MICROPROPAGATION OF JAPANESE PERSIMMON *(DIOSPYROS KAKI)*

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Abstract. A micropropagation method for Japanese persimmon, *Diospyros kaki*, is described. Of a range of cytokinins tested, only zeatin (Z) supported good shoot growth. Adenine sulphate (AdS) at 40 mg/l improved shoot growth in the presence of Z at 1 mg/l, but indolebutyric acid (IBA) had no effect at 0.1 mg/l and was deleterious at 1.0 mg/l. Best shoot growth occurred with Murashige and Skoog minerals. Rooting of shoots approximately 2 cm long was induced by dipping shoot bases into 1000 mg/l aqueous IBA before placing them in fine pumice. Bottom heat (26°C) and intermittent mist (2 sec/30 min) in a high humidity tent resulted in 80% rooting. Rooted cuttings became dormant or died when disturbed. The use of Rootrainers is being investigated to improve shoot growth following rooting.

Japanese persimmon (*Diospyros kaki* L.f.) is extensively grown in Japan and there appears to be good prospects for off-season production of this fruit in New Zealand. New selections are being tested for suitability to New Zealand growing conditions, but propagation using conventional methods has been slow. Attempts to root cuttings from adult trees have been unsuccessful. Seedling rootstocks of *D. kaki* take 2 to 3 years to reach graftable size.

Yokoyama and Takeuchi (7) reported callus and root and shoot induction from young embryos of *D. kaki*. Roots and abnormal structures, but not shoots, arose from cambial callus on mature twigs (8).

There did not appear to be any reported attempts to micropropagate this species. A micropropagation study was undertaken in an attempt to improve the rate of multiplication of new selections.

MATERIALS AND METHODS

For preliminary experiments, shoots were collected from field-grown plants of the cultivars Fuyu and Okame. All subsequent work used greenhouse-grown grafted plants of the following cultivars: Fuyu,, Gailey, Hiratanenashi, Izu, and Maekawa Jiro. To surface sterilize the shoots, leaves were removed and the shoots were dipped in 95% ethanol, followed by immersion in a solution containing 0.5% sodium hypochlorite with 0.05% multifilm X77 wetting agent for 30 min. For bud dissection the shoots were then rinsed in 95% ethanol and air dried before dissection of buds 0.5 to 2 mm long. For nodal

explants the shoots were rinsed with sterile distilled water and cut into single node sections approximately 15 mm long.

Dissected buds were placed into petri dishes, and nodal explants into 100 ml jars, both containing 20 ml of medium. Plates containing dissected buds were wrapped in aluminum foil for the first 2 to 3 days of culture. Buds and explants were grown at 25°C with 16 hours of light, 8 hours of darkness, using cool white fluorescent tubes at 5 μE m^{-2} sec^{-1} for the first month and thereafter at approximately 35 μE m^{-2} sec^{-1}.

Rooting experiments were conducted either *in vitro*, or by dipping shoots into hormone solutions or powders before placing them into a variety of potting media. In some experiments, trays containing microcuttings were placed in high humidity chambers under 70 μE m^{-2} sec^{-1} fluorescent lights at 26°C. In other experiments, trays were placed in high humidity tents in a shaded greenhouse with bottom heating of 26°C with or without intermittent mist.

RESULTS AND DISCUSSION

Preliminary experiments using dissected buds from field-grown plants compared shoot development on media containing either benzyl adenine (BA) or isopentenyl adenine (IPA). On the medium with BA the agar turned brown and the buds died, whereas on IPA buds began to expand and small leaves grew. However, black callus developed at the bud base, which eventually almost enveloped the explants. Explants also died on the media used by Yokoyama and Takeuchi (7,8).

Cytokinin Response. Cultures were established on IPA-containing media from greenhouse-grown shoots of 'Gailey' and 'Fuyu'. A trial was then set up comparing shoot growth induced by the cytokinins Z, kinetin (K), and benzyl-tetrahydropyranyl-adenine (SD8339), at 1 mg/l and IPA at 5 mg/l. Zeatin resulted in the best shoot development. Some elongation occurred with IPA, but a large basal callus developed and phenols leached into the medium. In the presence of other cytokinins the explants blackened and died.

Addition of adenine sulphate (AdS) at 40 mg/l to Z at 1 mg/l appeared to improve shoot growth. Increasing AdS to 80 mg/l was deleterious, so 40 mg/l has been adopted as a standard addition to all media.

An experiment was set up comparing growth on Z at concentrations ranging from 0.01 mg/l to 10 mg/l with either small whole shoots or single nodes as explants. At both 0.01 and 0.1 mg/l Z all explants died. At 1 mg/l Z all whole elongated, but no axillary buds developed. Single nodes pro-

duced vigorous single shoots. At 10 mg/l Z shoot elongation was markedly suppressed and a number of small axillary shoots grew at the base of the original shoot or, in the case of single nodes, at the base of the new shoot.

In a further experiment, growth was compared using Z at 1 mg/l and 3 mg/l. The results are shown in Table 1. No significant difference in response between 1 and 3 mg/l was found. However, using small whole shoots as explants, a multiplication rate of 1.6-fold was attained compared with a 3.5-fold increase when single nodes were used.

Table 1. Effect of zeatin concentration and explant type on multiplication of 'Gailey' persimmon after six weeks.

Zeatin concentration	Whole shoot explants[1] No. shoots/shoot	Single node explants[2] No. nodes/node	No. shoots/shoot
1 mg/l	1.55 ± 0.13[3]	3.72 ± 0.35	4.25 ± 0.25
3 mg/l	1.66 ± 0.16	3.25 ± 0.31	4.38 ± 0.38

[1]32 shoots per treatment.
[2]32 nodes from 8 shoots, per treatment.
[3]Mean ± standard error.

Zeatin is a very expensive cytokinin, so experiments were set up to determine whether part of the zeatin could be replaced with IPA. Although 1 or 10 mg/l IPA with 0.1 mg/l Z stimulated shoot growth, shoots equivalent to those which developed on 1 mg/l Z were not achieved.

In all subsequent experiments, single nodes have been used as explants (Figure 1) and 1 mg/l Z as the standard shoot growth medium.

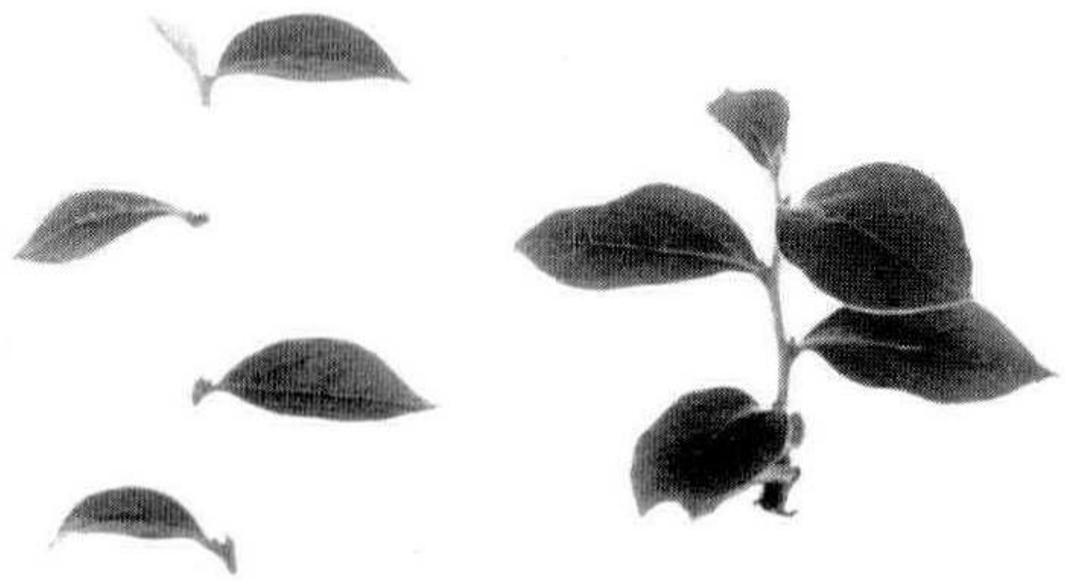

Figure 1. Single node explants (left) and shoot development after 6 weeks (right) on medium containing zeatin (1 mg/l).

Auxin Response. The effect of indolebutyric acid (IBA) at 0, 0.1, or 1 mg/l was checked in the presence of 1, 3 or 5 mg/l Z and 40 mg/l AdS. IBA at 1 mg/l was very inhibitory to shoot

growth, whereas at 0 and 0.1 mg/l growth was not significantly different.

Mineral Requirements. Up to this time all experiments had used either full or half strength Murashige and Skoog (MS) minerals. Shoot growth on these media were then compared with growth on a range of media which have been used for woody plant micropropagation in our laboratory (Table 2). Best growth occurred on the full MS medium. On 'Le Poivre', elongation was also good, but leaves were pale, narrow and small. Growth on B5, Knops, WPM, and ½MS was very poor (Figure 2).

Table 2. Comparison among inorganic salt formulations[1,2].

	MS	½MS	Le Poivre	B5	WPM	Knop
Composition in mg/l						
NH_4NO_3	1650	825	400	—	400	—
$(NH_4)_2SO_4$	—	—	—	134	—	—
KNO_3	1900	850	1800	2500	—	250
$K72SO_4$	—	—	—	—	990	—
$Ca(NO_3)_2.4H_2O$	—	—	1200	—	556	1000
$CaCl_2.2H_2O$	440	220	—	150	96	—
KH_2PO_4	340	170	270	—	170	250
$NaH_2PO_4.H_2O$	—	—	—	250	—	—
$MgSO_4.7H_2O$	730	185	360	—	370	250
H_3BO_3	6.2	3.1	6.2	3.0	6.2	6.2
$MnSO_4.4H_2O$	22.3	11.2	*1.0*	*13.2*[3]	*29.4*[3]	22.3
$ZnSO_4.7H_2O$	8.6	4.3	8.6	2.0	8.6	8.6
KI	0.83	0.42	0.08	0.75	—	0.83
$Na_2MoO_4.2H_2O$	0.25	0.13	0.25	0.25	0.25	0.25
$CuSO_4.5H_2O$	0.025	0.013	0.025	0.025	0.25	0.025
$CoCl_2.6H_2O$	0.025	0.013	0.025	0.025	—	0.025
$FeNa_2EDTA$	40	40	40	40	40	40
Balance of macronutrients (mM)						
NH_4^+	20.6	10.3	5.0	2.0	5.0	—
NO_3^-	*39.4*	*19.7*	*33.0*	*25.0*	*9.8*	*10.9*
Total N	*60.0*	*30.0*	*38.0*	*27.0*	*14.8*	*10.9*
K^+	20.3	10.2	19.8	25.0	12.7	4.2
Ca^{++}	3.0	1.5	10.2	1.0	3.1	4.3
Mg^{++}	1.5	0.8	3.0	1.0	1.5	2.0
PO_4^{3-}	1.5	0.8	2.0	1.1	1.3	1.8

[1] All formulations used with MS vitamins, 30 g/l sucrose, 1 mg/l Z and 40 mg/l AdS.
[2] References for media. MS (6), Le Poivre (5), B5 (3), WPM (4), Knop (2).
[3] The original recipe specified $MnSO_4.H_2O$ and the amount of $MnSO_4.4H_2O$ has been adjusted accordingly.

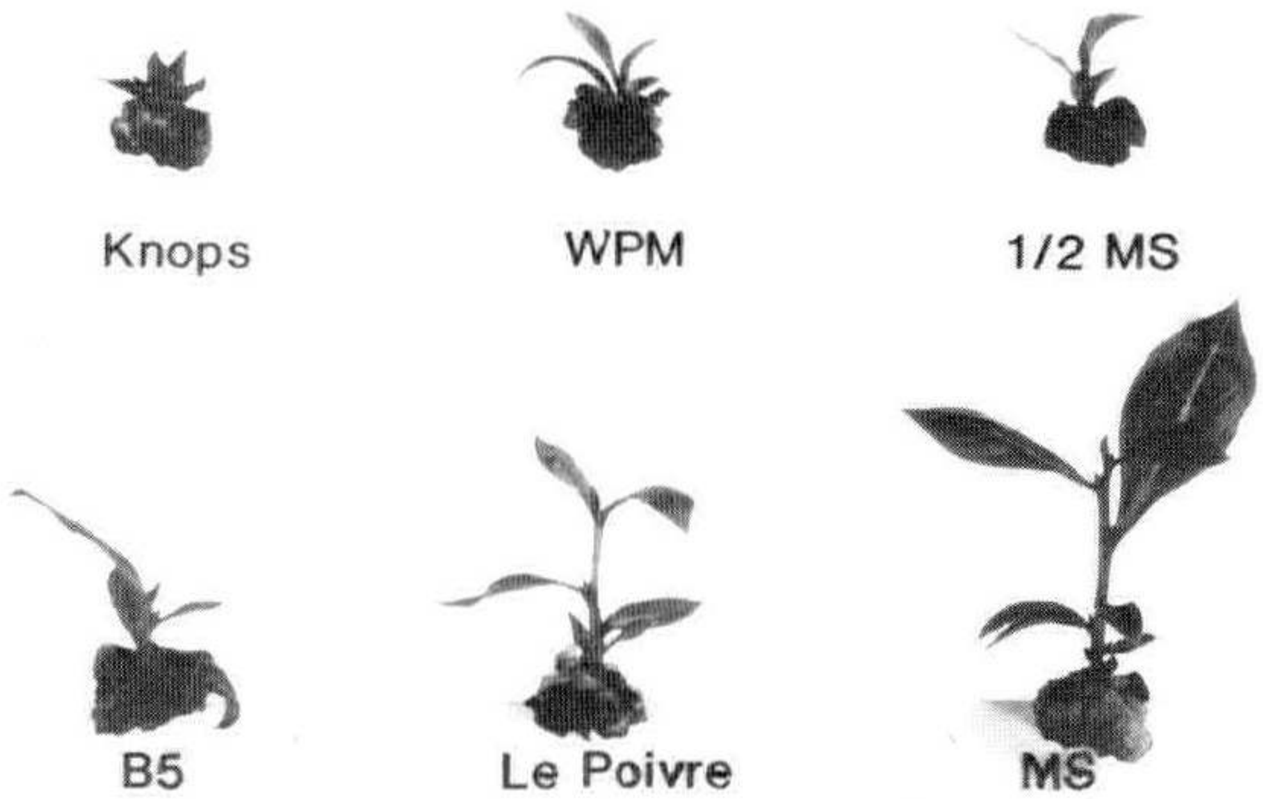

Figure 2. Representative shoots developing from nodal explants grown for 4 weeks on different media.

Shoot Water-soaking. Water-soaking or vitrification of shoots (1) was a problem in some experiments, particularly at levels of Z above 1 mg/l. This was overcome by increasing the agar concentration from 6 to 8g/l of Davis bacteriological agar, which did not reduce shoot growth. Davis agar at 8 g/l gave a firmer gel and less water-soaking than Difco Bacto agar at 8 g/l.

Cultivars. Clear differences in the response of different cultivars have been noted. Excellent shoot growth has been achieved with 'Gailey', 'Fuyu' and 'Maekawa Jiro', with multiplication rates of 4- to 6-fold every four weeks. Under similar conditions, multiplication of 'Hiratanenashi' has been approximately 2-fold, while growth of 'Ize' has been very poor.

Rooting. Attempts were made to root shoots *in vitro* using IBA or NAA at a range of concentrations from 0 to 10 mg/l in the presence or absence of zeatin. This always led to either heavy callus formation and/or senescence of the shoot. With other plants, the addition of charcoal to media often reduces callus formation and improved leaf expansion. With persimmon cultures, charcoal at 2.5 or 5.0 g/l led to leaf drop and death. Only one shoot has been rooted *in vitro* and this subsequently died.

Attempts were also made to root shoots directly into peat:pumice potting media following treatment with either hormone powders (Seradix 1, 2 and 3; May and Baker Ltd.) or a quick dip into aqueous auxin solutions. Using hormone powders some shoots formed roots but results using aqueous IBA at 1000 mg/l were more encouraging, with 66% rooting in one experiment. At 500 mg/l IBA or NAA less than 10% rooting occurred. Concentrations above 1000 mg/l IBA showed no

further stimulation. However, results were variable. The base of many of the cuttings collapsed and it was suspected that there might be something toxic in the peat:pumice medium used.

A comparison was made among different potting media in an attempt to improve overall rooting percentages and cutting survival. The results are shown in Table 3. After seven weeks, the fine pumice medium gave best results. It was apparent that wherever peat was present many cuttings turned black at the base and rooting percentages were lower (14%) than when peat was absent (61%).

Table 3. Effect of various potting media[1] on the rooting of 'Gailey' persimmon after seven weeks[2]; 12 cuttings per treatment.

Potting medium	pH	Rooting (percent)	Stem black (percent)	Death (percent)
Fine pumice	5.5	83	0	0
Coarse pumice	5.8	58	42	17
Sand	6.0	42	58	8
Peat	4.6	8	92	8
Peat:sand[3]	5.1	0	100	75
Peat:pumice[3]	5.1	33	58	8

[1] All potting media were sterilized.
[2] In high humidity chambers at 26°C, 70 μE m^{-2} sec^{-1} for 16 hours.
[3] 50:50 v/v.

Softwood cuttings from seedling persimmons root well using bottom heat of 26°C with air temperatures reaching 40°C (B. McKenzie, pers. comm.). This tolerance for high temperatures was also observed using tissue-cultured material. Shoots continued to grow and rooted well in the shaded (80%), high humidity tent with 26°C bottom heat, intermittent mist (2 sec/30 min), with air temperatures often up to 38°C in the afternoon.

Subsequent Growth. Persimmons are particularly prone to transplantation shock. When rooted shoots were transplanted to new potting media they usually went dormant or died. To overcome this problem, rooting directly into Rootrainers has been carried out and current trials are investigating the requirements for maintenance of shoot and root growth.

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IMPORTANCE OF EARLY NUTRITION IN PLANTS

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Abstract. Three studies were conducted to demonstrate the importance of nutrition on the growth and development of young seedlings and rooted plants from cuttings. Seedlings of four Curcurbiataceae species were grown in rockwool without nutrient for 14 days from sowing and then given one application of nutrient. Strong growth responses occurred within 3 days of nutrient application, an indication that the internal nutrient reserves had become exhausted even before the appearance of visual deficiency symptoms. *Gerbera jamesonii* seedlings were fed weekly for 10 weeks from sowing with nutrient solutions of different strength. Optimum growth was achieved when solutions had electrical conductance values between 2 and 3.9 mS cm^{-1}. *Daphne odora* cuttings were taken from mother stock of different vigour and struck in rockwool blocks, with and without nutrients. They were grown-on in rockwool then transplanted into scoria and grown hydroponically for one growth cycle. Absence of nutrients in the rockwool caused the production of very long roots which were prone to damage on transplant. After one growth cycle, best plants were from cuttings taken from strong healthy mother stock and supplied nutrients throughout.

INTRODUCTION

Plant propagators hold many opinions on the need to supply nutrients to newly germinated seedlings and cuttings prior to or at time of rooting. Some hold the view that the early application of nutrients is unnecessary because the internal reserves in the seed and cutting are adequate to fully sustain early stages of growth. Also, without nutrient application, algal