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IN VITRO PLANT REGENERATION OF LOCAL PUMMELO (*CITRUS GRANDIS* (L.) OSBECK.) VIA DIRECT AND INDIRECT ORGANOGENESIS

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Summary: The present study was aimed to micropropagate local pummelo trees using direct and indirect organogenesis methods. The results showed that the nucellar embryo cultured on MS medium supplemented with 2 or 4 mg L^{-1} BA plus 0.1 mg L^{-1} NAA gave adventitious shoots directly after eight weeks, but the cotyledonary segments cultured on MS medium supplemented with 4 mg L^{-1} BA plus 0.1 mg L^{-1} NAA gave white callus after eight weeks. Then, that formation callus sub-cultured on MS medium supplemented with 1 or 2 mg L^{-1} BA plus 0.1 mg L^{-1} NAA gave adventitious shoots indirectly after six weeks. The induction shoots cultured on half strength of MS medium supplemented with 0.2 mg L^{-1} NAA plus 0.1 mg L^{-1} BA gave roots after six weeks. The pummelo plantlets acclimatized successfully when cultured in plastic pots in a growth room under controlled conditions (temperature 27±2°C,16/8 h, light/dark photoperiod and light intensity 1500 Lux).

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Abbreviations: BA – 6-benzyl amino purine; MS – Murashige and Skoog medium; NAA – α-naphthaleneacetic acid.

INTRODUCTION

The genus *Citrus* has been recognized as one of the most economically important group of plants in the world (FAO, 2004). Pummelo trees (*C. grandis* L. Osbeck.), one of the citrus species, are widely grown in the tropical and sub-tropical areas (Nwaoguikpe and Braide, 2010). Plant tissue culture is an efficient method of vegetative propagation of various perennial trees. Different protocols of regeneration using various techniques and explants, including somatic embryogenesis and organogenesis have been reported for various citrus species (Al-Taha, 2009; Jajoo, 2010; Lombardo et al., 2011). Cotyledons have high potential of regeneration and represent a good source of tissue cultures. Organogenesis from

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cotyledons was successfully achieved in *C. clementtina* and *C. reticulata* (Burger and Hackett, 1982; Sarma et al., 2011). Jajoo (2010) found that 6-benzyl amino purine at a concentration of 2.22 mM induced the highest number of shoots/ explant from cultured nucellar embryos of *C. limonia* Osbeck. *in vitro*. The aim of this work was to study the efficacy of nucellar embryos and cotyledonary segments (explants), cultured *in vitro* for direct and indirect organogenesis.

MATERIALS AND METHODS

The experiments were carried out in the Plant Tissue Culture Laboratories, Agriculture College, Basrah University, Basrah, Iraq during 2011-2012. Healthy and ripe fruits were collected from local pummelo trees (*C. grandis* L. Osbeck.) from one orchard in Iraq. The mature and fresh seeds were isolated and washed thoroughly under tap water to remove mucus and sugars present on the seed coat. Then the seeds were sterilized with 1.05 % sodium hypochlorite solution with 3-4 drops of tween20 for 15 min and washed 3-4 times with distilled water inside the laminar air-flow cabinet. The seed coats were carefully removed using pointed forceps. Nucellar embryos were excised and surface sterilized by the same method of seed sterilization. The cotyledons were divided in 2-3 pieces and surface sterilized. The sterilized explants were placed in sterilized anti-oxidant solution (100 mg L^{-1} ascorbic acid and 150 mg L^{-1} citric acid), and incubated at 4°C in a refrigerator. The culture medium contained MS salts (Murashige and Skoog, 1962) and was supplemented with sucrose (30gm L^{-1}), polyvinylpyrrolidone (1 gm L^{-1}), glycine (2 mg L^{-1}) and thiamine HCl (2 mg L^{-1}). The pH of the medium was adjusted to 5.7- 5.8 and solidified by phyto-agar (6 gm L^{-1}).

Direct and indirect organogenesis

Two concentrations of BA (2 and 4 mg L^{-1}) with 0.1 mg L^{-1} NAA were tested in a basal medium for callus induction and organogenesis. In addition, 1 or 2 mg L^{-1} BA with 0.1 mg L^{-1} NAA was tested for shoot induction from callus.

Rooting of shoots

The rooting medium contained half strength MS medium supplemented with 0.2 mg L^{-1} NAA plus 0.1 mg L^{-1} BA.

Acclimatization

The rooted plantlets were transplanted into 15 cm in diameter pots containing a mixture of sand:peat moss (1:1), placed in a growth room under controlled conditions (temperature 27±2°C,16/8 h photoperiod and light intensity 1500 Lux).

Statistical analysis

Completely randomized design was used with 10 replicates. The data were subjected to the analysis of variance and mean values were compared using revised-LSD as described by Snedicor and Cochran (1980).

RESULTS AND DISCUSSION

Our results showed that local pummelo trees (*C. grandis* L. Osbeck.) can be clonally mass propagated by *in vitro* direct organogenesis using nucellar embryos and indirect organogenesis using cotyledonary segments as explants (Fig. 1). The nucellar embryo cultured on MS medium

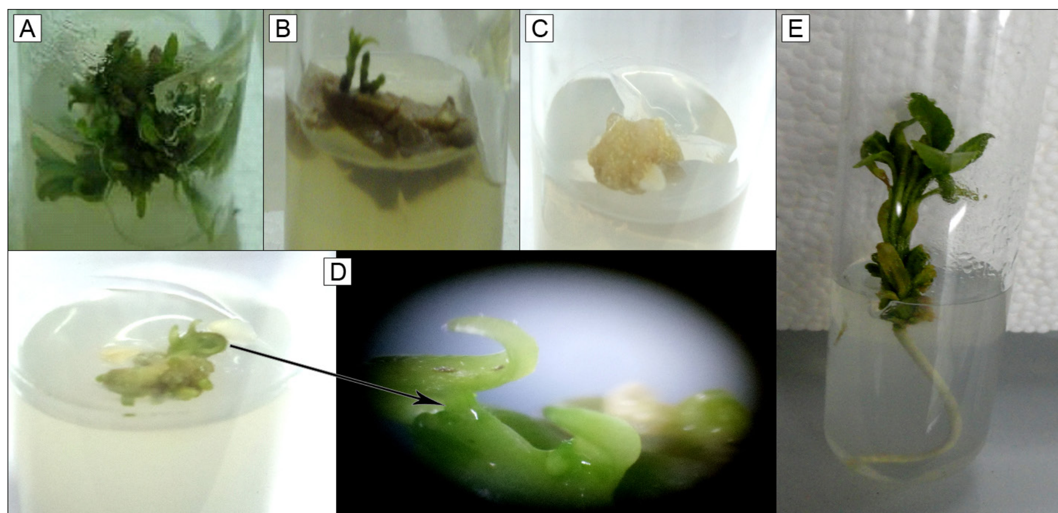

Figure 1. *In vitro* plant regeneration of local pummelo trees (*Citrus grandis* L. Osbeck) by organogenesis.
A: Direct shoots from nucellar embryo cultured on MS medium supplemented with 2 mg L^{-1} BA.
B: Direct shoots from cotyledonary segment cultured on MS medium supplemented with 2 mg L^{-1} BA.
C: Callus induction from cotyledonary segment cultured on MS medium supplemented with 4 mg L^{-1} BA.
D: Indirect shoots from callus cultured on MS medium supplemented with 1 mg L^{-1} BA.
E: For rooting, induction shoot cultured on half strength MS medium supplemented with 0.2 mg L^{-1} NAA + 0.1 mg L^{-1} BA.

supplemented with 2 or 4 mg L^{-1} BA and 0.1 mg L^{-1} NAA gave adventitious shoots directly after eight weeks, in particular when BA was added at 2 mg L^{-1} to the medium (Table 1). This concentration was optimal for adventitious shoot formation. The number and length of adventitious shoots and induction shoots percentage gave high values compared with 4 mg L^{-1} BA treatment (Table 1). Nucellar embryos were probably more meristematic and had more potential as compared with cotyledonary segments (Fig. 1A and B). However, with cotyledonary segments, the use of MS medium supplemented with 2 mg L^{-1} BA and 0.1 mg L^{-1} NAA produced adventitious shoots directly on the surface of the segments. A few adventitious shoots were produced (3.3 shoot). These cotyledonary segments enlarged and became brown in color (Fig. 1B and Table 1). When BA was added at 4 mg L^{-1} to the same culture medium (MS + 0.1 mg L^{-1} NAA), white compact callus was obtained (Fig. 1C). Similar results were obtained with cotyledonary segments of citrus (Begum et al.,2003; Al-Taha, 2009). The induction callus cultured on MS medium supplemented with 1 or 2 mg L^{-1} BA and 0.1 mg L^{-1} NAA developed and produced adventitious shoots on the callus surface (Fig. 1D). BA added to the MS medium at 1 mg L^{-1} gave the best adventitious shoots induction compared

Table 1. Effect of the type of explant and BA on vegetative characteristics of shoots induction by direct and indirect organogenesis of local pummelo.

Type of explant	BA [mg L^{-1}]	No. of shoots per explant	Shoot length [cm]	Shoot proliferation [%]
Direct shoots				
Nucellar embryo	2	9.33[a]	3.0[a]	88[a]
	4	6.66[b]	1.8[b]	85[b]
Cotyledonary segment	2	3.33[c]	1.2[b]	75[c]
	4	*	*	*
Indirect shoots				
Cotyledonary segment	1	6.33[a]	1.5[a]	80[a]
	2	4.33[b]	0.5[b]	70[b]

*Callus induction.
Different letters in each column are significantly different at $p \geq 0.05$ by RLSD Test.

with 2 mg L^{-1} BA (Table 1). Similar results were obtained by other researchers in their studies on pummelo and mandarin (Begum et al., 2003; Sarma et al., 2011). The formation shoots were cultured on rooting medium consisting of half strength MS supplemented with 0.2 mg L^{-1} NAA and 0.1 mg L^{-1}BA. Rooted shoots were obtained within six weeks (Fig.1E). The present results are consistent with other studies using half strength MS medium supplemented with low concentrations of NAA (Begum et al., 2003; Al-Taha, 2009). After rooting, regenerated plantlets were acclimatized by transferring to pots containing peat moss. They were further grown under controlled conditions (temperature 27±2°C,16/8 h, light/dark photoperiod and light intensity 1500 Lux).

CONCLUSION

Shoot induction in *C. grandis* L. Osbeck. cv. Local Pumello can be obtained through direct organogenesis using nucellar embryos as explants. Benzyladenine added at a concentration of 2.0 mg L^{-1} is essential to induce differentiation.

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