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# Micropropagation of Indian jujube (*Ziziphus mauritiana* Lam. cv. Zaytoni) through shoot tip culture

Muayed F. Abbas, Majid A.-H. Ibrahim, Abbas M. Jasim

College of Agriculture, Basrah University, Basrah, Iraq. Corresponding author: M. F. Abbas, muayedfadhil@yahoo.co.uk

**Abstract**. *In vitro* shoot multiplication were successfully achieved from shoot tips of *Ziziphus mauritiana* Lam. cv. Zaytoni by culturing the shoot tip explants on MS medium supplemented with various concentrations of 6-benzyl amino purine (BA) (0.5, 1.0 and 1.5 mg $L^{-1}$). BA at 1.0 mg $L^{-1}$ induced significantly highest number (4) of axillary shoots in comparison with other concentrations. The well developed plantlets were transferred to a potting mix containing sand and peat moss (2:1) and grown for 3 months, with an average survival rate of 66.7 %. The results of the present work, clearly demonstrated the efficient cloning of *Z. mauritiana* Lam. cv. Zaytoni, through shoot tip culture multiplication.
**Key Words**: shoot tip, micropropagation, plantlet regeneration, *Ziziphus mauritiana* (Lam.)

**Introduction**. Jujube (*Ziziphus* Mill.) it is a subtropical fruit tree native to northern hemisphere (Lyrene 1979). It is an increasingly important crop fruit in arid and semi-arid regions of the world (Pareek 2001). The fruit is a good source of ascorbic acid and carotenoids (Abbas 1997). The conventional method of vegetative propagation of jujube trees is through budding of elite cultivars on seedling rootstocks. However, the rate of multiplication is very low and therefore is not suitable for mass propagation of elite cultivars. Micropropagation provides an attractive and alternative method for large scale propagation and commercial production of jujube trees. Various *in vitro* protocols of regeneration through shoot tip cultures in Indian jujubes have been reported (Goyal & Ayra 1985; Mathur et al 1995; Rathore et al 1992; Sudhersan et al 2001; Sudhersan & Hussain 2003). The proliferation of axillary shoots from cultured shoot tips is greatly influenced by nature of the used culture medium. Accordingly, it is of paramount importance to standardize culture conditions for a particular cultivar.

The research described in this paper provides an efficient procedure for mass propagation of Indian jujube, cultivar Zaytoni, which is an excellent commercial cultivar and is widely grown in Basrah area (Abbas & Fandi 2002).

Abbreviations: BA - 6-benzyl amino purine; IBA - Indole-3-butyric acid; NAA - α-naphthalene acetic acid.

## Material and Method

The experiment to be described was carried out at the Plant Tissue Culture Laboratories, Date Palm Research Centre, Basrah University, Basrah, Iraq.

Shoot tips (1.0 cm) of *Ziziphus mauritiana* Lam. cv. Zaytoni was obtained from a healthy and well established fruit yielding mature trees growing in a private orchard. The shoot tips were then kept in anti-oxidant solution containing 100 mg $L^{-1}$ ascorbic acid and 150 mg $L^{-1}$ citric acid for 24 hours to avoid phenolic compounds exudation during explants culturing. The shoot tips were then rinsed with sterile distilled water for 3 times and surface sterilized with 20 % commercial chlorax solution containing 1.05 % sodium hypochlorite, and a drop of Tween 20 for 15 minutes. The shoot tips were rinsed in sterile distilled water for 3 times.

***Axillary shoot induction***. The shoot tip explants were cultured on full strength MS basal medium supplemented with various concentration of benzyl adenine (0.0, 0.5, 1.0, and 1.5 mg $L^{-1}$ BA). The pH of the media was adjusted to 5.7 with 0.1 N NaOH or HCl after adding 0.7 % agar, and before autoclaving for 20 minutes at 121 °C (15 psi nominal steam pressure). All media were dispensed in 25 x 150 mm test tubes containing 25 mL medium cultures, and were incubated under 1000 lux light intensity provided by white fluorescent lamps for 16 h at 26 ± 1 °C. The numbers of axillary shoots produced were recorded after 4 weeks from culture (Figure 1 A-B).

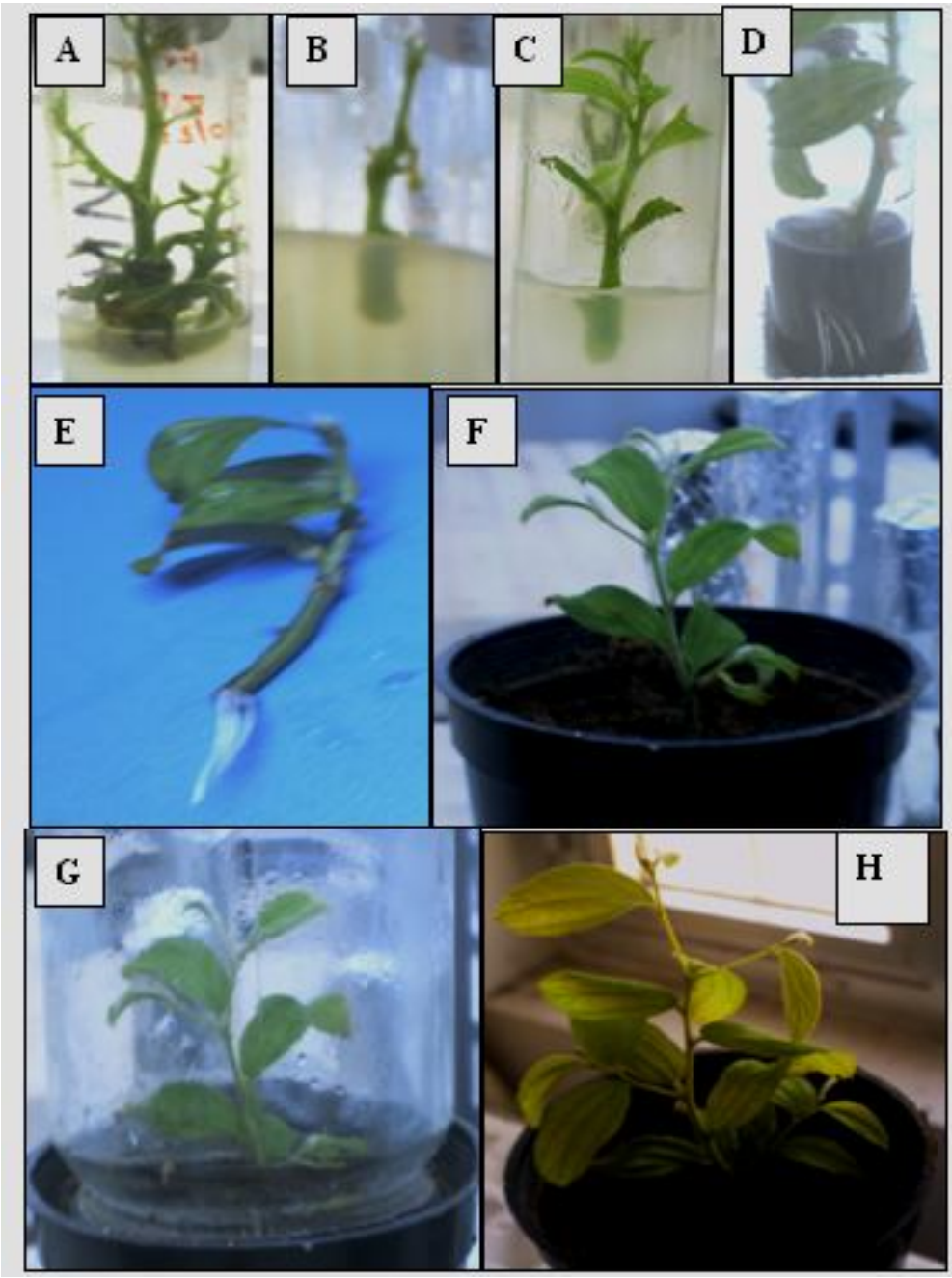


Figure 1. Micropropagation of jujube trees (*Ziziphus mauritiana* Lam. cv. Zaytoni) by shoot tip multiplication. A - Shoot tip multiplication on MS medium supplemented with 1.0 mg $L^{-1}$ BA. B - Shoot tip with no axillary shoots on MS without BA. C - No rooting of shoot tip on full strength MS. D - Rooted shoot cultured on half strength MS supplemented with 0.2 mg $L^{-1}$ NAA. E - Rooted plantlet. F, G and H - Stages of plantlet acclimatization.

***Rooting induction***. The isolated axillary shoots were planted on full strength MS medium containing the auxin either IBA or NAA at (0.0, 0.1, 0.5, 1.0, 5.0, 10.0 and 15.0 mg $L^{-1}$). Axillary shoots also planted on half strength MS medium containing the auxin NAA at 0.2 mg $L^{-1}$. Only axillary shoots planted on half strength MS with NAA at 0.2 mg $L^{-1}$ produced adventitious roots after 30 days of culture (Figure 1 C-D).

***Plantlet acclimatization***. The process of acclimatization was carried out on plantlets, 7.6 cm in length, with an average of 5.3 leaves and having a good root system. Plants were removed from the culture vessels and washed with sterilized water to clean the root system from the remains of the culture medium. The plantlets were then placed in glass tubes containing half strength MS medium and distilled water ensuring the submergence of the root system.

The glass tubes were then closed with thin aluminum foil and placed in a growth chamber for 24 h. Then, the plantlets were planted in an autoclaved soil mix containing sand and peat moss (2:1) and covered with a glass tube. The acclimatized plantlets were watered once a week with half strength MS medium, 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 acclimatization continued for 3 months, and the rate of survival was 66.7 % (Figure 1 F, G, H).

***Statistical analysis***. Data were statistically analyzed in a completely randomized design with ten replicates. Mean values were compared using revised LSD at 5.0 % (Steel & Torrei 1980).

**Results and Discussion**. It is evident from the results, that *Z. mauritiana* Lam. cv. Zaytoni can be clonally mass propagated *in vitro* using shoot tip culture. *In vitro* shoot multiplication had been reported in various cultivars of *Z. mauritiana* Lam. (Goyal & Ayra 1985; Mathur et al 1995; Rathore et al 1992; Sudhersan et al 2001; Sudhersan & Hussain 2003; Al-Sulaiman & Barakat 2010).

Table 1 shows the effect of BA concentration on adventitious shoot formation in Zaytoni jujube.

Table 1

The effect of concentration of benzyl adenine on number of axillary shoots produced by shoot multiplication of *Ziziphus mauritiana* Lam. cv. Zaytoni

| *Concentration of BA (mg $L^{-1}$)* | *No of axillary shoots* |
|---|---|
| 0.0 | 2.1 |
| 0.5 | 2.6 |
| 1.0 | 4.0 |
| 1.5 | 3.3 |
| Revised LSD (0.05) | 0.6 |

Each value is the mean of 10 replicates.

It is obvious, that the number of axillary shoots significantly increased with increase in BA concentration, with the highest number obtained at 1.0 mg $L^{-1}$. Such effects are due to the known effects of cytokinins in promoting axillary shoot production and its role in plant morphogenesis in plant tissue culture (Hopkins & Muner 2008). Similar results were obtained by other worker, using the cytokinin benzyl adenine with other jujube cultivars (Rathore et al 1992; Sudhersan & Hussain 2003; Al-Suliaman & Barakat 2010). The rate of multiplication of adventitious shoots obtained in the present work after six months of culture was 106 shoots, from a single shoot tip (Ibrahim 2008).

No rooting response was obtained when axillary shoots were planted on full strength MS medium containing various concentrations of IBA or NAA 0.0, 0.1, 0.5, 1.0, 5.0, 10.0 and 15.0 mg $L^{-1}$ (Figure 1C). However, a rooting response of 45 % was obtained, when the axillary shoots were cultured on half strength MS containing 0.2 mg $L^{-1}$

NAA (Figure 1D). Sudhersan et al (2001) working with *Z. mauritiana* Lam. cv. Umran, reported 30 % rooting after 30 days in the rooting medium. However, Al-Sulaiman & Barakat (2010) working with *Ziziphus spina-christi* (L.) Wild, reported 60 % rooting on media containing 0.5 and 1.0 mg $L^{-1}$ IBA. Ibrahim (2008) also working with *Z. spina-christi* (L.) Wild, reported 72 % rooting on half strength MS, containing 0.2 mg $Ll^{-1}$ NAA.

Table 2 shows various vegetative characteristics of *Z. mauritiana* Lam. cv. Zaytoni during acclimation. It is clear, that hardened plantlets showed normal pattern of the growth and development (Figure 1 F, G, H).

Table 2

Certain vegetative characteristics of *Ziziphus mauritiana* Lam. cv. Zaytoni plantlets, during the process of acclimation

| *Days from the beginning of acclimation* | *Plantlet height (cm)* | *No of leaves per plantlet* | *No of side shoot/plantlet* |
|---|---|---|---|
| 0 | 7.6 | 5.3 | 1.0 |
| 30 | 9.0 | 6.3 | 1.0 |
| 60 | 11.5 | 7.3 | 1.7 |
| 90 | 18.5 | 10.0 | 3.7 |
| Revised LSD (0.05) | 1.0 | 1.2 | 1.1 |

**Conclusion**. In conclusion, the results obtained in the present work clearly demonstrated the efficient cloning of *Z. mauritiana* Lam. cv. Zaytoni, through shoot tip culture multiplication.

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Authors:
Muayed Fadhil Abbas, College of agriculture, Department of Horticulture and Landscape Design, University of Basrah, Garmat Ali Campus, Basrah, Iraq, 0964319128, e-mail: muayedfadhil@yahoo.co.uk
Majid Abdul-Hameed Ibrahim, College of agriculture, Department of Horticulture and Landscape Design, University of Basrah, Garmat Ali Campus, Basrah, Iraq, 0964319128, e-mail: majidalbassiri@yahoo.com
Abbas Mehdi Jasim, College of agriculture, Department of Horticulture and Landscape Design, University of Basrah, Garmat Ali Campus, Basrah, Iraq, 0964319128, e-mail: abbasjasim5@yahoo.com

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