Appl Biochem Biotechnol (2009) 158:470–475
DOI 10.1007/s12010-008-8401-2

# Callus Induction and Plant Regeneration from Different Explants of *Actinidia deliciosa*

**Filiz Akbaş · Çiğdem Işikalan · Süreyya Namli**

Received: 6 June 2008 / Accepted: 14 October 2008 /
Published online: 31 October 2008


**Abstract** In this study, an efficient procedure was developed for callus induction and regeneration of kiwifruit (*Actinidia deliciosa*) using different organs of shoots developed under in vitro conditions. Effects of explants source and media ($M_1$, 1.0 mg $l^{-1}$ BA+2.0 mg $l^{-1}$ 2,4-D–$M_2$, 1.0 mg $l^{-1}$ NAA+2.0 mg $l^{-1}$ 2,4-D) on initiation of callus were examined in order to obtain callus for organogenesis. The best callus for plant regeneration was obtained from leaf explants on Murashige and Skoog's medium (MS) supplemented with $M_2$. Formation of callus from leaf of kiwifruit (*A. deliciosa*) was cultured in MS medium containing different concentration of $N^6$-benzylaminopurin (BA; 0.0, 1.0, 2.0, 4.0, 6.0, 8.0 mg $l^{-1}$) for callus proliferation and plant regeneration. Although the first shoot formation was appeared in medium containing 6.0 and 8.0 mg $l^{-1}$ BA, the best shoots formation was obtained in medium with 4.0 mg $l^{-1}$ BA.

**Keywords** Kiwifruit · Callus · Organogenesis · In vitro

## Introduction

Kiwifruit has gained enormous popularity in the recent years in many countries of the world and is in great demand due to its nutritional and medicinal value. For commercialization of this crop and to meet the growing demand for planting material, tissue and organ culture techniques are being used as alternative method for propagation in many countries [1].

Different explant types and culture media have been used as source of material for shoot–embryo induction, callus and cell culture, regeneration and efficient rooting for different kiwifruit genotypes [2–6].

Leaf explants offer the possibility to produce plants through indirect organogenesis of *Actinidia chinensis* [7], after intensive proliferation of callus cultures from leaf explants.

Young stem segments were reported as the most suitable for micropropagation followed by roots. Whereas, leaf blades produced few buds and petioles almost none [8]. Pais et al.

F. Akbaş (✉) · Ç. Işikalan · S. Namli
Department of Biology, Art & Science Faculty, Dicle University, Diyarbakır, Turkey
e-mail: fayaman@dicle.edu.tr

[9], found a suitable way to obtain meristem differentiation and later plant development from petiole segments of in vivo plants of *A. chinensis*. The same methodology was used successfully with taken from in vivo [10] and in-vitro-propagated shoots of *Actinidia deliciosa* cv. Hayward [5].

Organogenesis in vitro depends on the application of phytohormones and also on the ability of the tissues to respond to these hormonal changes during culture [11]. Specifically, the presence of auxins and cytokinins is necessary for 'indirect organogenesis. The procedure for plant multiplication involves: callus induction and formation from initial explant and shoot stimulation and development. During these two steps, the required levels of exogenous hormones may be different [12]. Chamail [13] studied on the variations in kiwifruit plants regenerated from callus. The author reported that the best callus growth was obtained on MS medium and was supplemented with 3 mg $l^{-1}$ BA and 1 mg $l^{-1}$ NAA. On the differentiation medium, maximum average number of shoots per callus was produced on medium containing 2 mg $l^{-1}$ BA and 0.06 mg $l^{-1}$ IBA. The least average number of shoots was produced on the medium containing 4 mg $l^{-1}$BA and 0.06 mg $l^{-1}$ IBA with the increase in concentration of BA the number of shoots formed/callus decreased.

Several cytokinins were effective in inducing organogenic cultures from *Actinidia* tissues. Thidiazuran was reported to be the most effective for shoot induction from callus of *A. deliciosa*, *Actinidia polygoma*, and *Actinidia kolomikta* [14].

In *A. deliciosa* tissues, development of callus from an initial explant and the induction of new organs from the newly formed callus depend on the different concentrations of the exogenously supplied BA and NAA [15]. The hormonal control of formation and development of initial callus from kiwifruit petioles has been previously studied [16, 17]. The authors reported that NAA and BA required for induction callus from petioles.

Moncaleon et al. [18] demonstrated that BA not only has on important effect on the different phases of the micropropagation, but also regulates the development of the regenerants. Zeatin [19] and zeatin+auxin combination [20, 21] seems to be the best in inducing regeneration of shoots from callus in cv. Hayward.

The aim of the present study is to develop an efficient procedure for callus induction and plant regeneration of kiwifruit (*A. deliciosa*) under in vitro culture conditions. In this study, the most suitable explant source and combination of plant growth regulators (PGRs) for callus induction and the best concentration of BA for shoot production were investigated.

## Materials and Methods

*Plant Material* In vitro shoots obtained from mature seeds of kiwifruit (*A. deliciosa*) were used as plant material. Commercial fruits of *A. deliciosa* were washed with 70% alcohol and the seeds isolated from the mature fruit. Sterile mature seeds were germinated in 1/4 MS medium [22] containing 30 g $l^{-1}$ sucrose and 7 g $l^{-1}$ agar (*w*/*v*). Shoots obtained from mature seeds were proliferated in MS medium supplemented with 1.0 mg $l^{-1}$ BA [23] (Fig. 1a).

### Callus Induction

The leaf explant (juvenile; smaller leaf, mature; bigger leaf), petiole, and stem (0.5 cm) isolated from in vitro shoots obtained from mature seeds were used to initiate callus formation. Isolated explants were cultured for initiation of callus formation on MS medium

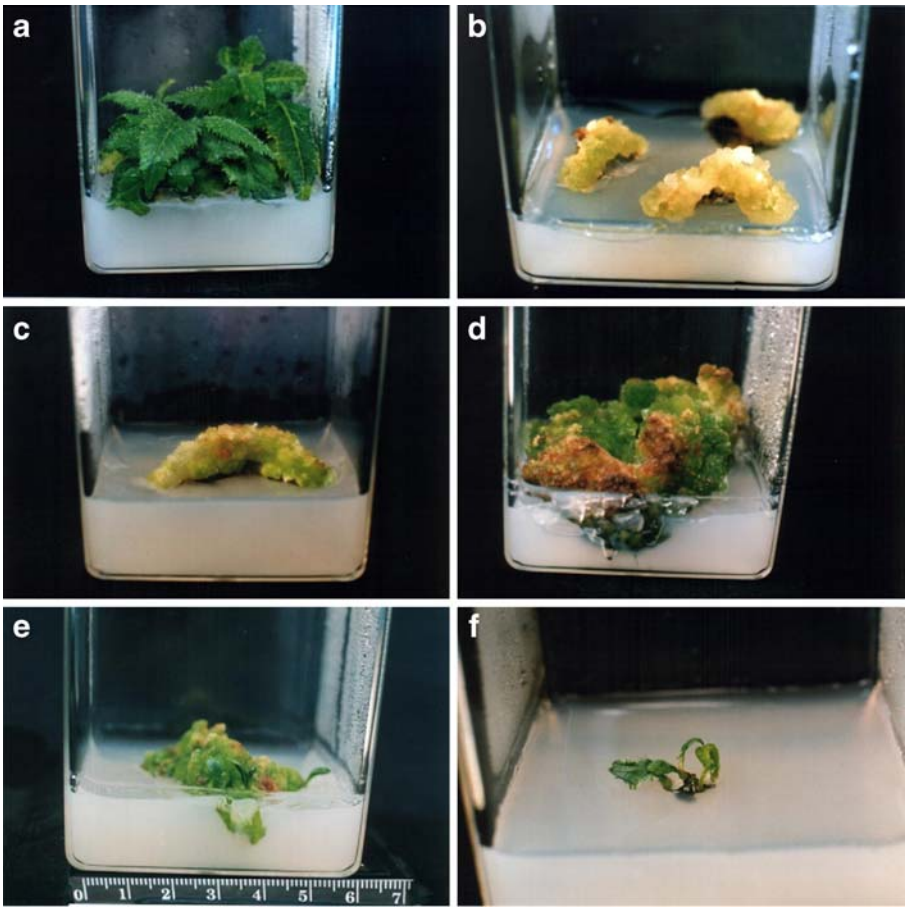


**Fig. 1** Callus induction and plant regeneration from different explants of *A. deliciosa*. **a** In vitro shoots obtained from mature seeds of kiwifruit. **b** Callus induction from leaf explants of kiwifruit on medium $M_2$. **c** Proliferation of callus on MS medium containing 4.0 mg $l^{-1}$ BA. **d** After subcultures, proliferation of callus on MS medium containing 4.0 mg $l^{-1}$ BA. **e** Shoot regeneration from callus on MS medium containing 4.0 mg $l^{-1}$ BA. **f** Microshoots excised from callus cultured on MS medium supplemented 1.0 mg $l^{-1}$ BA

supplemented with different combination of concentration of BA and α-naphthalene acetic acid (NAA) with 2,4-dichlorophenoxyacetic acid (2,4-D).

$$M_1: 1.0\text{mg}\,l^{-1}\text{BA} + 2.0\text{mg}\,l^{-1}2,4-\text{D} \qquad M_2: 1.0\text{mg}\,l^{-1}\text{NAA} + 2.0\text{mg}\,l^{-1}2,4-\text{D}$$

Callus percentage data were taken after 2 months on culture. Induced callus was categorized on a scale from 0 to 4 (0, 0% callus, 1, 25% callus (low), 2, 50% callus (good), 3, 75% callus (optimal), 4, 100% callus (excellent/very good)). Callus was maintained for its proliferation and plant regeneration experiments.

## Callus Proliferation and Plant Regeneration

Callus formed from leaf explants were cultured for callus and shoot proliferation on MS medium containing different concentrations of BA (0.0, 1.0, 2.0, 4.0, 6.0, 8.0 mg $l^{-1}$).

Callus was subcultured on fresh medium every 4–5 weeks until shoot was formed. The number of shoots was recorded after 2 months.

All inoculations were carried out under aseptic conditions in a sterile cabinet. The medium was adjusted to pH 5.8 prior to autoclaving at 121°C for 25 min. The cultures were maintained at 25±2°C with a 16-h photoperiod (40 μmol $m^{-2}$ $s^{-1}$) provided by mercury fluorescent lamps.

## Results and Discussion

### Callus Induction

The effect of initial explants on callus formation was investigated. The initial explants (petiole, stem, juvenile and mature leaf) were cultured separately on medium $M_1$–$M_2$ and results of callus induction rate are shown in Table 1.

Callus induction rates in the medium $M_1$ varied evidently among the tested explants. The highest callus induction in terms of both density and morphogenic properties was obtained from stem explants. The callus induction in the medium $M_2$ was generally observed in all explant types. Levels of callus formation on medium $M_2$ were also very good compared to medium $M_1$. Among all explants, the best callus induction rate in the medium $M_2$ was obtained from leaf (juvenile and mature) explants (Fig. 1b).

As a conclusion, the highest callus induction rate was obtained from leaf explants on the medium $M_2$. Therefore, leaf explants were chosen as an initial material for callus induction among all explant types tested. The results are supported by those of Ludvova and Ostrolucka [7], who reported that leaf explants offer the possibility to produce plants, after intensive proliferation of callus cultures from leaf explants.

The medium $M_2$ (1.0 mg $l^{-1}$ NAA+2.0 mg $l^{-1}$ 2,4-D) was also chosen as callus induction medium. However, Chamail [13] reported that the best callus growth in kiwifruit was obtained with 3 mg $l^{-1}$ BA+1 mg $l^{-1}$ NAA. Similar results with BA and NAA were also reported by Centeno et al. [15–17], in *A. deliciosa* petioles.

**Table 1** Effect of explants and medium $M_1$, $M_2$ on callus induction.

| Explants | Medium $M_1$ | | Medium $M_2$ | |
|---|---|---|---|---|
| | Callus coverage (0 to 4)[a] | Remarks | Callus coverage (0 to 4)[a] | Remarks |
| Juvenile leaf | 2 | Loose callus, light green in color | 4 | Moderate hard texture, light green in color |
| Mature leaf | 1 | Loose texture callus, yellowish in color | 3 | Moderate hard texture, light green in color |
| Stem | 2 | Moderate hard texture, light green in color | 2 | Loose texture, light green in color |
| Petiole | 0 | Yellowish callus | 1 | Loose texture, Yellowish in color |

[a] Classification of callus coverage on explants: 0: 0% callus, 1: 25% callus (low), 2: 50% callus (good), 3: 75% callus (optimal), 4: 100% callus (excellent/very good)

## Callus Proliferation and Plant Regeneration

The callus obtained from leaf explants on the medium $M_2$ were excised and cultured on different concentrations of BA (0.0–1.0–2.0–4.0–6.0–8.0 mg $l^{-1}$) for callus proliferation and plant regeneration. The data obtained from all tested treatments are shown in Table 2.

After 4 to 5 weeks of culture, proliferation of callus in all tested treatments occurred profusely. Callus formation on high concentration of BA (4.0–8.0 mg $l^{-1}$) was found to have higher organogenic potential with hard texture, granular-formed, and green-colored. Particularly, the best results for proliferation and growth of callus were determined in a medium containing 4.0 mg $l^{-1}$ BA (Fig. 1c and d).

The first shoot formation appeared in medium containing 6.0 and 8.0 mg $l^{-1}$ BA after 50–60 days of culture. Afterwards, shoot production occurred in all tested treatments, except in the media containing hormone-free and 1.0 mg $l^{-1}$ BA. These results demonstrated that the medium should be supplemented with exogenous hormones (PGRs) in order to shoot production from callus. These results are in agreement with those of Krikorian [12], who reported that during callus induction, shoot stimulation and development steps exogenous hormones are required.

BA used a cytokinine and was efficient for plant regeneration from callus. Parallel with our results, Centeno et al. [15] reported that for induction of new organs from callus of *A. deliciosa* tissues, the best results were obtained on the different concentrations of BA and NAA. But in contrast, Nayak and Beyl [14] reported that cytokinin such as thidiazuran was the most effective for shoot induction from callus of different three genotypes of *Actinidia*. Similar results with cytokinin (zeatin and zeatin+auxin combination) were also reported by Rugini et al. [19], Oliveira et al. [20], Raquel and Oliveira [21] in cv. Hayward.

It is clear from the data in Table 2 that the poor shoot development occurred with low concentrations of BA among the tested treatments. For both callus formation and plant regeneration, better results were obtained in high concentration of BA rather than low concentration of BA. Particularly, the maximum average number of shoots was produced on media containing 4.0 mg $l^{-1}$ BA (Fig. 1e).

**Table 2** Effect of different concentrations of BA on callus proliferation and plant regeneration.

| | Callus growth | | Shoot growth | |
|---|---|---|---|---|
| | Coverage (0 to 4)[a] | Remarks | Coverage (0 to 4)[a] | Shoot numbers |
| Treatment control | 2 | Compact granular callus formed & green in color | 0 | – |
| 1.0 mg $l^{-1}$ BA | 3 | Moderate hard, granular callus formed & light green in color | 0 | – |
| 2.0 mg $l^{-1}$ BA | 2 | Loose callus formed &light green in color | 1 | 2 |
| 4.0 mg $l^{-1}$ BA | 4 | Maximum callus formed, hard, granular & dark green in color | 4 | 15 |
| 6.0 mg $l^{-1}$ BA | 3 | Copious callus, hard, granular & dark green in color | 2 | 7 |
| 8.0 mg $l^{-1}$ BA | 3 | Profuse callus, hard, granular & dark green in color | 2 | 8 |

[a] Classification of callus and shoot coverage on explants: 0: 0% callus, 1: 25% callus (low), 2: 50% callus (good), 3: 75% callus (optimal), 4: 100% callus (excellent/very good)

Therefore, the medium containing 4 mg $l^{-1}$ BA was chosen as optimum media for plant regeneration in this study. Chamail [13], reported that maximum average number of shoots per callus was produced on medium containing 2 mg $l^{-1}$ BA+0.06 mg $l^{-1}$ IBA and also, the least average number of shoots were produced on the medium containing 4 mg $l^{-1}$ BA+ 0.06 mg $l^{-1}$ IBA.

As a conclusion, in the present study, the highest callus induction rate, the greatest percent regeneration, and number of shoots rate were obtained in medium with 4.0 mg $l^{-1}$ BA. Microshoots were developed from all tested treatments excised from callus and cultured on medium containing 1.0 mg $l^{-1}$ BA for micropropagation and development (Fig. 1f).

## References


1. Kumar, S., & Sharma, D. R. (2002). *The Journal of Horticultural Science & Biotechnology*, *77*(5), 503–508.
2. Canhoto, J. M., & Cruz, G. S. (1987). *Bol. Soc. Brot.*, *60*(2), 239–252.
3. Rey, M., Fernández, M. T., Gonzalez, M. V., & Rodriguez, R. (1992). *In Vitro Cellular Developmental Biology*, *28*, 148–152.
4. Cai, Q. Q., Qian, Y. Q., Ke, S. Q., & He, Z. C. (1993). In Y. P. S. Bajaj (Ed.), *Biotechnology in agriculture and forestry, 23* pp. 3–17. New York: Springer.
5. Gonzalez, M. V., Rey, M., & Rodriguez, R. (1995). *HortScience*, *30*, 1302–1303.
6. Rugini, E., & Gutierrez-Pesce, P. (2003). In S. M. Jain, & K. Ishii (Eds.), *Micropropagation of woody trees and fruits* pp. 647–669. Netherlands: Kluwer.
7. Ludvova, A., & Ostrolucka, M. G. (1998). *Acta Societatis Botanicorum Poloniae*, *67*, 217–222.
8. Augé, R., Vidalie, H., & Ginoux, R. (1982). E.N.I.T.A. Angers, 713–723.
9. Pais, M. S., Oliveira, M. M., & Barroso, J. (1987). *Acta Horticulturae*, *212*, 687–690.
10. Revilla, M. A., & Power, J. B. (1988). *Journal of Horticultural Science*, *63*, 541–545.
11. Sugiyama, M. (1999). *Current Opinion in Plant Biology*, *2*, 61–64. doi:10.1016/S1369-5266(99)80012-0.
12. Krikorian, A. D. (1995). In P. J. Davies (Ed.), *Plant hormones: Physiology, biochemistry and molecular biology* pp. 774–798. London: Kluwer.
13. Chamail, A. (1998). M.Sc. Thesis, Dr. Yashwant Singh Parmar University of Horticulture & Forestry, Solan.
14. Nayak, A. K., & Beyl, C. A. (1986). *47th Annual Meeting of Sothern Region of American Society for Horticultural Science.*
15. Centeno, M. L., Rodriguez, A., Feito, I., & Fernández, B. (1996). *Plant Cell Reports*, *16*, 58–62. doi:10.1007/BF01275450.
16. Centeno, M. L., Rodriguez, A. M., Albuerne, M. A., Feito, I., & Fernández, B. (1998). *Journal of Plant Physiology*, *152*, 480–486.
17. Centeno, M. L., Fernández, B., Feito, I., & Rodriguez, A. (1999). *Journal of Plant Growth Regulation*, *18*, 81–88. doi:10.1007/PL00007053.
18. Moncaleon, P., Rodriguez, A., & Fernández, B. (2001). *Plant Cell, Tissue and Organ Culture*, *67*, 257–266. doi:10.1023/A:1012732429147.
19. Rugini, E., Pellegrineschi, A., Mencuccini, M., & Mariotti, M. (1991). *Plant Cell Reports*, *6*(7), 291–295.
20. Oliveira, M. M., Borrosa, J. G., Martins, M., & Pais, M. S. (1994). In Y. P. S. Bajaj (Ed.), *Biotechnology in Agriculture and Forestry, 29* pp. 189–210. Berlin: Springer.
21. Raquel, M. H., & Oliveira, M. M. (1996). *Plant Science*, *121*, 107–114. doi:10.1016/S0168-9452(96)04509-8.
22. Murashige, T., & Skoog, F. (1962). *Physiol. Plant.*, *15*, 473–479. doi:10.1111/j.1399-3054.1962.tb08052.x.
23. Akbaş, A. F., Ç. Işıkalan, Ç., Namlı, S., & Başaran, D. (2007). *Int. J. Agri. Biol.*, *9*(3), 489–493.