MyrtusCommunis* L. Propagation of Ornamental Plants 8(2): 111-113.
14. Kongjika, E., Zekaj, ZH.,Caushi, E &Stamo, I. (2002). Bioteknologjia e Bimeve- Kulturat" in vitro". Akademia e shkencava,Tirane, pp. 97-105.
15. Damiano, C., Padro, M.D.A.& frattarelli, A. (2008). Propagation and Establishment in Vitro of Myrtle (*Myrtuscommunis* L.),Pomegranate (*punicagranatum* L.) and Mulberry (*morusalba* L.). Propagation of Ornamental Plants, 8 (1): 3-8.
16. NOBRE, J. (1997). Micropropagation of*MyrtusCommunis* L. (Mediterranean myrtle). In: Bajaj P.S. (Ed). Biotechnology in Agriculture and Forestry. Vol. 39, Hight-Tech and Micropropagation V, - Springer-VeriagPBerlin Heidelberg, pp. 127-134.
17. Pierik, R.L.M.(1987). Invitro Culture of Higher Plants.MartinusNijhoff PublishersNetherlands, Fourth Edition, pp. 213-220.
18. Ho-hyuk. J., Sung-Ho, A., Myung-Deak, k., Chan-wha, k., (2008). Use of Hydrogen Peroxide as an Effective Disinfectant to ActinobacillusUreae .Process Biochemistry. 43:225-228.