INTERNATİONAL JOURNAL OF AGRİCULTURE & BİOLOGY
1560–8530/2007/09–3–489–493
http://www.fspublishers.org

Micropropagation of Kiwifruit (*Actinidia deliciosa)*

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ABSTRACT

In this study, a method was developed for the micropropagation of kiwifruit (*Actinidia deliciosa*) using mature seeds. In the first stage, effect of various strength of Murashige and Skoog (MS) medium (1/4 - 1/2 - 1/1 & 2/1) and carbon source (30 g L^{-1} sucrose, maltose & dextrose) on shoot multiplication of micro shoot obtained from axenic germinated seeds of kiwifruit were investigated. The optimum results for shoot multiplication were obtained were 1/1 MS medium containing 30 g L^{-1} sucrose. In the second stage, effect of the 6-benzylaminopurine (BAP; 0.5 - 4.0 mg L^{-1}) and kinetin (0.5 - 4.0 mg L^{-1}) on shoot proliferation of kiwifruit were investigated. Although hormone-free medium seemed un-favourable for shoot growth, medium with cytokinin was the most effective in terms of new shoot multiplication and elongation. For shoot proliferation ın general, better results were obtained with BAP treatments rather than the ın kinetin treatments; the former was essential for shoot development during multiplication stage. Moreover, the best result was noticed in 0.5 mg L^{-1} BAP with a shoot number of 4.7 ± 1.08 per explants on the 28th day of culture. The shoots developed in *in vitro* conditions were rooted on MS medium with 1.0 mg L^{-1} α- napthaleneaceticacid. These plantlets were succesfully adapted to *in vivo* conditions.

Key Words: Kiwifruit; *Actinidia deliciosa; İn vitro; Micropropagation*

INTRODUCTION

In vitro multiplication is the most common proliferation tecnique for many fruit species like grape (Işıkalan *et al.*,1998; Adıyaman *et al*., 2004), almond (Işıkalan, 2003), Actinidia (Adıyaman, 2003) and pistachio (Onay, 2000; Tilkat, 2003; Onay *et al*., 2003). The genus *Actinidia* Lindl consists of 110 taxa and 60 species and consists of two family: *Actinidiaceae* that is widespread within the temperate and subtropical regions of the Asia continent. The best known species is the kiwifruit (*A. deliciosa*), which is widespread in China and is now cultivated in many other countries (Ferguson, 1990).

Kiwifruit is characterised by high amount of vitamin C with *A. deliciosa* containing 140 mg 100^{-1} g of pulp. Minerals are also high, especially potassium and magnesium as well as copper, zinc and manganese. Fruit is consumed fresh within 1 - 6 months after harvest. However, kiwifruit is also used in food industry, such as jams, juices and syrups (Rugini & Gutierrez-Pesce, 2003).

The expansion of the kiwifruit industry has led to an increasing demand for propagating material. The conventional propagation methods (cuttings & grafts) have been supplemented by *in vitro* micropropagation in some countries (Oliveira & Fraser, 2005). First micropropagation protocol for *A. deliciosa* was proposed by Harada (1975) and has been subsequently improved (Wang *et al.,* 1982; Standardi, 1983; Wessels *et al.,* 1984; Monette, 1986). Plant tissues used as starting material were shoot tips (Revilla *et al.,* 1992), shoot meristems (Standardi, 1981), the whole buds and nodal segments (Velayandom *et al.,* 1985). In *A. deliciosa* shoot meristems are a suitable starting material for micropropagation, since genetic mutations are limited and virus-free plants are obtained (Standardi, 1981). Leaf pieces, leaf petioles and zygotic embryos can be used to obtain new explants through organogenesis and somatic embryogenesis (Rugini & Gutierrez-Pesce, 2003). Different explant types and culture media have been used for shoot/embryo induction, regeneration and efficient rooting for different kiwifruit genotypes (Canhoto & Cruz, 1987; Rey *et al.,* 1992; Cai *et al.,* 1993; Gonzales *et al*., 1995), apical meristem culture (Standardi, 1981) and shoot tips (Monette, 1986).

Over the past years, more efforts have been focused on understanding the mechanism of uptake of plant growth regulators, especially cytokinins. The uptake and metabolism of 6- benzyladenine were studied in *A. deliciosa* explants grown in ventilated cultures as a system to avoid hyperhydric shoots when propagation was carried out in liquid medium. It was found that 65% of the initial amount of BAP disappeared in the first half hour of culture and then it was converted into 7 different glucosides (Cañal *et al.,* 2000). Soon after, Moncaleon *et al.* (2001) demonstrated that 6-benzylaminopurine (BAP) is not only important for different phases of the micropropagation, but also regulates the development of the regenerants. Highest quality shoots in terms of multiplication index, weight and length and callus, were obtained from culturing in the presence of 4.4 mg L^{-1} of BAP for one day. Zeatin seemed to be the best in inducing regeneration of shoots from callus in cv. Hayward (Rugini *et al.,* 1991).

The aim of the present study was to develop a reliable protocol for fast *in vitro* propagation of kiwifruit that is a new fruit species for Turkey and to boost/increase the production in our country.

MATERIALS AND METHODS

Mature seeds of *Actinidia deliciosa* were used for *in vitro* micropropagation. The seeds were excised from commercially available fruits of kiwifruit. Mature fruits, from which the outer pericarp and shells had been removed, were washed with 70% alcohol. After that the seeds were isolated from the mature fruit. MS basal medium (Murashige & Skoog, 1962) was supplemented with 3% sucrose (w/v) and agar (0.7% w/v) (Agar-agar, sigma). The medium was adjusted to pH 5.8 prior to autoclaving (120°C for 25 min). Plant growth regulators were added to the medium prior to adjustment of pH and sterilization. The cultures were maintained at 25 ± 2°C with a 16 h photoperiod (40 µmol m^{-2} s^{-1}) provided mercury fluorescent lamps. All the shoot tips used in the subsequent experiments were proliferated from mature seed. For *in vitro* culture initiation, modified MS medium supplemented with 30 g L^{-1} sucrose, 7 g L^{-1} agar and 1.0 mg L^{-1} BAP was used.

For *in vitro* proliferation of shoots, axenically germinated stem explants with apical bud was aseptically cut and cultured in Magenta GA-7 Vessel containing 50 mL of basal MS medium with 7 g L^{-1} agar with the same media as above. Effect of MS density (2/1, 1/1, ½, ¼ MS), types of carbonhydrate (sucrose, maltose & dextrose at 3% concentration) and different concentrations of BAP and kinetin (Kin; 0.0, 0.5 - 1.0 - 2.0 & 4.0 mg L^{-1}) on shoot proliferation of kiwifruit were investigated. Explants were examined for shoot production after 4 weeks of culture.

Newly formed shoots from micropropagated seedlings (1,0 - 1,5 cm) were excised and used for the induction of *in vitro* rooting. For root initiation, the explants were incubated in magenta GA-7 Vessel, each containing 50 mL MS medium with α- napthaleneaceticacid (NAA) at 0.5 – 1.0 – 2.0 and 3% sucrose. MS medium without plant growth regulators served as control. The rooting response was scored after 40 - 45 days of culture.

In vitro rooted shoots were washed before being potted in 1:1 mixture of sand and soil. Plantlets were covered with a plastic beaker to maintain 90 ± 5% relative humidity for 4 - 5 weeks before transfered into the growth room. The growth room was illuminated by mercury fluorescent lamps (400 w). The plants were irrigated every 2 - 3 days with water and after 30 days were succesfully adapted at *in vivo* conditions. The data on the number of shoots obtained through apical bud proliferations shoot and node number per explants and shoot length were analysed using ANOVA. The student's *t*-test was applied at p = 0.05 probability level to find significant differences between the means.

RESULTS AND DISCUSSION

Effects of mineral medium strengths of MS on shoot multiplication of kiwifruit. The influence of strenghts of MS on shoot proliferation was investigated to determine the optimal medium for future use. Four strengths of MS medium were prepared half, quarter, full and double (1/2 - ¼ - 1/1 & 2/1). Mean shoot number and length were recorded after 28 days in culture. The significant differences were observed in both mean shoot number and mean shoot length between the tested MS medium (Table I).

The results in Table I show that new shoot production decreased with decreasing strength of MS (1/4 & 1/2). There was rapid development during the first two week culturing when the strength of the MS medium was doubled. However, two weeks after the culture shoot development decreased. As for shoot proliferation, the best result was obtained on 1/1 MS medium, with a mean of 4.7 shoots per explant, respectively. The differences between 1/1 MS and the other MS treatments were found to be significant ($p < 0.05$).

Effects of carbon source on shoot multiplication of kiwifruit. In this experiment, the effects of different carbon source (sucrose, maltose & dextrose at 3% concentration) on shoot production were investigated to determine the optimal carbohydrate types.

The shoots of kiwifruit placed on MS medium were

Fig. 1. Development shoots on MS medium with 0.5 mg/l BAP. x 1.14

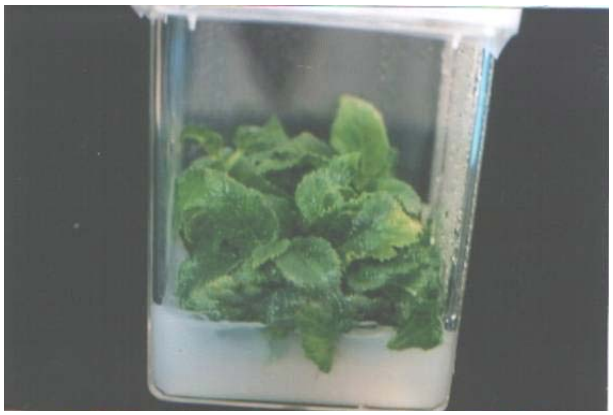

Fig. 2. Development shoots on MS medium with 0.5 mg/l kinetin. x 1.52

Table I. Effects of Strenght of MS on Shoot Multiplication of Kiwifruit.

Treatment	Shoot length (cm ± SE)	Shoots per explants ± SE	Nods per explants ± SE
1/4 MS	1.833 ± 0.221a	2.111 ± 0.309a	1.555 ± 0.175a
1/2 MS	2.388 ± 0.138a	3.000 ± 0.645a	2.000 ± 0.288a
1/1 MS	3.366 ± 0.226b	4.777 ± 0.433b	2.888 ± 0.260b
2/1 MS	3.200 ± 0.213b	2.555 ± 0.337a	2.666 ± 0.288b

Data recorded on the 28th day and presents an average of 9 replicates per treatment. Values followed by the same letter are no significantly different (p=0.05) according to student's *t*-test.

Table II. Effects of Carbon Source on Shoot Multiplication of Kiwifruit.

Treatment	Shoot length (cm ± SE)	Shoots per explants ± SE	Nods per explants ± SE
Maltose	3.000 ± 0.288a	3.000 ± 0.333a	2.555 ± 0.242a
Dexrose	3.111 ± 0.138a	2.444 ± 0.242a	3.111 ± 0.200b
Sucrose	4.800 ± 0.157b	4.666 ± 0.372b	3.666 ± 0.166c

Data recorded on the 28th day and presents an average of 9 replicates per treatment. Values followed by the same letter are no significantly different (p=0.05) according to student's *t*-test.

Table III. Effect of BAP on Shoot Multiplication of Kiwifruit.

Treatment	Shoot length (cm ±SE)	Shoots per explants ±SE	Nods per explants ±SE
Control	2.5 ± 0.28a	1.3 ± 0.23a	2.3 ± 0.23a
0.5 mg/l BAP	4.5 ± 0.28b	4.7 ± 1.08b	3.8 ± 0.39b
1.0 mg/l BAP	4.2 ± 0.41b	4.4 ± 0.47b	3.1 ± 0.20b
2.0 mg/l BAP	4.3 ± 0.39b	4.1 ± 0.20b	3.2 ± 0.27b
4.0 mg/l BAP	2.6 ± 0.30a	3.2 ± 0.55b	2.1 ± 0.39a

Data recorded on the 28th day and presents an average of 9 replicates per treatment. Values followed by the same letter are no significantly different (p=0.05) according to student's *t*-test.

Table IV. Effect of Kinetin on Shoot Multiplication of Kiwifruit.

Treatment	Shoot length (cm ±SE)	Shoots per explants ±SE	Nods per explants ±SE
Control	2.5 ± 0.28a	1.3 ± 0.23a	2.3 ± 0.23a
0.5 mg/l kin	4.3 ± 0.47b	4.0 ± 0.44b	3.5 ± 0.34b
1.0 mg/l kin	4.2 ± 0.42b	3.8 ± 0.47bc	3.8 ± 0.30b
2.0 mg/l kin	4.1 ± 0.25ab	3.1 ± 0.20c	3.6 ± 0.23b
4.0 mg/l kin	3.6 ± 0.16b	2.3 ± 0.21ac	4.5 ± 0.50b

Data recorded on the 28th day and presents an average of 9 replicates per treatment. Values followed by the same letter are no significantly different (p=0.05) according to student's *t*-test.

supplemented with 1.0 mg L^{-1} BAP and 0.7% agar. The average shoot lenght and number of the shoots produced were assessed after 28 days of culture. The results in Table II show that significant differences were observed in both mean shoot number and mean shoot length between carbon sources tested. Multiple shoots were obtained from all treatments tested but explants on MS medium with 3% sucrose produced shoots were significantly, compared to those on maltose and dextrose. Therefore, for shoot multiplication, the best results were obtained on MS medium, using 3% sucrose with a mean at 4.6 shoots per explants.

Effect of cytokinins on shoot multiplication of kiwifruit. In the present study, mature seeds of kiwifruit were axenically germinated on 1/4 MS medium containing hormone-free and newly formed shoots were used as starting material for micropropagation studies. Standardi (1981) reported that, in *A. deliciosa,* shoot meristems were suitable starting materials for micropropagation, since genetic mutations are limited and virus free plants are obtained. In this respect, Revilla *et al.* (1992) reported that suitable plant tissues used as starting material for kiwifruit are shoot tips, whole buds and nodal segments.

Using MS medium, the effects of BAP and kinetin on shoot proliferation were examined. The basal medium was supplemented with several concentrations of BAP and Kin (0.5 - 1.0 - 2.0 & 4.0 mg L^{-1}). The cytokinins (BAP or kinetin) were essential for shoot production since poor shoot development occurred on media without cytokinins (Table III & IV), but with significant differences ($p < 0.05$).

For micropropagation of kiwifruit, different concentrations of two cytokinins (BAP & kinetin) were examined. Results showed that cytokinins were essential for shoot production of kiwifruit. These results are consistent with the findings of Moncaleon *et al.* (1999), who reported that endogenous cytokinins levels were found to be insufficient in order to develop the explants of kiwifruit on *in vitro* conditions. Similar results were reported by Cañal *et al.* (2000) that plant growth regulators, especially cytokinins were efficient in the shoot development and growth of kiwifruit.

For shoot production better results were held in BAP treatments rather than ın kinetin treatments. As for shoot production, the best results were obtained from lower BAP concentrations. It appeared that shoot production had a tendency to decrease as BAP concentrations increase up to 4.0 mg L^{-1} (Table III). The best response was noticed in BAP (0.5 mg L^{-1}) with a shoot number of 4.7 per explant on the 28 days of culture (Fig. 1). A similar pattern was also observed in the colour of the shoots developing 0.5 and 1.0 mg L^{-1} BAP produced green and healthy shoots, whereas those developed at higher concentrations (2.0 & 4.0 mg L^{-1} BAP) had a greenish-yellow colour. In addition, large amounts of calli were observed at the base of the shoots developed on 1.0 mg L^{-1} BAP. Kinetin also induced multiple shoots, but not as effectively as BAP. The best results were obtained on 0.5 mg L^{-1} kinetin (Fig. 2) with a shoot number of 4.0 per explant on the 28th day of culture (Table IV). Shoot production was seriously reduced by higher kinetin concentrations (2.0 & 4.0 mg L^{-1}). A little callus was also observed at the base of the shoots developed on these tested concentrations. It is clear from the data in Table III and IV that the optimum results for shoot proliferation of kiwifruit were obtained with 0.5 mg L^{-1} BAP among all cytokinin concentration tested.

Fig. 3. The root formation on MS medium with 1.0 mg/l NAA. x 1.82

Fig. 4. The adaptation at *in vivo* condition of plantlet. x 0.48

In our experiments, shoot production resulted in a better BAP than the kinetin treatments. For shoot production of kiwifruit, the best result were obtained with on 0.5 mg L^{-1} BAP. In paralel with our studies, Moncaleon *et al.* (2001) reported that BAP not only has an important effect on the different phases of the micropropagation, but also regulates the development of the regenerants. In similar results on cv. Hayvard kiwifruit, zeatin seems to be the best in inducing regeneration of shoots from callus, while BAP is used normally for shoot proliferation(Rugini *et al*., 1991). Marino and Bertazza (1990) verified that BAP caused hyperhydricity of older leaves, on effect not observed with zeatin, but higher proliferation rates.

A high shoot proliferation rate was reported using liquid medium with 8.9 µm indolebutyricacid (IBA) and chilled lateral buds were the best for multiplication (Lionakis & Zirari, 1991). The other study of efficient proliferation has been achieved by using 50% MS macro- and micronutrients, full-strength MS vitamins 5 mg L^{-1} ascorbic acid as antioxidant and supplementation at 2.3 µm zeatin and 0.3 µm IAA during a 4- weeks subculture period (Pais *et al.*, 1987).

Rooting of *in vitro* shoot of kiwifruit. For *in vitro* rooting of shoots, different concentrations of NAA (0.5 - 1.0 & 2.0 mg L^{-1}) were used. The first roots were observed in 1.0 mg L^{-1} NAA concentration after three weeks of culture. However, other treatments resulted in poor roots development only on 42 day of culture. Optimal mean root number and lenght per shoot were obtained from media supplemented with 1.0 mg L^{-1} NAA (Fig. 3). The *in vitro* rooted plantlets were succesfully adapted at *in vivo* conditions (Fig. 4).

CONCLUSION

This study led to develop an effecvtive method for micropropagation of kiwifruit, which in turn may be applied to mature tissues of kiwifruit under *in vitro* conditions.

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(Received 03 January 2006; Accepted 28 December 2006)