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Effect of the application of benzyladenine pulse on organogenesis, acclimatisation and endogenous phytohormone content in kiwi explants cultured under autotrophic conditions

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Abstract

In traditional in vitro culture, explants grow enclosed in a non-ventilated vessel at high relative humidity with phytohormones continuously present and sucrose as the main energy source. Under such conditions explant growth is far from normal. In this paper, explants of *Actinidia deliciosa* were cultured in MS medium supplemented with sucrose, benzyladenine and gibberellic acid under autotrophic conditions in glass boxes flushed with air enriched with 600 μl l^{-1} CO_2 for the first 20 days and then transferred to MS medium until the end of the culture period. The effect of benzyladenine was assayed in two regimes of application: in cultures for 20 days in the medium or only 24 h in the presence of benzyladenine with the aim of improving shoot proliferation and acclimatisation. The longest explants were those grown under ventilation and pulsed for 24 h with benzyladenine. These explants also rooted spontaneously, whereas those grown with continuous benzyladenine under ventilation or without ventilation grew and rooted poorly. The highest amount of endogenous isoprenoid cytokinins were found in the longest explants grown under ventilation and pulsed for 24 h with benzyladenine; under these conditions zeatin riboside represented two thirds of the entire cytokinin pool. These explants presented the highest amount of indole-3-acetic acid, while abscisic acid content was high in explants cultured under non-ventilated conditions. No differences were observed between explants cultured under ventilation regardless of their exposure to benzyladenine. The longest explants, which also performed best in acclimatisation, also presented a high indole-3-acetic to abscisic acid ratio.

Keywords: Abscisic acid; Acclimatisation; Autotrophy; Cytokinins; Indoleacetic acid; In vitro culture; Kiwi (*Actinidia deliciosa*)

1. Introduction

Cytokinins regulate many physiological, metabolic and biochemical processes in plants, although it is not clear whether exogenously applied cytokinins (Cks) act directly on morphogenesis or their action is mediated by changes taking place in both the endogenous cytokinins as well as other plant growth regulators. In fact, modification of the level of endogenous cytokinins in transgenic plants containing a cytokinin biosynthesis gene showed that the actual level of endogenous cytokinin in cells is decisive for shoot formation [10].

In in vitro cultured explants, the absence of roots, the main site of Cks synthesis makes it necessary to add benzyladenine (BA) to the culture medium. Exogenous BA may act either directly in plant cells or through its control of the accumulation of other cytokinin compounds being the cause of differences in organogenic responses [3]. BA enhances shoot proliferation more effectively than zeatine (Z) or kinetine [23]. However, a high concentration and long periods of incubation of the explants in the presence of BA inhibits shoot elongation and leaf expansion and increases hiperhydricity [9].

Abbreviations: ABA, abscisic acid; BA, benzyladenine; C, non-ventilated explants cultured in MS medium with BA and GA3; Cks, cytokinins; DHZ, Dihydrozeatin; DHZR, dihydrozeatin riboside; GA3, gibberellic acid; IAA, indoleacetic acid; IBA, indole-3-butyric acid; iP, N^6-Isopentenyladenine; iPR, N^6-isopentenyladenosine; NP, ventilated explants cultured for 20 days with BA and GA3 in the medium and then transferred to fresh medium with no sucrose and no hormones; P, ventilated explants cultured for 24 h in the presence of BA and GA3 and then transferred for 19 days to medium without BA and subsequently transferred to fresh medium with no sucrose and no hormones; PPFD, photosynthetic photon flux density; Z, zeatin; ZR, zeatin riboside.

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In previous studies with *Actinidia deliciosa* explants cultured in liquid medium, it was observed that BA absorption reaches a peak in the first 30 min [26] and that 70% of the absorbed BA is metabolised in the first 24 h [12]. Moncaleán et al. [27] reported that reducing the period that the explants remain in the presence of BA in liquid medium to 24 h may be advantageous for the multiplication of *A. deliciosa*.

On the whole, it seems that 24 h in the presence of BA is sufficient to complete the entire process from hormonal induction of cell division (30 min) to the formation of shoot meristems [33].

In closed vessels the accumulation of gases in the internal atmosphere affects explant growth and development. If diffusion of gases is allowed through a bacteriological filter and sugar is removed from the culture medium, the explants become autotrophic, assimilating the carbon dioxide that is flushed inside the vessels [1,2].

In this study, we assayed pulsing in vitro cultured kiwi explants grown under autotrophic controlled conditions with BA for the first 24 h with the aim of improving shoot proliferation and to help the explants successfully withstand their acclimatisation to ex vitro conditions. We also measured the endogenous levels of Cks, indolacetic acid (IAA) and abscisic acid (ABA) at the end of multiplication phase in order to correlate the content of these regulators and the different types of nutrition (heterotrophic or autotrophic) with ex vitro plant performance.

2. Results

2.1. In vitro organogenesis

The longest shoots were those from explants grown in ventilated atmospheres enriched with 600 μl l^{-1} CO_2 and a pulse of BA for 24 h (P), the number of shoots and leaves being similar to explants cultured for 20 days in MS medium with BA (NP) (Table 1). Explants grown without ventilation (C) had fewer and shorter shoots and fewer leaves (Table 1).

P explants showed good spontaneous rooting (>30%), while rooting was poor (<10%) in NP and C explants. The number of roots per rooted explant was similar in all cases (Table 1).

Immersion in indole-3-butyric acid (IBA) and transfer to K(h) medium induced maximum rooting in P explants, although no differences were observed in the number of roots per rooted explant (Table 2). There were longer shoots and more leaves in ventilated NP and P explants than in non-ventilated C explants (Table 2). After 25 days in K(h) medium, the percentage of basal callus was highest (100%) in C explants (Table 2).

2.2. Ex vitro development

P explants were the longest, the number of leaves being similar to NP explants (Table 3). C explants had the lowest survival (Table 3), clear symptoms of desiccation being observed in most of the explants. In fact, the explants that survived were those with the poorest leaf development (data not shown).

Nearly all NP and P explants survived when transferred to the greenhouse (Table 3).

2.3. Endogenous phytohormones

Total isoprenoid Cks was higher in P explants (Table 4), no differences being observed between C and NP explants. N^6-Isopentenyladenine (iP) and N^6-isopentenyladenosine (iPR) content was similar in all cases, although iP was half of iPR (Table 4). Zeatin riboside (ZR) represented two thirds of the entire cytokinin pool (Table 4). Zeatin (Z) and derivatives were lower for C explants, except dihydrozeatin (DHZ).

Table 1
Organogenesis of kiwi explants after 45 days of culture

Treatments	Shoot length (cm)	Shoot number	Number of leaves ≤1 cm	Number of leaves >1–2 cm	Number of leaves >2–3 cm	Number of leaves >3 cm	% Rooted explants	Number of roots
C	2.85 ± 0.13a	2.39 ± 0.10a	5.67 ± 0.97a	2.92 ± 0.53a	1.23 ± 0.32a	0.31 ± 0.08a	6.25	2 ± 0a
NP	3.82 ± 0.20b	3.22 ± 0.14b	7.52 ± 0.86b	4.06 ± 0.40b	2.12 ± 0.25b	1.54 ± 0.13b	9.67	1.66 ± 0.33a
P	4.38 ± 0.20c	3.35 ± 0.15b	7.28 ± 0.91b	3.95 ± 0.43b	2.18 ± 0.24b	1.48 ± 0.11b	31.25	2.20 ± 0.20a

C, non-ventilated explants cultured in MS medium with BA and GA3; NP, ventilated explants cultured for 20 days with BA and GA3 in the medium and then transferred to fresh medium with no sucrose and no hormones; P, ventilated explants cultured for 24 h in the presence of BA and GA3 and then transferred for 19 days to medium without BA and subsequently transferred to fresh medium with no sucrose and no hormones. Means ± S.E. followed with the same letter are not significatly different at $P < 0.05$.

Table 2
Organogenesis of kiwi explants after 25 days in the rooting medium

Treatments	Shoot length (cm)	Number of leaves	% Basal callus	% Rooted explants	Number of roots
C	2.54 ± 0.24a	4.10 ± 0.64a	100	78.1	2 ± 0.1a
NP	3.16 ± 0.14b	6.40 ± 0.72b	81.3	87.5	2.1 ± 0.2a
P	3.19 ± 0.11b	6.94 ± 0.90b	59.4	100	2.3 ± 0.1a

C, non-ventilated explants cultured in MS medium with BA and GA3; NP, ventilated explants cultured for 20 days with BA and GA3 in the medium and then transferred to fresh medium with no sucrose and no hormones; P, ventilated explants cultured for 24 h in the presence of BA and GA3 and then transferred for 19 days to medium without BA and subsequently transferred to fresh medium with no sucrose and no hormones. Means ± S.E. followed with the same letter are not significatly different at $P < 0.05$.