African Journal of Biotechnology Vol. 8 (22), pp. 6168-6174, 16 November, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Effect of plant growth regulators on *in vitro* shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki

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Accepted 21 July, 2009

An efficient protocol was established for *in vitro* shoot multiplication from apical shoot tips derived from mature trees of almond (*Amygdalus communis* L.) cultivars, Yaltsinki. Explants were cultured on Murashige and Skoog (1962) (MS) medium containing various concentrations of 6-benzyladenin (BA) and kinetin (kin) for shoot multiplication. Shoot multiplication was best achieved from explant on MS medium containing 30 gl^{-1} sucrose, 7 gl^{-1} agar and 1.0 mgl^{-1} BA. This amount of BA (1.0 mgl^{-1}) gave the best multiple shoot formation response with an average of 16.10 shoots per explant. In addition, shoots were cultured on the media containing 1.0 mgl^{-1} BA and kin combined with three different auxins (0.25 and 0.5 mgl^{-1} of IAA, IBA and NAA) separately. It was noted that 1.0 mgl^{-1} BA and kin combinated with NAA had inhibitory effect on new shoot formation and no shoot formation was induced. However, explants cultivated on medium containing 1.0 mgl^{-1} BA and 0.5 mgl^{-1} IAA resulted in 11.25 shoots per explant. The effect of four different sucrose concentrations (20, 30, 40, 50 gl^{-1}) on the multiplication of shoots was also investigated. The best shoot multiplication was obtained in MS media containing 30 gl^{-1} sucrose with an average of 15.40 shoots per explant.

Key words: *Amygdalus communis* L.cv. Yaltsinki, PGRs, *in vitro*, shoot multiplication.

INTRODUCTION

Almonds are one of the oldest commercial nut crops of the world; from the Middle and West Asia. İt has diffused to other regions and continents which include the Middle East, China, the Mediterranean region and America (Ladizinsky, 1999). Besides its commercial use as a nut crop, the almond can be used for ornamental planting because it has beautiful flowers which are white or pale pink.

Since it is a cross-pollinated species, a continuous genetic variation and heterozygous individuals have occured. Thus the homogenity decreased in the traditional orchards, which has led to very different fruit yield and quality (Gülcan, 1976; Küden, 1998). This, therefore, makes the plant tissue culture techniques more valuable for the clonal propagation of almond trees (Henry et al.,1992; Gomez and Segura, 1995).

Compared to traditional propagation with *in vitro* propagation procedure, *in vitro* propagation has several potential advantages over the traditional procedure: For large-scale *in vitro* plant production the important attributes are the quality, cost effectiveness, maintenance of genetic fidelity, and long-term storage. Moreover, micropropagation may be utilized, in basic research, in production of virus-free planting material, cryopreservation of endangered and elite woody species, applications in tree breeding and reforestation (Mohan Jain and Häggman, 2007).

Micropropagation has become a reliable and routine approach for large-scale rapid plant multiplication, which is based on plant cell, tissue and organ culture on well defined tissue culture media under aseptic conditions. Up till now, micropropagation of many fruit trees including apricot (Perez-Tornero et al., 2000), mulberry (Anis et al., 2003), chestnut (Osterc et al., 2005), khinjuk pistachio

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Abbreviations: BA, Benzylaminopurine (N^6-benzyladenine); **kin**, kinetin; **IBA**, indole butyric acid; **NAA**, α-naphthaleneacetic acid; **IAA**, Indole-3-acetic acid; **MS**, Murashige and Skoog medium.

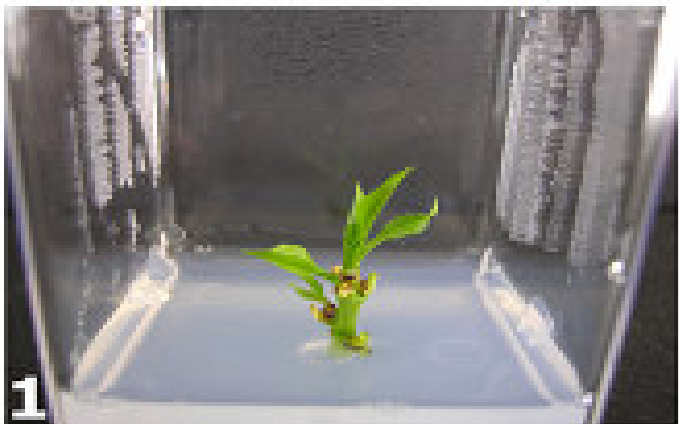

Figure 1. *In vitro* shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki using explants from mature (7 year old) trees. Apical shoot tips cultured on basal MS medium.

(Tilkat et al., 2005), sweet cherry (Canli et al., 2008), kiwifruit (Akbaş et al., 2009), almond (Ainsley et al., 2000; Channuntapipat et al., 2003; Yapar et al., 2006; Işıkalan et al., 2008) has been reported.

Any micropropagation system must produce large number of genotypically uniform plants similar to the original plant from which they were propagated. Optimization of culture conditions and media is important for micropropagation studies. To the best of our knowledge, only a single report is available as regards *in vitro* propagation of *A. communis* L. cv. Yaltsinki (Yapar et al., 2006). These researchers cultured embryos of *A. Communis* L. cv Yaltsinki cultured on MS media supplemented with several concentrations and combinations of cytokinins and auxins (IAA, BAP and Kinetin) for *in vitro* micropropagation. They reported that only root formation and stem elongation were obtained. Moreover, in the same study, the embryogenic callus formation was researched by using nodal segments and apical shoot tips as explant (Yapar et al., 2006).

Therefore, the objective of the present study was to develop a procedure for shoot multiplication from apical shoot tips derived from mature trees of cv. Yaltsinki.

MATERIALS AND METHODS

Young offshoots were collected from *Amygdalus communis* L. cv. Yaltsinki (7 year old), growing at the Botanical garden of the University of Harran, in Şanlıurfa, a province located in southeastern Turkey.

For establishment of *in vitro* shoot cultures, the leaves from young offshoots were eliminated and cutted 10 - 15 cm in length. The apical shoot tips of 'Yaltsinki' that was used as explants were washed with tap water for 5 - 10 min. Then, these were dipped in 70% ethanol for 30 s, surface-sterilized in 10% (w/v) commercial bleach solution (NaOCl) for 10 min, and rinsed five times with sterile distilled water (5 min per rinse). Prior to cultured, the explants were trimmed into 1 cm long pieces.

In the first stage of our study, the effect of different sucrose concentrations (20, 30, 40, 50 g l^{-1}) on the multiplication of shoots was investigated. In this experiment MS medium was supplemented with 1.0 mg l^{-1} BA in accordance with the study reported by Işıkalan et al. (2008).

In the second stage of our study, in order to test the effect of cytokinins on shoot multiplication, explants were cultured on basal MS (Murashige and Skoog, 1962) medium supplemented with different concentrations of (0.5, 1.0, 2.0, 4.0 mgl^{-1}) BA and kin (Sigma-Aldrich) separately (Figure 1). According to the results of our previous experiments, explants were cultured on the MS medium supplemented with different concentrations (0.25 and 0.5 mgl^{-1}) of IAA, IBA, NAA combinated with one concentration (1.0 mgl^{-1}) of cytokinins such as BA and kin.

In this study, all MS media were supplemented with 3% (w/v) sucrose (Merck) and solidified with agar (0.7 %, w/v, Agar-Agar, Sigma). All media were adjusted to pH 5.8 prior to autoclaving (120°C for 20 min), and *in vitro* cultures were maintained at 25 ± 2°C with 16 h photoperiod (40 µmol m^{-2} s^{-1}) provided with mercury fluorescent lamps.

RESULTS AND DISCUSSION

Micropropagation has become a reliable and routine approach for large-scale rapid plant multiplication, which is based on plant cell, tissue and organ culture on well defined tissue culture media under aseptic conditions.

In the present study, the type and concentration of cytokinin influenced the average number of shoots produced per explant as well as mean length of the shoots. There was no sign of growth when explants were cultured in the media without cytokinin or auxin (Tables 1 and 2).

All the investigated concentrations (0.5, 1.0, 2.0, 4.0 mgl^{-1}) of BA showed shoot production. Among various concentrations best response in terms of multiple shoot formation was observed on MS supplemented with 1.0 mgl^{-1} BA, which was followed by the media supplemented with 0.5 mgl^{-1} BA (Table 1). 1.0 mgl^{-1} BA, proved to be optimal, producing an average of 16.10 ± 4.22 shoots per explant with an average shoot length of 2.02 ± 0.47 cm (Figure 2).

Our results are in agreement with those of Tabachnick and Kester (1977) and Işıkalan et al. (2008). They reported that a cytokinin was necessary for development of *A. communis* L.cv. Nonpareil shoots and the best result for shoot proliferation was obtained from MS medium supplemented with 1.0 mgl^{-1} BA.

Explants did not respond significantly when cultured on the medium containing kin alone. At a low level of kin concentration (0.5 mgl^{-1}), fewer shoots were obtained (0.68 ± 0.70). Maximum shoot length (2.38 ± 0.71 cm) was obtained from explants on a MS medium with 1.0 mgl^{-1} kin (Figure 3), followed by 2.15 ± 0.53 shoots from 2.0 mgl^{-1} kin (Table 2). BA was found to be superior to kin in terms of the overall number of shoots produced per explant.

Kinetin alone or in combination of auxins (IAA, IBA) did not show any positive response on shoot formation and development (data not shown) (Figure 4). The combination of NAA+BA, IBA+BA and NAA+kin treatments also did not promote new shoot formation even when cultured for a

Table 1. Effect of concentrations of BA on shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki.

Concentrations of BA (mg l^{-1})	Average no. of shoots/explant (mean ± SE)	Average length of shoots (cm) (mean ±SE)
0.0	0.25 ± 0.44 c	1.76 ± 0.35 a
0.5	8.06 ± 4.26 b	1.91 ± 0.33 a
1.0	16.10 ± 4.22 a	2.02 ± 0.47 a
2.0	7.93 ± 6.42 b	1.98 ± 0.40 a
4.0	7.50 ± 2.50 b	1.83 ± 0.25 a

Data recorded on the 6 weeks; 16 replicates / treatment; repeated twice.
Values followed by the same letter are not significantly different (p = 0.05) according to student's t-test.

Figure 2. *In vitro* shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki using explants from mature (7 year old) trees. Multiple shoots grown on MS medium supplemented with 1.0 mgl^{-1} BA.

Figure 3. *In vitro* shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki using explants from mature (7 year old) trees. Aspect of shoots grown on MS medium with 1.0 mgl^{-1} kin.

Table 2. Effect of concentrations of Kin on shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki.

Concentrations of BA (mg l^{-1})	Average no. of shoots/explant (mean ± SE)	Average length of shoots (cm) (mean ±SE)
0.0	0.25 ± 0.44 b	1.76 ± 0.35 b
0.5	0.68 ± 0.70 b	1.71 ± 0.18 b
1.0	2.25 ± 2.17 a	2.38 ± 0.71 a
2.0	2.12 ± 2.06 a	2.15 ± 0.53 a
4.0	2.12 ± 1.82 a	1.77 ± 0.51 b

Data recorded on the 6 weeks; 16 replicates / treatment; repeated twice.
Values followed by the same letter are not significantly different (p = 0.05) according to student's t-test.

Figure 4. *In vitro* shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki using explants from mature (7 year old) trees. Aspect of shoots cultured on MS medium with 1.0 mgl^{-1} kin plus 0.25 mgl^{-1} IBA.

prolonged period (up to 6 week) (Figure 5). For this reason, these media were not suitable for the multiplication of shoots (Table 3).

Gürel and Gülşen (1998b) reported that the best result for shoot development and growth of almond was obtained from the combination of 0.1 mgl^{-1} IBA and 1.0 mgl^{-1} BA. Similar observations were recorded from Channuntapipat et al. (2003), who suggested that AP (Almehdi and Parfitt, 1986) medium containing 0.049 µM IBA and 3 µM BA was effective for propagating shoot tips of Nonpareil 15-1. In contrast, our results showed that the use of only BA on medium proved to be more beneficial than the combination of auxin and cytokinin for shoot proliferation.

Among the tested combinations of auxin plus cytokinin, BA (1.0 mgl^{-1}) plus IAA (0.5 mgl^{-1}) gave a higher rate of shoot formation (Figures 6 and 7) (Table 3).

Yapar et al. (2006) reported that nodal explant of mature shoots and shoot tips of almond cultivars Yaltsinki, were cultured on MS media supplemented with several concentrations and combinations of cytokinins (2.0 and 4.0 mgl^{-1} BA) and auxins (IAA), for *in vitro* micropropagation. They reported that only root and stem formation from embryo and non-embryogenic callus formation from shoot tips were obtained. However, they did not reported any data related to new shoot formation or multiplication.

Hisajima (1982) reported that the best results for proliferation of the almond were obtained from MS medium supplemented with 0.2 mgl^{-1} BA + 0.005 mgl^{-1} IBA. In contrast, our study determined that the shoot number is considerably reduced when the auxin was added to the nutritient media, and using 1.0 mgl^{-1} BA alone was more economical and effective.

Data in Table 4 showed the effect of different concentrations of sucrose on average number of shoots/explant

Figure 5. *In vitro* shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki using explants from mature (7 year old) trees. Aspect of shoots. cultured on MS medium with 1.0 mgl^{-1} BA plus 0.50 mgl^{-1} IBA.

Table 3. Effect of cytokinins plus auxins on shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki.

Growth regulators (mg l^{-1})					Average no. of shoots/explant (mean ± SE)	Average length of shoots (cm) (mean±SE)
BA	Kin	IAA	IBA	NAA		
1.0	-	0.25	-	-	9.16 ± 4.01 ab	3.51 ± 0.39 b
1.0	-	0.50	-	-	11.25 ± 3.44 a	4.45 ± 0.92 a
1.0	-	-	0.25	-	6.83 ± 4.08 b	2.67 ± 1.20 c
1.0	-	-	0.50	-	-	-
1.0	-	-	-	0.25	-	-
1.0	-	-	-	0.50	-	-
-	1.0	0.25	-	-	-	-
-	1.0	0.50	-	-	-	-
-	1.0	-	0.25	-	-	-
-	1.0	-	0.50	-	-	-
-	1.0	-	-	0.25	-	-
-	1.0	-	-	0.50	-	-

Data recorded on the 6 weeks; 12 replicates / treatment; repeated twice.
Values followed by the same letter are not significantly different (p = 0.05) according to student's t-test.

and shoot length of cv. Yaltsinki. As for the effect of different concentrations it can be noticed that, 30 gl^{-1} gave the highest significant number of shoots and shoot length (Figure 8).

Conclusion

Methods was developed for *in vitro* shoot multiplication of *A. communis* L. cv. Yaltsinki using apical shoot tips from mature (7 year old) trees. Cytokinin BA was found to be essential for shoot multiplication from mature explant on MS medium. BA at 1.0 mgl^{-1} gave the best results for the proliferation of cultures from explant among the tested cytokinins (BA, kin). Inclusion of auxins as applied to the best cytokinin treatment was not effective for further shoot multiplication.

To our knowledge the present study is the first report for *in vitro* shoot multiplication of *A. communis* L. cv. Yaltsinki. The procedure described here provides a rapid

Figure 6. *In vitro* shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki using explants from mature (7 year old) trees. Development of shoots on MS medium with 1.0 mgl^{-1} BA plus 0.50 mgl^{-1} IAA.

Figure 7. *In vitro* shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki using explants from mature (7 year old) trees. Formation of longer shoots on MS medium with 1.0 mgl^{-1} BA plus 0.50 mgl^{-1} IAA.

Table 4. Effect of concentrations of sucrose on shoot multiplication of *Amygdalus communis* L. cCv. Yaltsinki.

Sucrose ($g\ l^{-1}$)	Average no. of shoots/explant (mean ± SE)	Average length of shoots (cm) (mean ±SE)
0	0.30 ± 0.48 c	1.06 ± 0.49 c
20	6.80 ± 4.07 d	1.82 ± 0.53 b
30	15.40 ± 2.41 a	3.32 ± 1.03 a
40	9.30 ± 2.45 b	1.56 ± 0.46 b
50	8.90 ± 2.60 bd	1.66 ± 0.42 b

Data recorded on the 6 weeks; 10 replicates / treatment; repeated twice.
Values followed by the same letter are not significantly different (p = 0.05) according to student's t-test.

Figure 8. *In vitro* shoot multiplication of *Amygdalus communis* L. cv. Yaltsinki using explants from mature (7 year old) trees. Aspect of shoots grown on MS medium with 30 gl^{-1} sucrose.

and prolific micropropagation system.

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