ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 5 (1): 49-52, 2006
ISSN 1682-296X

Application of *in vitro* Tissue Culture Techniques for Propagation of *Amygdalus communis* L. Cultivars, Garrigues and Yaltsinki

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Abstract: Almond (*Amygdalus communis* L.) is planted and naturally spreads in the Southeastern Anatolian region where some hybrid varieties are cultivated and the adaptation studies have still been continued. Although being easily propagated through grafting, plantation in large areas may have some problems, due to the environmental stress. Embryos, nodal segmets of mature shoots and shoot tips of two *A. communis* L. cultivars, Garrigues and Yaltsinki, were cultured on MS media supplemented with several concentrations and combinations of cytokinins and auxins, for *in vitro* micropropagation. The best root formation in embryo cultures for both cultivars was obtained in the media without growth regulators. The highest stem development was obtained with 4.0 mg L^{-1} of IAA in Garrigues. Callus developed from cultured shoot tips. MS media supplemented with 4.0 mg L^{-1} BAP induced fast growing-fragile callus growth. The best nodal explant development occurred with MS contained 2.0 mg L^{-1} BAP and 0.5 mg L^{-1} IAA. Results obtained demonstrated that the nodal explants could be used for direct embryogenesis of Garrigues and Yaltsinki varieties.

Key words: Clonal propagation, *Amygdalus communis,* Garrigues, Yaltsinki

INTRODUCTION

Almond (*Amygdalus communis* L.) is a nut tree, which has been grown in large areas in the world due to having climatic tolerance and having many microclimates suitable for almond growth (Dokuzoğuz and Gülcan, 1979). Almond seedlings have been used in production for centuries (Edstrom and Viveros, 1996). In Turkey, the production budding and/or grafting are not common; and instead seed has been usually used for production. Since it is a cross-pollinated species, a continuous genetic variation and heterozygous individuals have occurred. Thus the homogenity decreased in the traditional orchards, which has led to very different fruit yield and quality (Gülcan, 1976; Küden, 1998). In Turkey, almond has been grown mostly in the Aegean, Marmara and Mediterranean regions. Recently, almond production has been launched in many microclimates of the South Eastern Anatolian region and Eastern Anatolia, due to the encouragement of the relevant units of the Agriculture and Village Affairs Ministry, Turkey. The first outcome from the region holds promise.

Micropropagation of many fruit trees were reported including peach (Hammerschlag, 1982), cherry (Borkowska and Szczerba, 1991; Deogratias *et al*., 1989) and almond (Channuntapipat *et al*., 2003; Caboni, 1994; Tabachnik and Kester, 1977; Rugini and Verma, 1983). However scarce information is available for *A. communis* L. cultivars, Garrigues and Yaltsinki.

Heterozygous individuals generates great problem in the almond production and the establishment of orchards. In order to overcome this problem, it was aimed to develop a reliable protocol for clonal propagation Garrigues and Yaltsinki varieties that were recently introduced to the Southeastern Anatolian region of Turkey. Clonal propagation through plant tissue culture techniques with different explant types and achievements in *in vitro* propagation of Garrigues and Yaltsinki varieties were explored in this study.

MATERIALS AND METHODS

This study was undertaken during 2002-2004. The plant materials, *Amygdalus communis* L. cv. Yaltsinki and cv. Garrigues, were kindly provided by Pistachio Research Institute, Ministry of Agriculture, Gaziantep-Turkey. Research was conducted at 3 stages according to explant types.

Experiment I: Embryos of cv. Yaltsinki and cv. Garrigues were used. Seeds were first washed with tap water for 10 min. Then they were surface-sterilized in 70% of absolute ethanol for 2 min, in 5% NaOCl for 10 min, in 70% of absolute ethanol for 1 min, followed by three rinses

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in sterile distilled water. And then the plant materials were drained on the sterile Whatmann 3 MM paper aseptically. The embryos were cultured on Murashige and Skoog (MS) medium 12 (Murashige and Skoog, 1962), MS media supplemented with Indole-3-aceticacid (IAA) and 6-benzylaminopurine (BAP) at 0.0, 0.5, 1.0, 2.0, 4.0 mg L^{-1} concentrations and IAA/Kinetin at 0.0, 2.0, 4.0 mg L^{-1} concentrations.

Experiment II: Shoot tips of cv. Yaltsinki and cv. Garrigues were used. The explants were first agitated in water with detergent for 5 min and then rinsed in tap water for 5 min, followed by sterilization in 5% NaOCl for 5 min. The explants were then rinsed in sterile distilled water, put in 10% NaOCl for 5 min and rinsed twice in sterile distilled water, dried in filter papers and were placed on MS medium supplemented with IAA/BAP (0.0, 0.5, 2.0, 4.0 mg L^{-1}) combinations and concentrations.

Experiment III: Nodal explants of cv. Yaltsinki were used and sterilization procedure was the same as those described for the shoot tips. The nodal explants were cultured on MS medium supplemented with various concentrations and combinations of IAA and BAP. Explants were cultured on MS medium containing 3% sucrose, solidified with 0.8% agar and adjusted to pH 5.7. Hormones were added to medium prior autoclaving.

Incubation conditions and assessments: Cultures were incubated at 26±2°C under white fluorescent tubes with 16/8 h (light/dark) photoperiod.

The data were analyzed using the Duncan ($p< 0.05$) Multiple Comparative Test.

RESULTS

Experiment I: Different combinations and concentrations of IAA and BAP were used and lengths of root and stem originated from cultured embryo were recorded. In cv. Yaltsinki, two statistically different groups were constituted at root and stem length values. The best result for root development was obtained with MS basal medium. When root lengths were compared, there were no significant differences among combinations of 2.0 or 4.0 mg L^{-1} BAP with 0.5 mg L^{-1} IAA. The best stem elongation was observed on medium supplemented with 0.5 mg L^{-1} IAA (Table 1). In cv. Garrigues, different groups were obtained. The highest proliferation of root was obtained on MS basal medium. No root formation was observed on medium supplemented with 4.0 mg L^{-1} BAP+0.5 mg L^{-1} IAA. Besides, both rooting and stem elongation were never observed on medium combined with 4.0 mg L^{-1} BAP, 0.5 mg L^{-1} IAA and 1.0 mg L^{-1} IAA (Table 1). Two groups in root lengths and 3 groups in stem lengths were determined in experiment using embryos of cv. Yaltsinki with IAA and KIN hormones. The best root formation was observed on hormone-free MS medium and MS supplemented with 2.0 mg L^{-1} Kinetin and there is no significant different between them. However, the best stem growing was produced on MS basal medium (Table 2). Three different groups for root and 2 different groups for stem were provided in cv. Garrigues. The best results were obtained from MS basal medium at root growing and medium supplemented with 4.0 mg L^{-1} IAA at stem length (Table 2).

Table 1: Means of root and stem lengths (in cm) of embryos of *Amygdalus communis* cv. Yaltsinki and Garrigues*

Hormones (mg L^{-1})		cv. Yaltsinki**		cv. Garrigues	
BAP	IAA	Root	Stem	Root	Stem
0.00	0.00	6.75b	1.50ab	2.80c	1.50b
2.00	0.00	2.25a	1.25ab	1.50b	1.50b
4.00	0.00	0.75a	2.00ab	0.00a	0.00a
0.00	0.50	2.16a	3.16b	0.00a	0.00a
2.00	0.50	0.50a	1.50ab	0.82ab	1.40b
4.00	0.50	0.00a	0.00a	0.00a	0.85ab
0.00	1.00	5.92b	2.82ab	0.00a	0.00a
2.00	1.00	2.27a	1.57ab	0.80ab	0.60ab
4.00	1.00	0.85a	1.00ab	0.50ab	1.30ab

*The evaluations were done 60 days after culturing, **The lower capitals next to root and stem lengths indicates different groups determined by Duncan's Multiple Comparison Tests ($p<0.05$)

Table 2: Means of root and stem lengths (in cm) of embryos of *Amygdalus communis* cv. Yaltsinki and cv. Garrigues on MS media supplemented with IAA and Kinetin growth regulators*

Hormones (mg L^{-1})		cv. Yaltsinki**		cv. Garrigues	
IAA	Kinetin	Root	Stem	Root	Stem
0.00	0.00	9.00b	3.40c	5.33c	1.13ab
0.00	2.00	15.00b	2.50bc	0.92ab	0.80a
0.00	4.00	1.10a	1.86a-c	1.85ab	1.25ab
2.00	0.00	0.00a	0.00a	2.15ab	1.55ab
2.00	2.00	1.00a	2.25a-c	1.76ab	1.80ab
2.00	4.00	1.10a	1.00ab	1.50ab	0.60a
4.00	0.00	12.50b	1.80a-c	3.25b	2.85b
4.00	2.00	0.00a	0.00a	1.50ab	1.70ab
4.00	4.00	0.00a	0.00a	0.10a	0.50a

*The evaluations were done 60 days after culturing, **The lower capitals next to root and stem lengths indicate different groups determined by Duncan's Multiple Comparison Tests ($p<0.05$)

Considering the data from this experiment, for 'Yaltsinki', the highest root length (15.0 cm) and the highest stem elongation (3.40 cm) occurred on medium supplemented with 2.0 mg L^{-1} Kinetin and hormone-free MS medium, respectively. For 'Garrigues', the highest root length (5.33 cm) and the highest stem elongation (2.85 cm) were observed on hormone-free MS medium and on 4.0 mg L^{-1} IAA containing MS medium, respectively.

Experiment II: In this experiment no embryogenesis from shoot tips occurred, only non-embryogenic callus formed.

Table 3: Means of callus weight (in mg) originated from shoot tips of *Amygdalus communis* cv. Yaltsinki and cv. Garrigues*

Hormones (mg L^{-1})			
IAA	BAP	cv. Yaltsinki**	cv. Garrigues
0.00	0.00	0.191ab	0.139a
0.00	2.00	0.195ab	0.156a
0.00	4.00	0.428b	0.171a
0.50	0.00	0.252ab	0.130a
0.50	2.00	0.000	0.145a
0.50	4.00	0.385ab	0.209a
1.00	0.00	0.171ab	0.00
1.00	2.00	0.148a	0.210a
1.00	4.00	0.308ab	0.192a

*The evaluations were done 60 days after culturing, **The lower capitals next to root and stem lengths indicate different groups determined by Duncan's Multiple Comparison Tests ($p<0.05$)

Table 4: Means of nodal explant growth of *Amygdalus communis* cv. Yaltsinki*

Hormones (mg L^{-1})		
IAA	BAP	Shoot length (cm)**
0.00	0.00	0.683ab
0.00	2.00	0.625ab
0.00	4.00	0.500a
0.50	0.00	0.000
0.50	2.00	1.750b
0.50	4.00	0.783ab
1.00	0.00	0.150a
1.00	2.00	0.000
1.00	4.00	0.200a

*The evaluations were done 60 days after culturing, **The lower capitals next to root and stem lengths indicate different groups determined by Duncan's Multiple Comparison Tests ($p<0.05$)

The highest calli formation was observed on medium supplemented with 4.0 mg L^{-1} BAP. There were no significant difference on amount of callus growth between on MS basal medium and MS supplemented with 2.0 mg L^{-1} BAP, 0.5 mg L^{-1} IAA, 0.5 mg L^{-1} IAA+4.0 mg L^{-1} BAP (Table 3). As shown in Table 3, callus was not obtained on medium supplemented with 1.0 mg L^{-1} IAA in cv. Garrigues.

Experiment III: A new shoot developed from each nod of cv. Yaltsinki and these shoots were compared one another in terms of shoot lengths (cm). The best result was obtained from the medium supplemented with 0.5 mg L^{-1} IAA and 2.0 mg L^{-1} BAP (Table 4).

DISCUSSION

Explant selection is very important for success of *in vitro* studies (Tisserat, 1991). Embryo, shoot tip and nodal explants were used in this study as explant sources. Root and stem formation from embryo, shoot formation from nodal tips and callus formation from shoot tips were obtained.

Depending on species or cultivars, the most important part of the success obtained in the propagation of many plant materials through tissue cultures has been frequently based on the successful adjustment of the type and combination of plant growth regulators (Tran Thanh Van, 1981; Murashige, 1990). Of these, cytokinins and auxins are of extreme importance for shoot and root development in plant tissues (Jones, 1985; Baraldi *et al*., 1988). Auxins are known to induce cell division in plant tissues and, thus, result in a large amount of callus production in many species (Peterson, 1975; Philip and Padikkala, 1989). In this study, use of higher level of cytokinins than those of auxins was more effective on callus formation from shoot tips of 'Yaltsinki and the best media was a cytokinin (4 mg L^{-1} BAP) containing MS. These results were in agreement with reports by Gürel and Gülsen (998) in which BAP was determined to be required for shoot growth and that the high level of BAP (2.0 or 3.0 mg L^{-1}) induced callus formation.

Caboni *et al*. (1994) reported the highest rooting percentage of almond cultivar M51 on media supplemented with IAA. In this study the best root formation occurred on the hormone-free MS media and the media supplemented with 2.0 mg L^{-1} Kinetin in cv. Yaltsinki and on the hormone-free MS media in cv. Garrigues.

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