

## SOMATIC EMBRYOGENESIS AND PLANTLET REGENERATION IN INDIAN JUJUBE (*ZIZIPHUS MAURITIANA* LAMK.) CV. ZAYTONI

*Ibrahim M. A., A. M. Jasim, M. F. Abbas\**

*College of Agriculture, Basrah University, Basrah, Iraq*

Received: 10 November 2011 Accepted: 16 January 2012

**Summary.** Somatic embryogenesis and plantlet regeneration were achieved in callus culture derived from shoot tips of Indian jujube (*Ziziphus mauritiana* Lamk. cv. Zaytoni). It is for the first time shown that somatic embryogenesis and plantlet regeneration were successfully achieved in Indian jujube using explants from vegetatively propagated trees. Callus was induced from shoot tips (1.0 cm) of Zaytoni jujube on MS medium supplemented with either NAA or IBA and BA. Somatic embryos were developed on half strength MS medium supplemented with BA only. Embryo maturation took place on MS medium supplemented with NAA and BA. Plantlets were regenerated from these somatic embryos on half strength MS medium free of plant growth regulators. The well developed plantlets were transferred to a potting mix containing sand and peat moss (2:1) and grown for three months with an average survival rate of 58%.

**Keywords:** Callus; plantlet regeneration; shoot tip; somatic embryogenesis; *Ziziphus mauritiana* Lamk.

**Abbreviations:** BA – N<sup>6</sup>-Benzyl aminopurine; IBA – Indole butyric acid; NAA –  $\alpha$ -Naphthalene acetic acid.

## INTRODUCTION

Jujube (*Ziziphus* Mill.) is a sub-tropical fruit tree native to the northern hemisphere (Lyrene, 1979). It is an increasingly important fruit crop in semiarid regions of the world (Anon, 1998). The fruit is generally eaten fresh and is a good source of ascorbic acid and carotenoids (Abbas, 1997). Although it is possible to multiply

Indian jujube trees through budding of selected genotypes on seedling rootstocks, the rate of multiplication is very low and therefore is not suitable for mass propagation to meet the demands of planting materials.

In species where a clonal propagation is normal, rather than an exception, cloning through tissue culture could be

---

\*Corresponding author: [muayedfadhil@yahoo.co.uk](mailto:muayedfadhil@yahoo.co.uk)

highly desirable. Although various *in vitro* protocols of regeneration through shoot tip culture and nodal stem segments in Indian jujube have been reported, however, low rooting efficiency has been reported as the major problem for *in vitro* production of jujube plants using such techniques (Goyal and Arya, 1985; Mathur et al., 1995; Rathore et al., 1992; Sudharsan, 2001; Sudharsan and Hussain, 2003).

Somatic embryogenesis using explants taken from vegetatively propagated mature trees is an efficient method of plant regeneration, allowing rapid production of large number of plants within a short period. The present paper describes the induction of embryogenic callus from shoot tip, somatic embryogenesis and subsequent plantlet regeneration in Indian jujube cv. Zaytoni, which is an excellent commercial cultivar widely grown in the Basrah area (Abbas and Fandi, 2002).

## MATERIALS AND METHODS

The experiment was carried out in the Plant Tissue Culture Laboratories, Date Palm Research Centre, Basrah University, Basrah, Iraq.

Shoot tips (1.0 cm) of *Ziziphus mauritiana* Lamk. cv. Zaytoni were obtained from healthy and well-established fruit yielding mature trees growing in a private orchard. The shoot tips were then kept in an antioxidant solution containing 100 mg l<sup>-1</sup> ascorbic acid and 150 mg l<sup>-1</sup> citric acid for 24 h to avoid phenolic compounds exudation during explants culturing. The shoot tips were rinsed 3 times with sterile distilled water and then surface sterilized with 20% commercial Chlorax solution containing 1.05% sodium hypochlorite and a drop of Tween 20 for 15 min. The shoot

tips were rinsed in sterile distilled water 3 times.

### Callus induction

Shoot tips were cultured on full strength MS basal media supplemented with either IBA (10 mg l<sup>-1</sup>) or NAA (15 mg l<sup>-1</sup>). 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<sup>-2</sup> for 15 min. All media were dispensed in 25x150 mm test tubes containing 25 ml medium. The cultures were incubated at a light intensity of 1000 lux provided by white fluorescent lamps for 16 h photoperiod at 26±1°C. After 60 days, white globular callus was formed at the base of the shoot tip (Fig. 1, A).

### Embryogenic callus induction

The white globular callus was divided and incubated on full strength MS medium supplemented with BA at a concentration of 5.0 mg l<sup>-1</sup> for callus proliferation. This process continued 120 days with subculturing every six weeks to obtain a sufficient amount of embryogenic callus (Fig. 1, B).

### Induction of somatic embryos

The embryogenic callus obtained at the previous step was incubated on half strength MS media supplemented with BA at a concentration of 1.0 mg l<sup>-1</sup> for the induction of somatic embryos. Somatic embryos were obtained after 60 days of culture (Fig. 1, C, D and E).

### Embryos maturation

Somatic embryos were cultured on MS media supplemented with BA and NAA at concentrations of 0.1 mg l<sup>-1</sup> and 0.1 mg l<sup>-1</sup>, respectively for embryo maturation. This

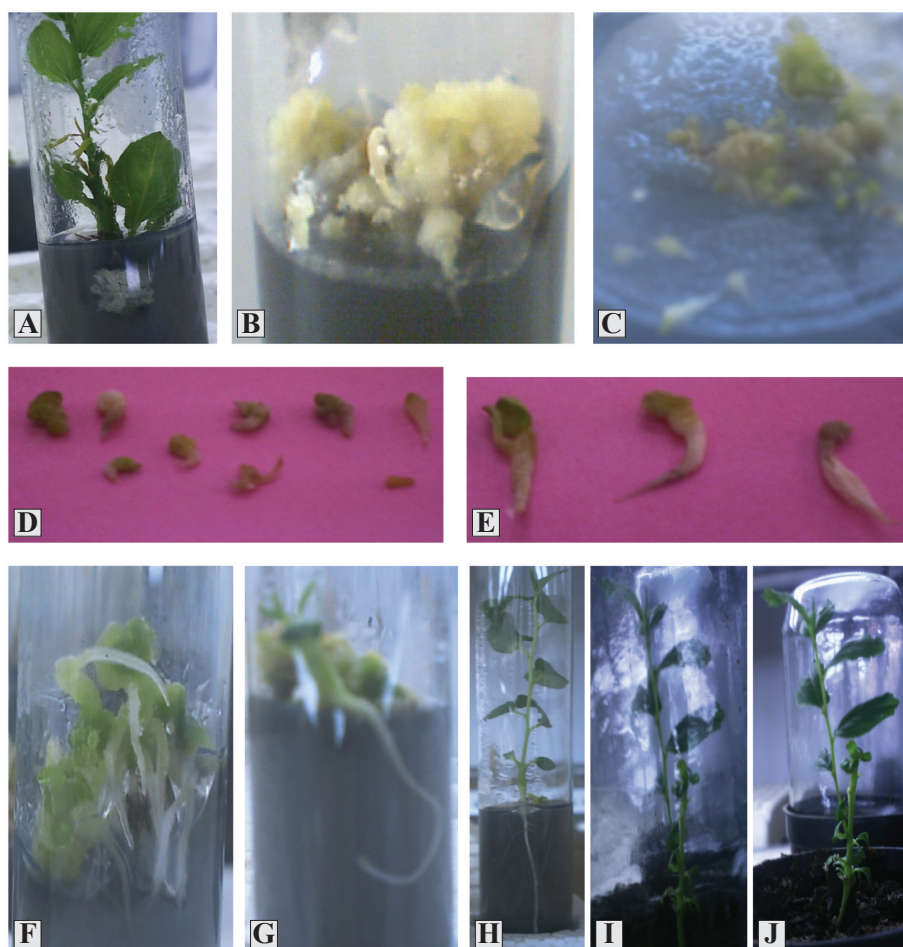


Fig. 1. Regeneration of plantlets through somatic embryogenesis in *Ziziphus mauritiana* Lamk. cv. Zaytoni. A – Callus induction; B – Callus proliferation; C, D, E – Embryogenesis; F – Embryo development; G – Mature embryo; H – Mature plantlet; I, J – Acclimatized plantlet.

step took about 60 days (Fig. 1, F).

### Plantlet regeneration

The fully developed embryos were cultured, each in a test tube containing half strength MS medium free of plant growth regulators. This step took about 60 days, after which the plantlets were ready for acclimation (Fig. 1, H).

### Plantlet acclimation

The process of acclimation was carried out on plantlets (8.0 cm in height) having an average of five leaves and well developed

root system.

Plantlets were removed from the culture vessels and washed with sterilized water to clean the root system from the remains of the growth 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. The plantlets were planted in an autoclaved soil mix containing sand and peat moss (2:1). The plantlets were then

covered with glass covers. The acclimized 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 process of acclimation continued 3 months, at the end of which the average height of the plantlet was 16.8 cm with 12.6 leaves and 2.8 auxiliary shoots (Fig. 1, I and J). The rate of survival was 58%.

## RESULTS AND DISCUSSION

It is evident from Fig. 1, that *Ziziphus mauritiana* Lamk. cv. Zaytoni can be clonally mass propagated *in vitro* using somatic embryogenesis from shoot tip derived callus. To our knowledge, this is the first report on the use of somatic embryogenesis for *in vitro* propagation of Indian jujube using explants taken from vegetatively propagated trees. Earlier, several authors used shoot tips and nodal stem segments for *in vitro* propagation of Indian jujube of various cultivars, but the major problem was low rooting efficiency (Goyal and Arya, 1985; Mathur et al., 1995; Rathore et al., 1992; Sudharsan et al., 2001; Sudharsan and Hussain, 2003).

Callus initiation occurred at the cut end of the shoot tip explants as a white globular mass on MS media containing 5.0 mg l<sup>-1</sup> BA + 10.0 mg l<sup>-1</sup> IBA within 8 weeks. Profuse callus was obtained on MS media supplemented with BA at a concentration of 5.0 mg l<sup>-1</sup>. The results obtained in the present work were similar to those reported for other jujube cultivars, regarding the importance of auxins for callus induction and cytokinins for callus proliferation

(Rathore et al., 1992; Mitrofanova et al., 1997; Sudharsan et al., 2001; Sudharsan and Hussain, 2003; Asserah and Sardabi, 2005).

It is obvious from the present work, that half strength MS medium supplemented with BA at a concentration of 1.0 mg l<sup>-1</sup> induced the formation of somatic embryos (Fig. 1, C, D and E).

The importance of the cytokinin BA for the induction of somatic embryos has been reported for *Z. jujuba* Mill. (Mitrofanova et al., 1997) and also for citrus (Praveen and Ahlawat, 2003).

Embryo germination and maturation occurred on half strength MS medium supplemented with BA (0.1 mg l<sup>-1</sup>) and NAA (0.1 mg l<sup>-1</sup>). Similar results were obtained by other authors for Chinese jujube, citrus and date palm (Mitrofanova et al., 1997; Miah et al., 2002; Bhargava et al., 2003). After embryo germination and maturation, they were transferred to half strength MS medium free of plant growth regulators. Complete plantlets (8.0 cm in height, having an average of 5.0 leaves) were obtained within 60 days (Fig. 1, G), which were ready for acclimation. The plantlets obtained through somatic embryogenesis were transferred into soil mix (sand: peat moss, 2:1), and the rate of survival was 58 %.

In conclusion, the results obtained in the present work demonstrate efficient cloning of *Ziziphus mauritiana* Lamk. cv. Zaytoni through somatic embryogenesis using explants from vegetative organs. Furthermore, protocols for *in vitro* multiplication of commercial jujube cultivars using shoot tips, nodal stem segments and organogenesis have been developed in our laboratory and the results will be reported soon.

## REFERENCES

- Abbas, MF. 1997. Jujube. In: Post-harvest physiology and storage of Tropical and Sub tropical Fruits (Mitra, K.S., ed.), CAB International, Wallingford, pp 405–415.
- Abbas, MF, BS Fandi, 2002. Respiration rate, ethylene production and biochemical changes during fruit development and maturation of jujube (*Ziziphus mauritiana* Lamk.). J. Sci. Food Agric., 82: 1472–1476.
- Anonymous, 1998. Fruits for the future. International Centre for Underutilized Crops. Institute of Irrigation and Development studies, University of Southampton, Southampton, England.
- Assareh, MH, H Sardabi, 2005. Macro propagation and micro propagation of *Ziziphus spina-christi*. Pesq. Agropec. Bras. Brasilia, 40: 549–465.
- Bhargava, SC, SN Saxena; R Sharm, 2003. *In vitro* multiplication of *Phoenix dactylifera* (L.). J. Plant Biochemistry and Biotechnology, 12: 43–47.
- Goyal, Y, HC Arya, 1985. Tissue culture of desert trees II. Clonal multiplication of *Ziziphus in vitro*. J. Physiol., 119: 398–404.
- Lyrene, PM, 1979. The jujube tree (*Ziziphus jujuba* Mill.) Fruit Var. J., 33: 100–104.
- Mathur, N, KG Ramawat, D. Nandwani, 1995. Rapid *in vitro* multiplication of jujube through mature stem explants. Plant Cell Tissue and Organ Culture, 43: 75–77.
- Miah, MN, S Islama, S Hadiuzzaman, 2002. Regeneration of plantlet through somatic embryogenesis from nucellus tissues of *Citrus macroptera* Mont. Plant Tissue Cult., 12: 167–172.
- Mitrofanova, IV, OV Mitrofanova, DK Pandey, 1997. Somatic embryogenesis and plantlet regeneration in *Ziziphus jujuba* Mill *in vitro*. Russian J. Plant Physiology, 44: 94–99.
- Praveen, S, V Pawar, S Ahlawat, 2003. Somatic embryogenesis and plant regeneration in Kinnow Mandarin. J. Plant Biochemistry and Biotechnology, 12: 163–165.
- Rathore, TS, RP Singh, NS Dora; NS Shekhawat, 1992. Clonal propagation of *Ziziphus* species through tissue culture. Scientia Hortic., 51: 165–168.
- Sudharsan, S, M. Aboel-Nil, J Hussian, 2001. *In vitro* propagation of *Ziziphus mauritiana* cultivar Umran by shoot tip and nodal multiplication. Current Science, 80: 290–292.
- Sudharsan, S, J Hussian, 2003. *In vitro* clonal multiplication of multipurpose tree, *Ziziphus spina-christi* (L.). Desf. Turk. J. Bot, 27: 167–171.