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ORGANOGENESIS IN ENDOSPERM OF *ACTINIDIA DELICIOSA* CV. HAYWARD CULTURED IN VITRO

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An efficient procedure for shoot regeneration was obtained by endosperm culture in *Actinidia deliciosa* cv. Hayward. Mature endosperm cultured on MS medium supplemented with 2 mg/l 2,4-D and 5 mg/l kinetin developed callus with 80% efficiency. Callus was transferred on MS medium containing different plant growth regulators (2,4-D, TDZ, IAA, NAA, BAP, kinetin, 2iP) for regeneration. There were significant differences in regeneration response between medium supplemented with TDZ and medium with other hormones. Only medium containing TDZ stimulated shoot induction. The highest efficiency of shoot regeneration (avg. 6.2 shoots per culture) was on medium supplemented with 0.5 mg/l TDZ. The ploidy of callus and organs formed in endosperm culture was examined by flow cytometry. The results, peaks corresponding to 3C DNA amounts, confirmed the endospermal origin of callus, roots and shoots. Aneuploid and polyploid cells were found in endosperm-derived callus and regenerated organs.

Key words: *Actinidia*, endosperm culture, regeneration, tissue culture, ploidy.

INTRODUCTION

Endosperm plays an important role in the life cycle of angiosperm plants. It maintains an optimal physiological environment and supplies nutrition for the developing embryo. In most angiosperm plants, endosperm is formed during the process of double fertilization as a result of the fusion of three haploid nuclei: two originating from the embryo sac (polar nuclei) and one from male gametes; hence it is triploid.

Triploid plants display some features important for commercial use. For example, triploids may form fruits without seeds (e .g., banana, citrus fruits, melon) or may taste better than their diploid ancestors (e.g., potato). Because of this, development of efficient procedures for triploid production is important for commercial as well as research purposes.

Triploid plants may be obtained by sexual reproduction when tetraploid plants are crossed with diploids, but this procedure is complicated and time-consuming, and in many cases it fails (Bhojwani, 2004). A potentially more efficient and simpler way of triploid production is in vitro endosperm culture followed by plant regeneration. The first attempts at endosperm culture were in the 1930s. It has proved successful in many taxa (Straus and LaRue, 1954; Nostog, 1956; Tamaoki and Ullstrup, 1958; Rangaswamy and Rao, 1963; Nakano et al., 1975; Seghal and Khurana, 1985; Thomas et al., 2000; Chaturvedi et al., 2003; for review, see: Johri and Srivastava, 1973; Srivastava, 1982; Bhojwani, 2004).

Kiwifruit (*Actinidia deliciosa* var. *deliciosa*), an important commercial plant, is hexaploid (2n = 6x = 174; McNeilage and Considine, 1989). The first reports on endosperm culture and embryoid formation in this species were by Gui et al. (1982), and triploids have been induced in some related taxa (Gui et al., 1982, 1993; Kin et al., 1990). Endosperm culture followed by organogenesis was also achieved in several *Actinidia* interspecific hybrids (Kin et al., 1990). Later, Machno and Przywara

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Abbreviations: MS – Murashige and Skoog (1962) medium; 2,4-D – 2,4-Dichlorophenoxyacetic acid; TDZ – 1-Phenyl-3-(1, 2, 3-thiadiazol-5-yl) urea; IAA – Indole-3-acetic acid; IBA – 3-indolebutyric acid, NAA – alpha-Naphthaleneacetic acid; BAP – 6-Benzylaminopurine; 2iP – 6-(γ, γ-Dimethylallylamino) purine.

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TABLE 1. Effect of different auxins and cytokinins on root formation (mean number of roots/culture) from endosperm-derived calli

	BAP			KIN			2iP		
	0.1 mg/l	1 mg/l	5 mg/l	0.1 mg/l	1 mg/l	5 mg/l	0.1 mg/l	1 mg/l	5 mg/l
					IAA				
0.1 mg/l	2.3	4.3	8	4	2.7	1.3	0.7	0	1
1 mg/l	6.7	1	3	4.3	3	8.7	11.3	2.7	0
5 mg/l	2.7	5	3	2.7	8	7	1	2.3	0.3
					NAA				
0.1 mg/l	3.0	0	0.3	1.3	4	1.3	0	0	1.7
1 mg/l	1.3	15.3	0	0	0	0	4.3	3.3	0.7
5 mg/l	0.7	0	0.3	0	0	0	0.7	3.7	1.3
No auxins	1	0	0.7	1.7	3	1.3	4.3	5	3.3

(1997) established a protocol for efficient callus induction and proliferation from mature kiwifruit endosperm. After 8 months of culture on induction medium containing 2iP, many roots and sporadic shoots were obtained.

In the present study we aimed to find a more efficient combination of plant hormones for induction of organogenesis and production of triploid plants from endosperm-derived callus of *Actinidia deliciosa* cv. Hayward.

MATERIALS AND METHODS

PLANT MATERIAL

Commercial fruits of *Actinidia deliciosa* cv. Hayward were kept at room temperature to allow softening. Pieces of fruit (3 × 3 cm) were surface-sterilized for 7 min in Ace commercial bleach diluted 1:1 with distilled water and rinsed three times in sterile distilled water. The seeds were excised from the fruit, and the endosperm was isolated after the embryos were discarded or used for embryo culture.

CALLUS INDUCTION

The media and the procedure for callus induction were based on the protocol described by Machno and Przywara (1997). Isolated endosperm (143 Petri dishes; 10 explants per Petri dish) and embryos (41 Petri dishes; 10 explants per Petri dish) were cultured on *Actinidia* endosperm medium (AEM) based on MS medium (Murashige and Skoog, 1962) with kinetin (5 mg/l) and 2,4-D (2 mg/l) at 28 ± 1°C in darkness. Induced callus was continuously subcultured on fresh medium every three weeks.

ORGANOGENESIS

Root and shoot induction was attempted only with endosperm-derived callus. In preliminary studies we applied 63 MS media supplemented with different combinations and concentrations of BAP, kinetin and 2iP with IAA and NAA (Tab. 1). Each medium was tested with 3 Petri dishes each containing 5 explants (7-month-old endosperm calluses ~9 mm in diameter).

For regeneration, 9-month-old calli were subcultured on 13 different MS-based regeneration media (RM) supplemented with different concentrations of 2,4-D and TDZ (Tab. 2).

Cultures on RM were incubated at 25 ± 3°C under a 16 h photoperiod (cool-white fluorescent tubes, 60–90 µmol photons $m^{-2}s^{-1}$). Five explants (callus derived from endosperm) per culture (Petri dish) were inoculated, in five replicates per variant. Callus was subcultured on fresh RM every three weeks. Observations including root and shoot counts were made after two months of culture on RM.

To induce rooting, shoots 10–15 mm long were excised from the callus and the cut end was soaked in a solution of IBA (100 mg/l) according to the procedure of Kin et al. (1990). Plantlets were then transferred to 1/2 MS medium without any plant growth regulators.

HISTOLOGICAL ANALYSIS

The material for sectioning (fresh fragments of 7–14-day-old endosperm and 7-month-old endosperm-derived callus on AEM) was prepared by embedding tissues in Technovit 7100 (2-hydroxyethyl-methacrylate) (Heraeus Kulzer). Materials were fixed in glutaraldehyde for 24 h, washed four times in phosphate buffer (PBS), and dehydrated in a graded ethanol series (10%, 30%, 50%, 70%, 96%) for 15 min at each concentration and kept overnight in absolute ethanol. Later the samples were infiltrated in a mixture of absolute ethanol and Technovit in 3:1, 1:1 and 1:3 (v/v) proportions, 1 h in each mixture, and stored for 12 h in pure Technovit. The resin was polymerized with the addition of hardener. The material was sectioned 5 µm with a rotary microtome (Microm, Adamas Instrumenten), stained with toluidine blue and mounted in Entellan (Merck).

Microscopic sections were photographed with a Zeiss Axio Cam MRe digital camera. The images were processed with Zeiss Axio Vision 3.0 software.

ESTIMATION OF PLOIDY

Flow cytometry was used to determine the ploidy of callus and regenerated organs. The following types of samples were analyzed: (i) leaves or seedlings obtained in vivo from seeds and fresh isolated embryos, used as an external standard for the fluorescence intensity corresponding to 2C; (ii) freshly isolated endosperm used as an external standard for the fluorescence intensity corresponding to 3C; and (iii) cultured callus derived from embryos and endosperms, or shoots and roots regenerated from endosperm-derived callus.

Cell ploidy in tissues formed during culture was determined by comparison with the 2C and 3C amounts of DNA present in the cells of corresponding tissues. For flow cytometry analyses, samples were chopped with a sharp razor blade in nuclei extraction buffer, filtered through a 30 m nylon sieve (Partec CellTrics) and stained with DAPI (4',6-diamidino–2-phenylindole). A Partec CyStain UV precise P kit was used. Samples were measured with a DAKO Galaxy flow cytometer equipped with an HBO–100 mercury lamp and a set of filters and mirrors (KG1, UG1, BG38, TK420, GG435).

RESULTS

CALLUS INDUCTION AND CULTURE

Endosperm cultured on AEM medium showed small amounts of callus three weeks after culture, and 80% of the cultured endosperm responded within about two months. In the following weeks the callus grew quickly. When kept in darkness the calli remained white or cream-colored. The callus turned green when subcultured on regeneration medium under a normal photoperiod. Dark spots appeared on the surface of some firm, nodular calli, the recognized initials of root formation.

ORGANOGENESIS

The type and efficiency of organogenesis observed in culture depended on the growth regulator combination present in the medium.

During preliminary studies in which media containing different combinations of BAP, kinetin and 2iP with IAA and NAA were tested, we did not obtain shoot regeneration, but rhizogenesis was observed frequently. The most efficient root formation medium contained 1 mg/l NAA and 1 mg/l BAP; the average number of roots per culture was 15.3. Medium supplied with 1 mg/l IAA and 0.1 mg/l 2iP induced 11.3 roots per culture (Tab. 1).

In the next stage of the experiment, both shoot formation and rhizogenesis were observed on media supplemented with TDZ and 2,4-D. There were striking differences in regeneration response between media supplied with TDZ and 2,4-D and media containing TDZ alone. Rhizogenesis occurred on media containing TDZ or TDZ combined with a small amount of 2,4-D (0.1 mg/l), but shoot formation occurred exclusively on media without 2,4-D. Roots developed from intensively pigmented (black, brown or purplish) spots that appeared on the surface of callus tissue (Figs. 1, 2). Shoot growth was accompanied by development of leaves typical in morphology (Fig. 3), and abnormal leaves also formed.

TABLE 2. Effect of different concentrations of 2,4-D and/or TDZ on shoot and root regeneration from endosperm-derived calli in A. *deliciosa* cv. Hayward

Plant growth regulators (mg/l)		Organogenesis	
2,4-D	TDZ	Shoots/culture	Roots/culture
0	0.1	1	5.6
0	0.25	0	1.6
0	0.5	6.2	3.2
0	1	4.2	2.6
0	2.5	3.8	6.0
0.1	0.1	0	0.4
0.1	0.5	0	2
0.1	2.5	0	4.8
0.5	0.1	0	0
0.5	2.5	0	0
2.5	0.1	0	0
2.5	0.5	0	0
2.5	2.5	0	0

The efficiency of shoot formation on media containing TDZ depended on its concentration (Tab. 2). Low concentrations of TDZ (0.1 or 0.25 mg/l) induced only few (1 shoot per culture) or no shoots, respectively. Higher concentrations of TDZ increased the efficiency of shoot formation in culture. The highest number of shoots per culture (6.2) was noticed on medium containing 0.5 mg/l TDZ. Efficiency of shoot formation was lower on media supplemented with 1 and 2.5 mg/l TDZ: average 4.2 and 3.8 shoots per culture, respectively. Shoots regenerated from callus, soaked in IBA solution and then transferred to 1/2 MS medium formed roots in 3 weeks (Fig. 4).

Even small amounts of 2,4-D inhibited shoot formation, but a low concentration (0.1 mg/l) did not suppress rhizogenesis, especially when 2,4-D was accompanied by a high amount of TDZ (2.5 mg/l, 4.8 roots per culture). Fewer roots formed on media containing 0.1 mg/l 2,4-D and low amounts of TDZ (0.1 mg/l, 0.4 roots per culture; 0.5 mg/l, 2 roots per culture).

Neither shoots nor roots formed when higher amounts of 2,4-D were used in the TDZ-supplied media.

HISTOLOGICAL STUDIES

Sections of freshly isolated endosperm showed tissue with small intercellular spaces and cells filled with

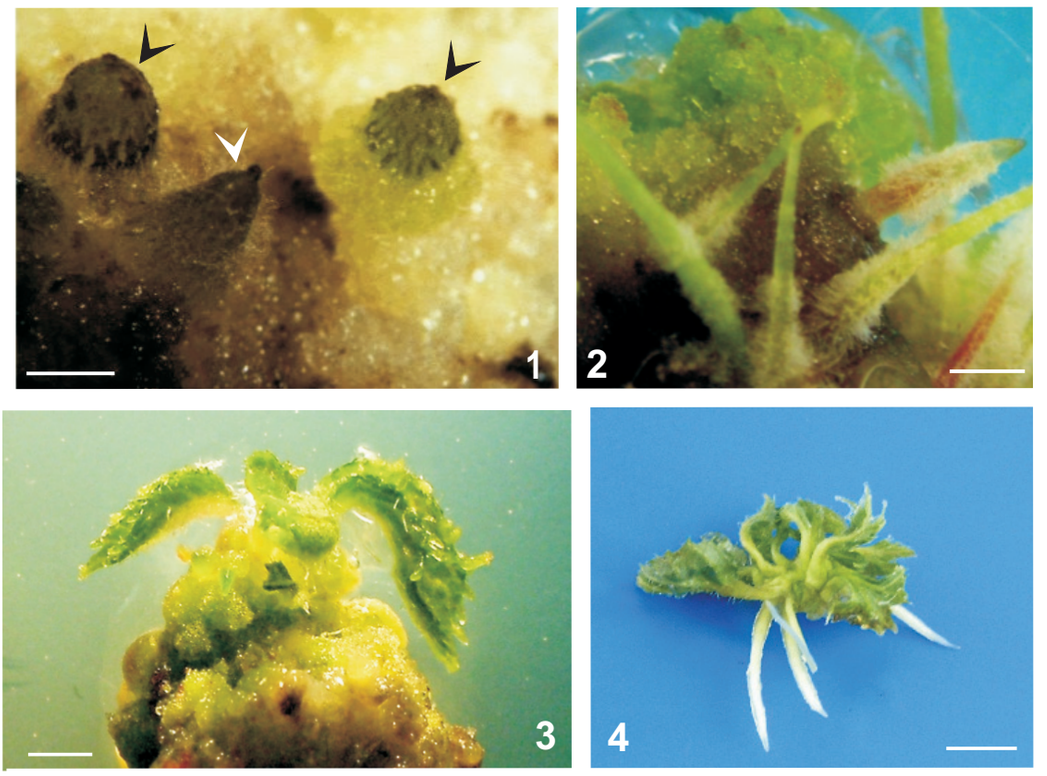


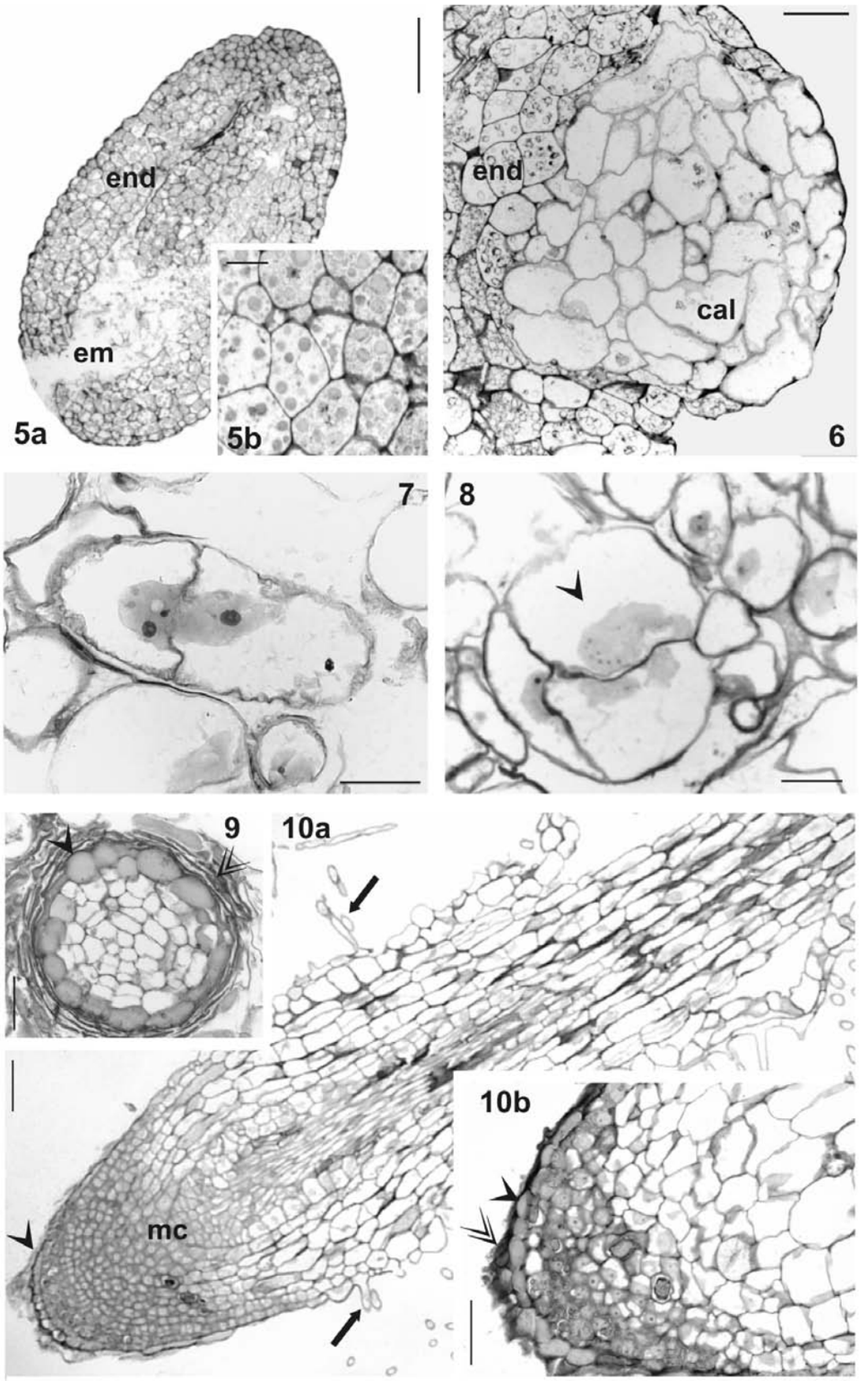
Fig. 1. Root meristems (arrowheads) forming on callus after 2 months of culture on regeneration medium supplied with 0.1 mg/l TDZ and 0.1 mg/l 2,4-D. Bar = 5 mm. **Fig. 2.** Roots formed on callus cultured for 2 months on regeneration medium supplied with 0.25 mg/l TDZ. Bar = 5 mm. **Fig. 3.** Shoot regeneration from calli cultured on MS medium supplemented with 0.5 mg/l TDZ, 2 months after culture. Bar = 5 mm. **Fig. 4.** Rooted shoot obtained from endosperm-derived callus (after treatment with IBA solution and 3 weeks of culture on 1/2 MS medium). Bar = 10 mm.

storage materials (Fig. 5a, b). Calli formation on the surface of the endosperm tissue was observed in sections from 7- and 11-day-old culture (Fig. 6). Cells of calli were larger than those of the initial explant, were vacuolated, and lacked storage materials.

Seven-month-old fragments of endosperm-derived calli cultured on AEM showed some characteristic features of calli tissue. Daughter nuclei attached to newly formed cell walls (Fig. 7) were often observed, suggesting disturbances of cell division. The cells differed in size and shape and contained nuclei with variable numbers of nucleoli (Fig. 8).

Transverse sections of the dark spots formed in endosperm-derived callus showed groups of small, vacuolated, densely packed cells, surrounded by a layer of dark-staining cells filled with granular or uniform dark-staining matrix. This group of cells was surrounded by a layer of squashed cell remnants (Fig. 9).

Fig. 5. Longitudinal sections of fresh isolated endosperm (end). (**a**) Explant obtained after seed coat and embryo removed; note space (em) after embryo dissection, (**b**) Part of endosperm tissue; visible are thick-walled endosperm cells with storage material. Bar = 200 µm in Fig. 5a, 100 µm in Fig. 5b. **Fig. 6.** Section of endosperm tissue 2 weeks after culture on AEM medium; visible are cells of endosperm (end) containing storage materials and vacuolated cells of callus (cal). Bar = 100 µm. **Figs. 7,8.** Sections of endosperm-derived callus cultured on induction medium AEM after 7 months. **Fig. 7.** Disturbed cell divisions: two daughter nuclei attached to newly formed cell wall separating two descendant cells of unequal size. **Fig. 8.** Cells differing in size and shape; note group of three cells with large nuclei and several nucleoli (arrowhead). Bars = 50 µm. **Fig. 9.** Transversal section of dark spot formed in endosperm-derived callus cultured on AEM medium; note dark-staining cells (arrowhead) and layer of cell remnants (double arrowhead). Bar = 200 µm. **Fig. 10.** Longitudinal sections of apical part of root obtained from endosperm-derived callus cultured on medium with 1.0 mg/l NAA and 0.1 mg/l kinetin; note meristematic center (mc) protected by root cap with outer layer consisting of dark-staining cells (arrowhead in b) and squashed remnants of cells (double arrowhead in b); visible are root hairs (arrows). Bar = 100 µm.


end
em
5a
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cal
6
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8
9
10a
mc
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Sections of regenerated roots confirmed that the dark spots were root initials. The layers distinguished in dark spot sections corresponded to root meristematic and root cap layers (Fig. 10a, b).

PLOIDY ANALYSIS

Histograms of external standards revealed mainly peaks corresponding to 2C and a very small one indicating 4C (Fig. 11a), except for histograms obtained from fresh isolated endosperms with the peak showing fluorescence intensity indicating 3C (Fig. 11c).

Cells of embryo-derived callus showed four different ploidy levels, from 2C to 16C (Fig. 11b); three different ploidy levels were observed in cells of endosperm-derived callus: 3C, 6C and 9C (Fig. 11d). Nuclei with DNA amounts between 6C and 12C were also observed in endosperm-derived callus.

A total of 59 samples of organs (mainly leaves) regenerated from endosperm-derived callus were analyzed. Of these samples, 45.7% showed fluorescence intensity peaks corresponding exactly to 3C (Fig. 11e). Part of the samples (42.2%) revealed peaks of fluorescence intensity representing C-values between 2C and 4C, but not exactly 3C. Only 8.4% of the samples indicated 2C DNA content, and one sample showed 6C DNA content.

DISCUSSION

Machno and Przywara (1997) established an efficient system for induction of callus from endospermal tissue of *Actinidia deliciosa*. In that work, shoots formed on medium supplemented with 2iP, but at very low frequency. The main aim of our experiment was to determine more efficient phytohormone combinations for regeneration of plants from kiwifruit callus. First we tried MS media supplemented with different combinations of BAP, kinetin and 2iP with IAA and NAA, but only roots were induced. In the next step we focused on combinations of 2,4-D with thidiazuron (TDZ), a plant growth regulator that has proved to be an effective factor in organogenesis of many plant species (Seelye et al., 1994; Shan et al., 2000; Srivatanakul et al., 2000; Qu et al., 2002; Mei-Chun, 2004; Prado et al., 2005).

Callus cultured on media containing 2,4-D, even at the lowest concentration, did not form any shoots. Our observations are in line with those of other authors who reported that although 2,4-D is an efficient auxin in callus induction and growth, it is not suitable for regeneration (Kin et al., 1990; Zheng and Konzak, 1999). In our experiments, root formation occurred on

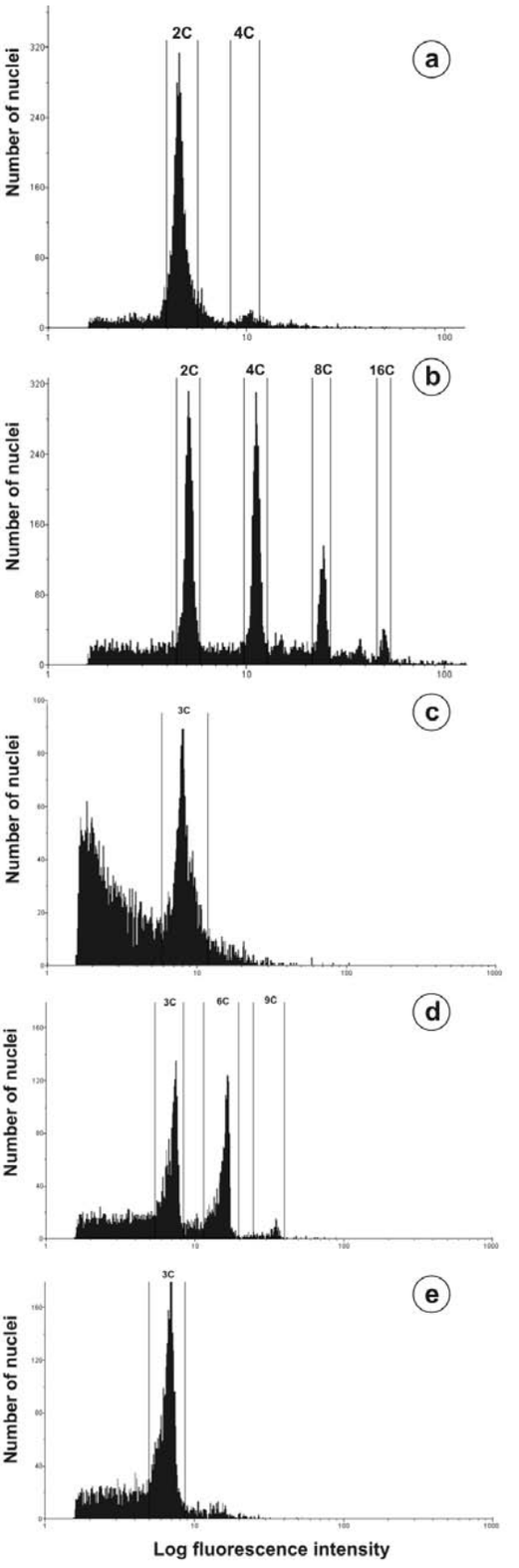

Fig. 11. Histograms of fluorescence intensity (log value of channel number) of nuclei extracted from fresh isolated embryos (**a**), embryo-derived callus (**b**), fresh isolated endosperm (**c**), endosperm-derived callus (**d**) and plant obtained from endosperm-derived callus (**e**).

media with low 2,4-D (0.1 mg/l), but was completely suppressed at higher concentrations.

TDZ, on the other hand, proved to be an efficient phytohormone for plant regeneration. TDZ alone stimulated shoot formation on all media except for medium with 0.25 mg/l TDZ. The lowest concentration of TDZ (0.1 mg/l) stimulated only 5 shoots per 25 explants. Shoot efficiency increased with higher concentrations; for example, 0.5 mg/l yielded 31 shoots per 25 calluses and was optimum for shoot formation. Above that level (1 mg/l, 2.5 mg/l) the organogenic efficiency of TDZ declined (21 and 19 shoots per 25 calluses, respectively). These results indicate that shoot formation efficiency on media containing TDZ was significantly better than previously reported.

Rooting of kiwifruit shoots has usually been achieved by soaking or immersion in an IBA solution followed by culture on rooting medium or by direct potting (Barbieri and Morini, 1987).

In our experiment, shoots obtained on TDZ-enriched medium were soaked in a solution of IBA and transferred to hormone-free 1/2 MS medium. Rooting initiation occurred after about 3 weeks.

Freshly isolated endosperm tissue consisted of thick-walled cells filled with storage materials. Callus induced on the surface of endosperm 5–7 days after endosperm inoculation was formed by larger, vacuolated cells differing in size and shape and without storage material. Variability of cell and nuclei size and shape is widely known as an effect of mitotic disturbances in cultures in vitro. The large cells with giant nuclei observed in endosperm-derived callus were probably the result of endoduplication or restitution processes. The high polyploidy of nuclei obtained from endosperm-derived callus was confirmed by flow cytometry analysis. The specific unequal and probably amitotic nuclei and cell divisions reported in endosperm development both in vivo (Rychlewski, 1969) and in vitro (Gu et al., 1991) can explain the aneuploidy of tissues obtained from in vitro culture.

Histological analysis revealed that the first step of organogenesis started even on the induction medium (AEM) in the form of dark spots on the callus surface. The inner part of these structures was meristematic, with small cells lacking intercellular spaces. The dark-staining cells surrounding the meristematic tissue probably contained a large vacuole with anthocyanins or tannins. Similar cells accumulating pigments observed on the surface of *A. deliciosa* callus were interpreted as initials of abnormal shoots (Oliveira and Pais, 1992). In *Brassica napus*, Klimaszewska and Keller (1985) noted dark green or purplish meristematic centers on the calli surface, growing into leafy structures. Our morphological observations indicated that most of the dark spots developed into roots, mainly after transfer to regeneration medium. Histological analysis of roots formed in culture showed that the root cap consists of dark-staining cells and squashed remnants of cells.

Although numerous shoots and roots were obtained in the experiment, confirmation of their endospermal origin was needed. The most reliable method is to check the ploidy of the organs formed in culture. It is technically difficult and time-consuming to accurately determine ploidy in *Actinidia deliciosa* tissues by standard chromosome counts, because condensed chromosomes are small and numerous ($2n = 6x = 174$) (McNeilage and Considine, 1989). The more efficient method is flow cytometry, which can assess the ploidy of a much more abundant nuclei population faster than would be possible with traditional techniques.

Histograms of DNA amounts from leaves and isolated embryos of kiwifruit suggested low frequency of cell divisions. Cells at G1 phase with 2C levels of DNA are specific to some mature plant tissue, especially tissue of nonpolysomatic species, and embryos isolated from "not quite germinated" seeds. Analysis of the data obtained from samples of fresh isolated endosperm indicated peaks corresponding to 3C.

The corresponding DNA histogram analysis showed that 45.7% of the samples of nuclei from cells of shoots and roots developing from endosperm-derived callus had amounts of DNA very similar to those of freshly isolated endosperm tissue containing nuclei with 3C DNA content. Moreover, the peaks that indicated the amount of DNA in callus were located between 2C and 4C values. These results confirmed the 3C ploidy of plants obtained in culture, and their endospermal origin.

In addition to nuclei with 3C ploidy, some peaks indicated nuclei with ploidy that did not fit 2C, 3C or 4C. These peaks may mark structural and numerical chromosome aberrations and disturbances in mitotic divisions, which can occur as a result of spindle failure or chromosome lagging at anaphase. Such phenomena are well known in culture of plant materials in vitro (for review, see: Bayliss, 1975; D'Amato, 1995).

A considerable part of the samples of embryo- and endosperm-derived callus (42.2%) clearly showed DNA amounts corresponding to either 2C or 3C. These data imply that almost half of the obtained plants could be aneuploid. A low percentage (8.4%) of samples indicated amounts corresponding to 2C, and only one sample was 6C. Similar ploidy variation was found in *Actinidia* taxa (Kin et al., 1990) and other species raised from endosperm culture (Gu et al., 1987). Some of the present morphological observations may point to a kind of genetic balance in the obtained plants. Samples from leaves typical in shape for kiwifruit were determined mainly as 3C, whereas aneuploidy was often found in samples taken from atypical leaves. However, further studies are needed to confirm suspicions of the aneuploidy of regenerated plants.

This study established an efficient procedure for shoot production from endosperm, as confirmed by flow

cytometry. The time required for shoot formation on regeneration media is comparatively short but still time-consuming; the described procedure for callus induction and multiplication requires several months. Also, as described above, prolonged culture in vitro may result in ploidy changes. An interesting option is direct regeneration of plants from freshly isolated endosperm tissue cultured on regeneration media. These experiments are in progress in our laboratory.

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REFERENCES

BARBIERI C, and MORINI S. 1987. Plant regeneration from *Actinidia* callus cultures. *Journal of Horticultural Science* 62: 107–109.

BAYLISS MW. 1975. The effects of growth *in vitro* on the chromosome complement of *Daucus carota* (L.) suspension cultures. *Chromosoma* 51: 401–411.

BHOJWANI SS. 2004. Production of triploid plants. *Encyclopedia of plant and crop science*. Digital Publisher Marcel Dekker, http://dekker.com

CHATURVEDI R, RAZDAN MK, and BHOJWANI SS. 2003. An efficient protocol for the production of triploid plants from endosperm callus of neem, *Azadirachta indica* A. Juss. *Journal of Plant Physiology* 160: 557–564.

D'AMATO F. 1995. Aneusomaty *in vivo* and *in vitro* in higher plants. *Caryologia* 48: 85–103.

GU SR, GUI YL, and XU TY. 1987. Induction of endosperm plants in *Lycium chinensis* and examination of chromosome ploidy. *Acta Genetica Sinica* 14: 37–41.

GU SR, GUI YL, LIU SQ, and XU TY. 1991. Suspension culture of endosperm callus cell and amitosis in vitro. *Acta Botanica Sinica* 33: 478–481.

GUI YL, MU SK, and XU TY. 1982. Studies on morphological differentiation of endosperm plants of chinese gooseberry in vitro. *Acta Botanica Sinica* 24: 216–221.

GUI YL, HONG S, KE S, and SKIRVIN RM. 1993. Fruit and vegetative characteristics of endosperm-derived kiwifruit (*Actinidia chinensis* F.) plants. *Euphytica* 71: 57–62.

JOHRI BM, and SRIVASTAVA PS. 1973. Morphogenesis in endosperm cultures. *Zeitschrift für Pflanzenphysiologie* 70: 285–304.

KIN MS, FRASER LG, and HARVEY CF. 1990. Initiation of callus and regeneration of plantlets from endosperm of *Actinidia* interspecific hybrids. *Scientia Horticulturae* 44: 107–117.

KLIMASZEWSKA K, and KELLER WA. 1985. High frequency plant regeneration from thin cell layer explants of *Brassica napus*. *Plant Cell, Tissue and Organ Culture* 4: 183–197.

MACHNO D, and PRZYWARA L. 1997. Endosperm culture of *Actinidia* species. *Acta Biologica Cracoviensia Series Botanica* 39: 55–61.

MCNEILAGE MA, and CONSIDINE JA. 1989. Chromosome studies in some *Actinidia* taxa and implications for breeding. *New Zealand Journal of Botany* 27: 71–81.

MEI-CHUN L. 2004. High frequency plant regeneration from callus culture of *Pleione formosana* Hayata. *Plant Cell, Tissue and Organ Culture* 78: 93–96.

MURASHIGE T, and SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* 15: 473–497.

NAKANO H, TASHIRO T, and MAEDA E. 1975. Plant differentiation in callus culture induced from immature endosperm of *Oryza sativa* L. *Zeitschrift für Pflanzenphysiologie* 76: 444–449.

NOSTOG KJ. 1956. Growth of rye-grass endosperm in vitro. *Botanical Gazette* 117: 253–259.

OLIVEIRA MM, and PAIS MSS. 1992. Somatic embryogenesis in leaves and leaf-derived protoplasts of *Actinidia deliciosa* var. *deliciosa* cv. Hayward (kiwifruit). *Plant Cell Reports* 11: 314–317.

QU L, CHEN J, HENNY RJ, HUANG Y, CALDWELL RD, and ROBINSON CA. 2002. Thidiazuron promotes adventitious shoot regeneration from pothos (*Epipremnum aureum*) leaf and petiole explants. *In Vitro Cellular and Development Biology-Plant* 38: 268–271.

PRADO MJ, HERRERA MT, VAZQUEZ RA, ROMO S, and GONZALEZ MV. 2005. Micropropagation of two selected male kiwifruit and analysis of genetic variation with AFLP markers. *Hortscience* 40: 740–746.

RANGASWAMY NS, and RAO PS. 1963. Experimental studies on *Santalum album*: Establishment of tissue culture of endosperm. *Phytomorphology* 13: 450–454.

RYCHLEWSKI J. 1969. Kariologia endospermy roślin okrytonasiennych. II. Procesy różnicowania jąder. *Wiadomości Botaniczne* 13: 43–53.

SEELYE JF, MADDOCKS DJ, BURGE GK, and MORGAN ER. 1994. Shoot regeneration from leaf discs of *Limonium perigrinum* using thidiazuron. *New Zealand Journal of Crop and Horticultural Science* 22: 23–29.

SEGHAL CB, and KHURANA S. 1985. Morphogenesis and plat regeneration from cultured endosperm of *Embilica officinalis* Gaertn. *Plant Cell Reports* 4: 263–266.

SHAN X, LI D, and QU R. 2000. Thidiazuron promotes in vitro regeneration of wheat and barley. *In Vitro Cellular and Development Biology-Plant* 36: 207–210.

SRIVASTAVA PS. 1982. Endosperm culture. In: Johri BM [ed.], *Experimental embryology of vascular plants*, 175–193. Springer Verlag, Berlin, Heidelberg, New York.

SRIVATANAKUL M, PARK SH, DANDERS JR, SALAS MG, and SMITH RH. 2000. Multiple shoot regeneration of kenaf (*Hibiscus cannabinus* L.) from shoot apex culture system. *Plant Cell Reports* 19: 1165–1170.

STRAUS J, and LARUE CD. 1954. Maize endosperm tissue grown in vitro I. Culture requirements. *American Journal of Botany* 41: 687- 694.

TAMAOKI T, and ULLSTRUP AJ. 1958. Cultivation in vitro of excised endosperm and meristem tissues of corn. *Bulletin of the Torrey Botanical Club* 85: 260–272.

THOMAS TD, BHATNAGAR AK, and BHOJWANI SS. 2000. Production of triploid plants of mulberry (*Morus alba* L) by endosperm culture. *Plant Cell Reports* 19: 395–399.

ZHENG MY, and KONZAK CF. 1999. Effect of 2,4-dichlorophenoxyacetic acid on callus induction and plant regeneration in anther culture of wheat (*Triticum aestivum* L.). *Plant Cell Reports* 19: 69–73.