COBISS Code 1.03

Agrovoc descriptors: ziziphus, adventitious roots, stems, meristem culture, callus, in vitro regeneration, explants, callogenesis

Agris category code: F62

In vitro plant regeneration of Indian jujube (*Ziziphus mauritiana* Lamk.) cv. Zaytoni via indirect organogenesis

Majid Abdel IBRAHIM, Abbas Mehdi JASIM and Muayed Fadhil ABBAS*

Received November 10, 2011; accepted February 21, 2012.
Delo je prispelo 10. novembra 2011, sprejeto 21. februarja 2012.

ABSTRACT

In vitro plant regeneration was achieved in *Ziziphus mauritiana* Lamk., through *de novo* formation of meristems in callus obtained from the shoot tip cultured on MS medium supplemented with either IBA (10.0 mg.l^{-1}) or NAA (15.0 mg.l^{-1}) and 5.0 mg.l^{-1} BA. Organogenic callus was obtained when the primary callus was cultured on MS medium supplemented with BA at 5.0 mg.l^{-1} .Adventitious shoots were obtained when the organogenic callus was incubated on MS medium supplemented with BA at 1.0 mg.l^{-1} and NAA at 0.1 mg.l^{-1}. Whole plants were developed when the adventitious shoots were transferred to half strength MS medium supplemented with NAA at 0.2 mg.l^{-1}.

Key words: *Ziziphus mauritiana* Lamk., adventitious shoots, shoot tip explants

IZVLEČEK

***IN VITRO* REGENERACIJA INDIJSKE ŽIŽOLE (*Ziziphus mauritiana* Lamk.) CV. ZAYTONI S POSREDNO ORGANOGENEZO**

In vitro regeneracija poganjkov je bila dosežena pri indijski žižoli (*Ziziphus mauritiana* Lamk.) iz meristemskih izsečkov preko kalusa na MS gojišču z dodatkom bodisi hormona IBA (10,0 mg l^{-1}) ali NAA (15,0 mg l^{-1}) in 5.0 mg l^{-1} BA. Organogeni kalus je bil dobljen, ko se je primarni kalus prestavilo na MS gojišče z dodatkom 5,0 mg l^{-1} hormona BA. Adventivni poganjki so nastali iz organogenega kalusa, ki je bil gojen na MS gojišču z dodatkom hormonov BA (1.0 mg l^{-1}) in NAA (0.1 mg l^{-1}). Adventivne poganjke se je koreninilo na polovični koncentraciji MS gojišča z dodatkom hormona NAA (0.2 mg l^{-1}).

Ključne besede: žižola, *Ziziphus mauritiana* Lamk., meristemski izsečki, adventivni poganjki

1 INTRODUCTION

The jujube belongs to the genus *Ziziphus* Mill., which is in the *Rhamanaceae* or bulk thorn family. The genus include about 40 species of plants in tropical and sub-tropical regions of the northern hemisphere (Lyrene,1979), of which the species *Z. jujaba* Mill. and *Z. mauritiana* Lamk. were the most important in the terms of distribution and economic significance. Four species are native to Iraq, but only two, namely *Ziziphus spina-christi* (L.) Wild. and *Z. mauritiana* Lamk. are of economic importance and are grown mainly in the Basrah region (Abbas,1997).

NAA: α- naphthalene acetic acid.
IBA: Indole-3- butyric acid.

Jujube fruits have a sponge, sweet-testing pulp, and are an excellent source of ascorbic acid and carotenoids (Abbas,1997).

Although, it is possible to multiply Indian jujubes through budding of selected genotypes on seedling rootstocks, the rate of multiplication is very low and therefore is not suitable for mass production to meet the demands of planting materials. Thus to meet the demand of planting material, it is necessary to obtain a true to the type plants through a method of rapid vegetative propagation.

Plant tissue culture is in efficient method of vegetative propagation of various perennial trees. Various protocols of regeneration through shoot tip *in vitro*

* muayedfadhil@yahoo.co.uk, College of Agriculture, Basrah University, Basrah, Iraq

culture and nodal stem segments in Indian jujubes have been reported (Goyal & Arya,1985; Mathur *et al*.,1995; Rathore *et al*.,1992; Sudhersan *et al*.,2001; Sudhersan & Hussain,2003). So far, no information is available on the *in vitro* propagation of Indian jujubes through organogenesis (direct or indirect).

The present paper describes the induction of organogenic callus from shoot tip, organogenesis and subsequent plantlet regeneration in Indian jujube cv. Zaytoni which is an important commercial cultivar and its fruits are of excellent quality.

2 MATERIALS AND METHODS

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

Shoot tips (1.0 cm) of *Ziziphus mauritiana* Lamk. 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 antioxidant 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 3 times.

2.1 Callus induction

Shoot tips were cultured on full strength MS basal media, supplemented with either IBA (10 mg l^{-1}) or NAA (15 mg l^{-1}) and 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 at 1.04 Kg cm^{-2} for 15 minutes. All media were dispensed in 25X150 mm test tubes containing 25 ml medium. Cultures were incubated under 1000 lux light intensity provided by white fluorescent lamps for 16 hrs photoperiod at 26 ± 1°C. After 60 days, white globular callus were formed at the base of the shoot tip (Fig.1 A and B).

2.2 Organogenic callus induction

The white globular callus was divided and incubated on full strength MS medium supplemented with BA at 5.0 mg l^{-1} for callus proliferation. This process continued for 120 days to obtain sufficient amount of organogenic callus (Fig.1 C), with sub-culturing every six weeks.

2.3 Adventitious shoot proliferation

The organogenic callus obtained from previous step was incubated on half strength MS supplemented with BA at 1.0 mg.l^{-1} and NAA at 0.1 mg.l^{-1} for the induction of adventitious shoots on the surface of the callus. New shoots were developed on the surface of the callus within six weeks of culture (Fig.1 D, E, F, G).

2.4 Induction of rooting

The newly formed shoots obtained in the previous step were separated with a small amount of callus and transferred to a rooting medium consisting of half strength MS supplemented with NAA at 0.2 mg.l^{-1}. Rooted shoots were obtained with in six weeks of culture on this medium (Fig.1 H, I).

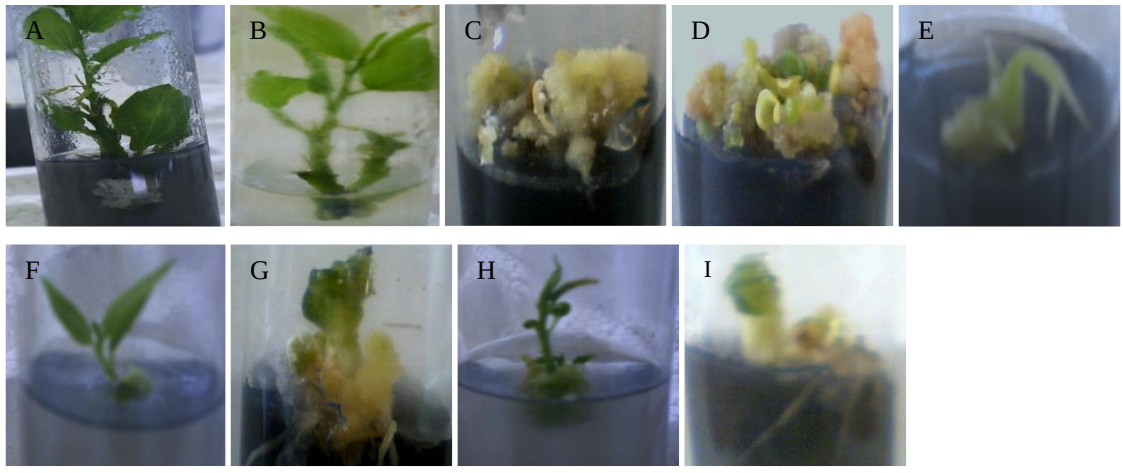

Figure 1: Indirect organogenesis and plantlet regeneration in Indian jujube (*Ziziphus mauritiana* Lam. cv. Zaytoni): A, B: Callus formation; C: Callus proliferatio; D,E,F,G: Adventitious shoot proliferation; H,I: Rooted shoot.

3 RESULTS AND DISCUSSION

It is evident from Fig. 1, that *Ziziphus mauritiana* Lamk. cv. Zaytoni can be clonally mass propagated *in vitro* using indirect organogenesis from shoot tip derived callus. To our knowledge, this is the first report on the use of indirect organogenesis for *in vitro* propagation of Indian jujube.

Earlier several authors have used shoot tip culture and auxiliary meristem culture for *in vitro* propagation of Indian jujube of several cultivars, but the major problem was low rooting efficiency (Goyal & Arya,1985; Mathur *et al*.,1995; Rathore *et al*., 1992; Sudhersan *et al*.,2001). In the closely related species, *Ziziphus sativa* L. (wild jujube) and *Z. jujuba* Mill. (Chinese jujube), *in vitro* plant regeneration have been achieved using indirect and direct organogenesis but the source of explants were zygotic embryos and seedling trees (Kim *et al*.,1987; Gu & Zhang,2005).

Callus initiation occurred at the cut end of the shoot tip explants, as white globular mass on MS medium containing 5.0 mg l^{-1} BA + 10.0 mg l^{-1} IBA within 60 days. Profuse callus was obtained on MS medium supplemented with BA at 5.0 mg l^{-1}. The results obtained in the present work are similar to those reported for other jujube cultivars, regarding the importance of auxins for callus induction and cytokinins for callus proliferation (Kim *et al*.,1987; Rathore *et al*.,1992; Mitrofanova *et al*.,1997; Gu & Zhang,2005). It is obvious from the present work, that half strength MS medium supplemented with BA at 1.0 mg l^{-1} and NAA at 0.1 mg l^{-1} promoted adventitious shoot regeneration (Fig.1). Similar results were obtained by Kim *et al*.(1987) for *Z. sativa* L. and Gu & Zhang (2005), for *Z. jujuba* Mill., who demonstrated the importance of cytokinins/auxins ratio in adventitious shoot proliferation during *in vitro* indirect and direct organogenesis. The importance of appropriate ratio of cytokinins: auxins for the production of adventitious shoots *in vitro* is well documented for a wide range of plant species (Collins & Edwards,1998).

Regenerated shoots rooted (100%) when they transferred to half strength MS medium supplemented with NAA at 0.2 mg l^{-1} (Fig. 2).

In conclusion, the results obtained in the present work demonstrate efficient cloning of *Z. mauritiana* Lamk. cv. Zaytoni via indirect organogenesis.

4 REFERENCES

Abbas, M.F. (1997). Jujube. In: Post-harvest physiology and storage of Tropical and Sub tropical Fruits (Mitra, K.S, ed.), CAB International, Wallingford, pp 405-415.

Collins, H.A. and Edwards, G.S. (1998). Plant cell culture. Bios Scientific publishers, Oxford, England.

Goyal , Y. and Arya, H.C. (1985). Tissue culture of desert trees II. Clonal multiplication of *Ziziphus in vitro*. J. Physiol., 119: 398-404.

Gu, X.F. and Zhang, J.R. (2005). An efficient adventitious shoot regeneration system for Zhanhua winter jujube (*Ziziphus jujuba* Mill.) using leaf explants. Plant Cell Reports, 23: 775-779.

Kim, S.K.; Hwang, J.K.; Peak, K.Y.; Han, B.H. and Hwang, J.H. (1987). *In vitro* callus formation responses of jujube as affected by growth regulators and levels of organic nutrient. J. Korean Soc. Hort. Sci., 28: 131-136.

Lyrene, P.M. (1979). The jujube tree (*Ziziphus jujuba* Mill.) Fruit Var. J., 33: 100-104.

Mathur, N.; Ramawat, K.G. and Nandwani, D. (1995). Rapid *in vitro* multiplication of jujube through mature stem explants. Plant Cell Tissue and Organ Culture., 43: 75-77.

Mitrofanova, I. V.; Mitrofanova, O.V. and Pandey, D.K. (1997). Somatic embryogenesis and plantlet regeneration in *Ziziphus jujuba* Mill. *in vitro*. Russian J. Plant Physiology, 44: 94-99.

Rathore , T.S.; Singh, R.P.; Dora, N.S. and Shekhawat, N.S. (1992). Clonal propagation of *Ziziphus* species through tissue culture. Scientia Hortic., 51: 165-168.

Sudhersan, S.; Aboel-Nil, M. and Hussian, J. (2001). *In vitro* propagation of *Ziziphus mauritiana* cultivar Umran by shoot tip and nodal multiplication. Current Science, 80: 290-292.

Sudhersan, S. and Hussian, J. (2003). *In vitro* clonal multiplication of multipurpose tree, *Ziziphus spina-christi* (L.). Desf. Turk. J. Bot. , 27: 167-171.