Effect of some growth regulators on proliferation of myrtle (*Myrtus communis* L. cv. Local) plant by in vitro culture

[1]Majid A. Ibrahim, [2]Haleemah J. Al-Aradi, [3]Khayun A. Muhsen

[1] Department of Horticulture and Landscape Design, College of Agriculture, University of Basra, Basra, Iraq; [2] Marine Science Center, University of Basra, Basra, Iraq; [3] Date Palm Research Center, University of Basra, Basra, Iraq. Corresponding author: M. A. Ibrahim, majidalbassiri@yahoo.com

Abstract. The study was carried out at Plant Tissue Culture, Marine Sciences Center, University of Basra, Iraq. The aim of this study is micro propagation of myrtle plant by shoot multiplication technique. The results showed that shoots were formed on MS medium supplemented with 1.0 mg.L^{-1} BA and 1.0 mg.L^{-1} kinetin after ten weeks from culture (7.0 shoots). Also, that shoot length and number of leaves/shoot were reached 4.5 cm and 8.20 leaves/shoot, respectively after ten weeks from culture. As well as the MS medium supplemented with 5.0 mg.L^{-1} NAA and 5.0 mg.L^{-1} IBA gave the highest rooting ratio (100%). Acclimatization was successful for 100% of the rooted plants. The results showed that plant height and number of leaves per shoot were increased to 6.67 cm and 10.00 leaves/shoot, respectively after two weeks from plant acclimatization. Then, shoots increased with increasing age of plant and reached the highest values after six weeks from acclimatization (10.33 cm and 13.20 leaves/shoot, respectively).
Key Words: acclimatization, callus, explants, shoot multiplication, shoots.

Introduction. The myrtle plant belongs to the Myrtaceae family, which includes 100 species and about 3000 species. This ornamental plant grows in temperate, tropical and semi-tropical regions (Traveset et al 2001). The myrtle plant belongs to the evergreen shrubs that grow to a height of about 1-5 m. The oppositely arranged leaves are ovate-lanceolate, 2-5 cm long, coriaceous, glabrous, punctuate-glandular and entire. When crushed, they have a delicate aromatic odor (Davis 1982). This plant is an important source of active substances used in the pharmaceutical and medical industries (Flamini et al 2004). The myrtle plant is propagated with seed and cutting methods (Khosh-Khui & Bassiri 1976). Micro propagation of myrtle were used different explants such as apical meristem (Parra & Amo-Marco 1998), nodal segments (Parra & Amo-Marco 1996), shoot tip culture (Khosh-Khui et al 1984) and axillary shoots (Ruffoni et al 2003). Scarpa et al (1994) found that MS medium containing 30 G.L^{-1} + 8 G.L^{-1} + 0.5 or 1.0 mg.L^{-1} BA + 0.05 or 0.1 mg.L^{-1} NAA, gave the best shoot multiplication rate. Also, they found that MS medium containing 0.5 mg.L^{-1} BA + 0.05 mg.L^{-1} NAA was resulted in root differentiation. Ruffoni et al (2009), noted that shoot tip cultured on MS medium supplemented with vitamins + 0.5 mg.L^{-1} BA + 0.2 mg.L^{-1} IAA gave the highest number of shoot rate. IAA or IBA at 0.5 mg.L^{-1} concentration increased the rooting percentage and noticed differences in root number and length. In one study conducted on the micro propagation of myrtle plant found that MS medium supplied with 0.3 mg.L^{-1} thidiazuron (TDZ) and 0.1 mg.L^{-1} NAA gave the highest number of shoots (4.0 shoots). Also, IBA applications induced more rooting than NAA. As well as the MS medium supplemented with 1.0 mg.L^{-1} and activated charcoal gave the highest rooting ratio (80%). Acclimatization was successful for 86% of the rooted plants (San et al 2015).

The aim of this study is micro propagation of myrtle plant by shoot multiplication technique.

Material and Method. The study was carried out at Plant Tissue Culture, Marine Sciences Center, University of Basra, Iraq.

Source of explants. The explants were taken from shoot tips (1.0 cm length) of healthy myrtle (*Myrtus communis* L.) plant cv. Local. The shoot tips were rinsed with sterile distilled water for 3 times and then surface sterilized with 20% commercial chlorax solution containing 1.05% sodium hypochlorite, and a drop of Polysorbate 20 for 15 minutes. The sterilized shoot tips were rinsed in sterile distilled water for 3 times.

Shoot proliferation. Using full strength MS (Murashige & Skoog 1962) basal medium supplied with 1.0 $mg.L^{-1}$ BA (Benzyl adenine) + 1.0 $mg.L^{-1}$ Kinetin + 1.0 $mg.L^{-1}$ for each of vitamins and glutamin + 30 $G.L^{-1}$ sucrose + 2 $G.L^{-1}$ poly vinyl pyrrolidone (PVP). The pH of the media was adjusted to 5.7 with 0.1 N NaOH or HCl after adding 5% agar, and before autoclaving at 1.04 $Kg.cm^{-2}$ for 15 minutes. All media were dispensed in culture tubes containing 15 mL medium cultures. Shoot tips explants were cultured on these medium and incubated at a temperature of 27±2°C and light intensity less than 1000 Lux light intensity was provided by white fluorescent lamps for 16 hrs (Figure 1A). The explants were re-cultured to a fresh medium after eight weeks intervals. The number of produced lateral shoots/explant, shoot length (cm) and number of leaves/shoot were recorded after ten weeks from culture.

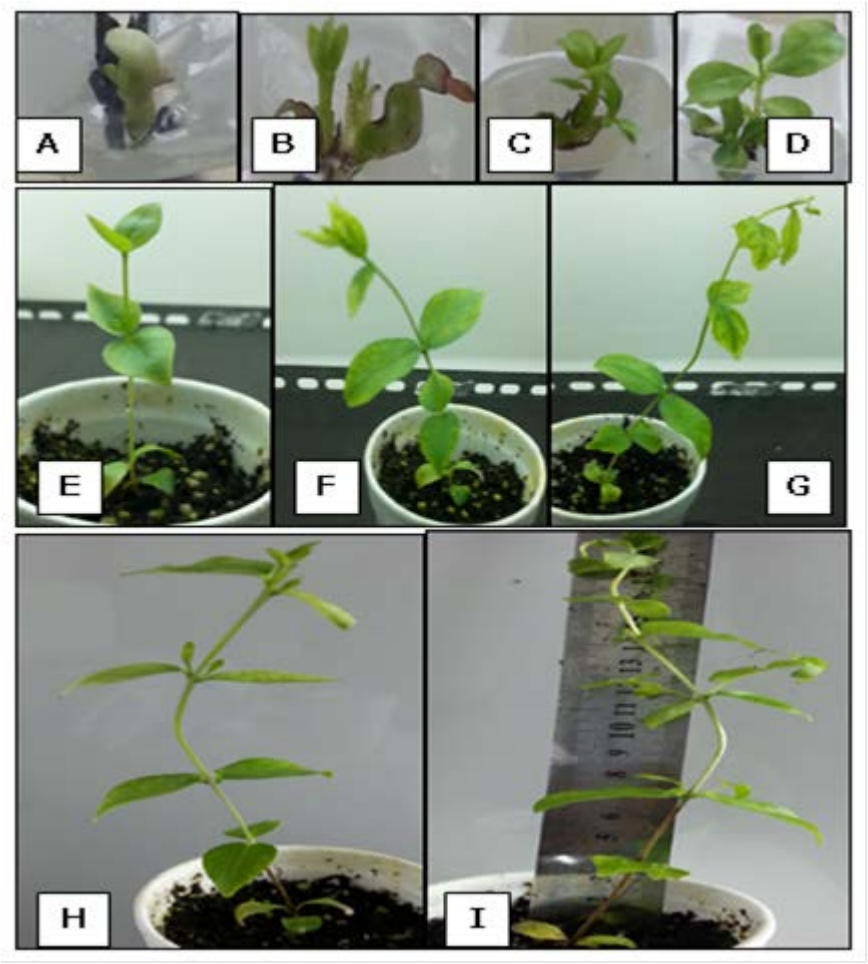

Figure 1. In vitro culture of myrtle plant (*Myrtus communis* L. cv. Local) by shoot multiplication method. A - Shoot tip cultured on MS medium supplemented with 1.0 mg L^{-1} BA and 1.0 mg L^{-1} Kinetine. B, C and D - Shoot proliferation on MS supplemented with 1.0 mg L^{-1} BA and 1.0 mg L^{-1} Kinetine. E, F, G, H and I - Stages of plantlet acclimatization cultured on pots contain fibers of coconut fruit.

Rooting shoot. The newly formed lateral shoots obtained in the previous step were separated and transferred to a rooting medium consisting of MS medium. This medium was supplemented with NAA and IBA at 5.0 $mg.L^{-1}$ concentrations for each of them. The cultures were incubated in a growth chamber on the same conditions as referred to above. The rooted shoots were obtained within 4-6 weeks of culture on this medium.

Plantlets acclimatization. The process of acclimatization was carried out on plantlets, 8-10 cm in length, with an average of 6-8 leaves and having a good root system. Plants were removed from the culture tube and washed with sterilized water to clean the root system from the remains of the culture medium. The plantlets were placed in glass flasks containing half strength of MS salts and distilled water ensuring the submergence of the root system. The glass flasks were closed with thin aluminum foil and placed in a growth chamber for 24 hours. Then, the plantlets were planted in plastic pots containing fibers of coconut fruit and covered with a glass tube. The acclimated plantlets were watered once a week with half strength MS salts, and distilled water was added to the pots as required. The plantlets were misted regularly with distilled water and the inner surface of the glass cover to achieve optimum humidity to prevent wilting of the plantlets. The plant height (cm) and number of leaves/plant were recorded after six weeks from culture.

Statistical design and analysis. Completely randomized design was used with five replicates. The data were subjected to the analysis of variance and mean values were compared using revised LSD at 5% (Snedecor & Cochran 1986).

Results and Discussion. The results presented in Table 1, showed that shoots were formed on MS medium supplemented with 1.0 $mg.L^{-1}$ BA and 1.0 $mg.L^{-1}$ kinetin after seven weeks from culture reached 2.6 shoots (Figure 1B, C and D). The number of formation shoot increased with increasing age and reached the highest rate after 10 weeks from culture (7.00 shoots). The shoot length and number of leaves/shoot were reached 1.4 cm and 4.33 leaves/shoot, respectively after seven weeks from culture. Those two characteristics were increase to highest values after ten weeks from culture (4.5 cm and 8.20 leaves/shoot, respectively). The known effects of cytokinins in promoting axillary shoot production and its role in plant morphogenesis in plant tissue culture (Hopkins & Muner 2008). The results of the study agreed with what Ruffoni et al (2009), and San et al (2015) found through their studies on the micro propagation of myrtle plant. They observed that cytokinin led to the best proliferation rate of shoot. As well as the MS medium supplemented with 5.0 $mg.L^{-1}$ NAA and 5.0 $mg.L^{-1}$ IBA gave the highest rooting ratio (100%) after 2-4 weeks from culture. The results of the study were agreed with the results of several studies conducted on the same plant studied (Ruffoni et al 2009; San et al 2015). The reason for this is due to role of auxins such as NAA and IBA in cell division and root formation.

Acclimatization was successful for 100% of the rooted plants (Figure 1E, F, G, H and I).

Table 1

Some growth characteristics of *Myrtus communis* shoot formed on MS medium supplemented with 1.0 $mg.L^{-1}$ BA + 1.0 $mg.L^{-1}$ Kinetin after 10 weeks from shoot tip culture

Weeks after culture	*Number of shoot formation*	*Shoot length (cm)*	*Number of leaves/shoot*
7	2.60	1.40	4.33
8	3.00	2.20	7.33
9	4.20	3.80	8.00
10	7.00	4.50	8.80
R-LSD (0.05)	0.14	0.31	0.11

The results of Table 2 showed that plant height and number of leaves per shoot were increased to 6.67 cm and 10.00 leaves/shoot, respectively after two weeks from plant acclimatization. Then, shoots increased with increasing age of plant and reached the highest values after six weeks from acclimatization (10.33 cm and 13.20 leaves/shoot, respectively). Similar results were obtained by other authors for ornamental myrtle (San et al 2015).

Table 2

Some growth characteristics of *Myrtus communis* acclimated on plastic pots containing fibers of coconut fruit, after six week from culture

Weeks after culture	*Plant height (cm)*	*Number of leaves/shoot*
2	6.67	10.00
4	7.67	12.40
6	10.33	13.20
R-LSD (0.05)	0.58	0.42

Conclusions. MS medium supplemented with 1.0 $mg.L^{-1}$ BA and 1.0 $mg.L^{-1}$ Kinetin gave the highest value of shoot formation after ten weeks from culture. As well as the MS medium supplemented with 5.0 $mg.L^{-1}$ NAA and 5.0 $mg.L^{-1}$ IBA gave the highest rooting ratio after 4-6 weeks from culture.

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Authors:
Majid Abdulhameed Ibrahim, University of Basra, College of Agriculture, Department of Horticulture and Landscape Design, Iraq, Basra, Garmat Ali Campus, 0964319128, e-mail: majidalbassiri@yahoo.com
Haleemah Jabbar Al-Aradi, University of Basra, Marine Sciences Center, Iraq, Basra, Garmat Ali Campus, 0964319128, e-mail: halema.gabar@yahoo.com
Khayun Ali Muhsen, University of Basra, Date Palm Research Center, Iraq, Basra, Garmat Ali Campus, 0964319128, e-mail: khaunali2000@yahoo.com

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