Ann. Bot. Fennici 36: 21–31
Helsinki 7 April 1999
ISSN 0003-3847

Micropropagation of several Japanese woody plants for horticultural purposes

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Received 11 May 1998, accepted 2 December 1998

Kärkönen, A., Simola, L. K. & Koponen, T. 1999: Micropropagation of several Japanese woody plants for horticultural purposes. — *Ann. Bot. Fennici* 36: 21–31.

Axillary buds of 43 Japanese broad-leaved species were collected and used to initiate micropropagation cultures. Four nutrient media (MS, DKW, WPM, A3) were tested with different combinations of growth regulators (BAP or 2iP alone or together with IBA or IAA). Ten species (*Callicarpa dichotoma* (Lour.) K. Koch, *Chosenia arbutifolia* (Pallas) B. V. Skvortz., *Lonicera chamissoi* Bunge, *Maackia amurensis* Rupr. & Maxim., *Morus alba* L., *Populus maximowiczii* Henry, *Prunus nipponica* Matsum., *Ribes japonicum* Maxim., *Salix sachalinensis* F. Schmidt, *Toisusu urbaniana* (Seemen) Kimura) produced shoot cultures which could be used for micropropagation of numerous plantlets. The shoots were rooted on IBA supplemented medium and transplanted to a peat-sand mixture; most of them survived under the greenhouse conditions. Many species have survived the following winters out of doors, but some of them have shown cold damage. It is apparent that the long-day conditions in south Finland (60°N) affect the development of dormancy of Japanese woody plants (Hokkaido 42–43°N), but some of them may be used for horticultural purposes.

Key words: acclimatization, bud, Finland, Japan, micropropagation, woody plant

INTRODUCTION

To establish the Japanese collection in the new botanical garden of the University of Helsinki, an excursion was organized to Japan in September of 1993, when most plants have ripe seed. It was not possible to collect seeds of species in genera such as *Populus*, *Salix* and *Ulmus*, which ripen in spring or early summer, and had already been shed. Therefore, micropropagation of bud material was attempted as a method of introduction of woody plants to the Botanical Garden. Details of the excursion, seed material collected and its horticultural consequences are to be found in Koponen and Koponen (1994, 1995, 1997) and Koponen (1998).

Micropropagation of a range of woody plants has been successfully carried out (Bonga & von

Aderkas 1992), however, numerous forest trees and bushes are still recalcitrant to establishment *in vitro*. Micropropagation of many broad-leaved species including *Prunus* has been accomplished (Quoirin & Lepoivre 1977, Chalupa 1983, Bouza *et al.* 1992). Multiple efforts have also been carried out with aseptic cultures of *Ribes* (Welander 1985). Most poplar species of economic importance have been cultured *in vitro* both from juvenile material or from that taken from adult trees (Rutledge & Douglas 1988, Coleman & Ernst 1989). Different species of the genus *Salix* are cultivated mainly due to their fast growth and considerable biomass production. Micropropagation is used as a method for mass propagation of those salicaceous species having difficulties in rooting (Chalupa 1983, Bergman *et al.* 1984). Micropropagation of *Betula pendula* Roth f. *purpurea* (Andraé) A. & G. from leaf callus has been successful (Simola 1985). While the explants originated from an open-cultivated several-year-old plant of German origin (Botanical Garden, University of Turku), during the very hard winter in Finland (–30°C, 1986–1987) most of the young trees were frost-bitten. For some genera tested in the present work no previous information existed in the literature, so modifications of methods have been designed for transferring Japanese bud material for micropropagation. Plantlets were transplanted out of doors in the Botanical Garden in Helsinki and in some town gardens in south Finland and studied for acclimatization in long-day conditions.

MATERIAL AND METHODS

Twigs (ca. 7 cm) were collected from field-grown adult trees in Japan. The number of buds on each twig varied from 5 to 20 depending on the internodal length of the species. Leaves were removed and the twigs packed in plastic 14 ml test tubes. The tubes were sealed tightly with plastic caps to avoid moisture loss and mailed in letter envelopes to Helsinki. No ingredients were added to prevent fungal infections or other damage and no surface sterilization was carried out. The time for the material to reach Helsinki was ca. 4 days. The twigs were stored in test tubes at +4°C in dark until used (from one day up to four weeks). Dormant buds were used to establish micropropagation cultures. The total number of samples sent or brought from Japan was 52 representing 43 woody species. In our list of material, detailed information on collecting localities is given only for those taxa micropropagated successfully. In the list below, the number is the database file number of the Botanical Garden of the University of Helsinki.

- *Chosenia arbutifolia* (Pallas) B. V. Skvortz., female tree (Salicaceae), 1993–692
- *Chosenia arbutifolia* (Pallas) B. V. Skvortz. (Salicaceae), 1993–694
- *Salix sachalinensis* F. Schmidt (Salicaceae), 1993–693
- *Toisusu urbaniana* (Seemen) Kimura, male tree (Salicaceae), 1993-691
  Tokachi District. 20 km S of Obihiro City, *Salix*, *Chosenia*, *Alnus hirsuta* forest at Satsunai River, alt. 100 m, temperate zone. 42°57´N, 143°12´E.
- *Lonicera chamissoi* Bunge (Caprifoliaceae), 1993-613
- *Prunus nipponica* Matsum. (Rosaceae), 1993-616
  Kamikawa District. Yamabe, Furano, Tokyo Univesity forest in Hokkaido. Mt. Dairoku. Mountain top with low *Pinus pumila*, *Picea glehnii*, *Sorbus matsumurana* shrub, alt. 1 450 m, upper oroboreal zone. 43°22´N, 142°23´E.
- *Maackia amurensis* Rupr. & Maxim. (Fabaceae), 1993–778
- *Ribes japonicum* Maxim. (Grossulariaceae), 1993–770
- *Callicarpa dichotoma* (Lour.) K. Koch (Verbenaceae), 1993–780
  Hidaka District. Samani, Nikanbetsu River 18 km NW of Cape Erimo. Secondary (cut and planted) mixed hardwood (*Acer* spp., *Cercidiphyllum*, *Tilia*); *Abies sachalinensis* forest, alt 100–230 m, temperate zone. 42°04´N, 143°09´E.
- *Morus alba* L. (Moraceae), 1993–664
  Kamikawa District. Yamabe, Furano, Tokyo Univesity forest in Hokkaido. *Abies sachalinensis* plantations with original mixed hardwood trees, alt. 450–500 m, orohemiboreal zone. 43°22´N, 142°23´E.
- *Populus maximowiczii* Henry (Salicaceae), 1993–686
  Kamikawa District. Yamabe, Furano, Tokyo Univesity forest in Hokkaido. *Abies sachalinensis* plantations with original mixed hardwood trees, alt. 500–600 m, orohemiboreal zone. 43°22´N, 142°23´E.

Methods for micropropagation

Abbreviations: A3 = Anderson medium; BAP = 6-benzylaminopurine; DKW = Driver and Kuniyuki medium; IAA = indole-3-acetic acid; IBA = indole-3-butyric acid; 2iP = isopentenyladenine; MS = Murashige and Skoog medium; PVP = polyvinylpyrrolidone; WPM = woody plant medium.

In vitro cultures were established by use of axillary buds as initial explants (Fig. 1A). First, twigs were soaked in tapwater for at least two hours. They were then cut into short nodal segments and surface sterilized in 70% ethanol for 1 min followed by 10 to 18 min in sodium hypochlorite (2% available chlorine). The explants were then rinsed five times in sterile distilled water. The bud scales were aseptically removed and the buds placed in Petri dishes containing ca. 20 ml of culture initiation medium. Four basal media

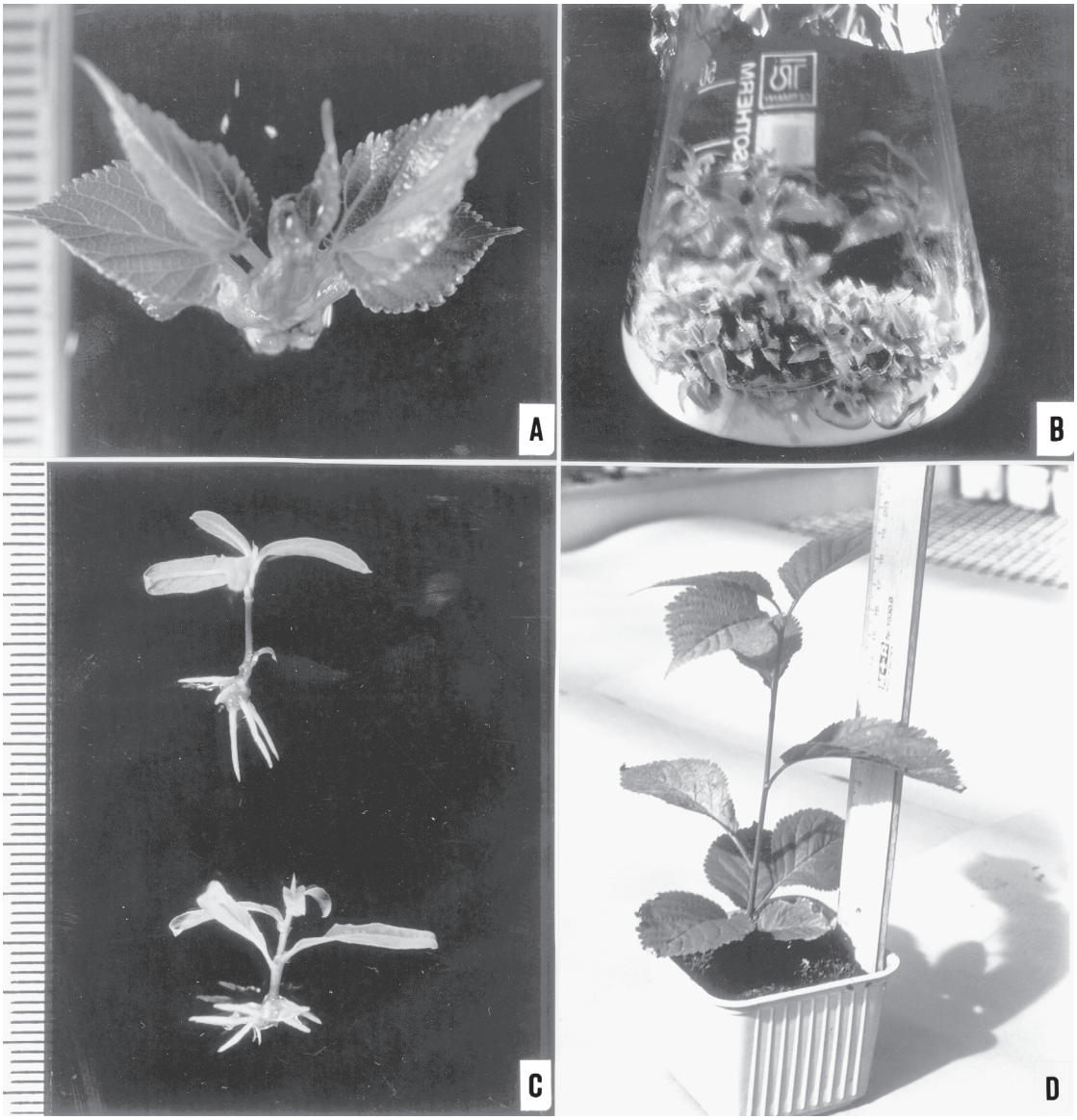

Fig. 1. Plantlet formation in *in vitro* cultures. — A: Sprouting bud of *Morus alba* on MS medium with 2.5 μM BAP and 0.5 μM IBA. — B: Multiplication phase of *Callicarpa dichotoma* on MS medium supplemented with 1 μM BAP. — C: Rooted shoots of *Toisusu urbaniana*. Rooting was achieved on $^1/_2$MS supplemented with 2.5 μM IBA. — D: Plantlet of *Prunus nipponica* growing in the greenhouse.

were used for bud cultures: 1) Murashige and Skoog medium, MS (1962), 2) woody plant medium, WPM (Lloyd & McCown 1980), 3) Driver and Kuniyuki medium, DKW (1984), 4) Anderson medium, A3 (Anderson 1984). All media were supplemented with various concentrations of 6-benzylaminopurine (BAP, Sigma: 1, 2.5 or 4.4 μM) or isopentenyladenine (2iP, Sigma: 25 or 74 μM). The media with 2.5 μM BAP were also supplemented with 0.5 μM indole-3-butyric acid (IBA, Fluka). Sucrose was supplied at 87.6 mM. Ascorbic acid (28.4 μM) was added as an antioxidant to all media. Prior to autoclaving at 120°C for 20 min, the pH of the media was adjusted to 5.7, except for the A3 medium whose pH was adjusted to 4.8; the media were then solidified with Difco Bacto agar (7 g l^{-1}). The cultures were incubated at 23°C day/20°C night with a 16 h photoperiod (Fluora, L 36 W Osram and warm white deluxe lamps, 36 W Airam, 70–80 μmol m^{-2} s^{-1} photosynthetically active radiation). If any exudation was observed in the nu-

trient medium the explants were transferred to fresh medium.

After a few weeks, explants showing growth were transferred to new media. Elongated shoots were subcultured in 50 ml Erlenmayer flasks containing 20 ml of nutrient medium, with four explants cultured per jar. The flasks were sealed with aluminium foil. Further shoot production was performed by subculturing individual shoots every 4–5 weeks (Fig. 1B). After four subcultures, the multiplication rates were recorded by determining the number of new microcuttings that had proliferated from the initial single shoots. This was done at least five times during successive subcultures. Shoots were rooted on WPM or MS medium with half-strength macroelements and sucrose ($^1/_2$WPM, $^1/_2$MS) supplemented with IBA (1 or 2.5 µM; Fig. 1C) or without growth regulators (*Morus alba*). On average, 75–300 shoots of each strain were used in each rooting experiment. Plantlets were transplanted to a peat-sand mixture (or to pure sand in the case of *Toisusu urbaniana*) and protected against drying with transparent plastic covers. After 1–2 weeks, the plantlets began to form new leaves and could tolerate greenhouse conditions. Plantlet survival was recorded after 3 weeks (Fig. 1D). Some of the micropropagated plantlets were planted in the Botanical Garden and in some town gardens in south Finland in summer 1994, the majority in summer 1995. The winter hardiness of the plantlets was monitored in the following years (Fig. 2).

RESULTS

Of 52 Japanese specimens, 29 (56%) showed some response (bud-sprouting, callus growth or shoot proliferation) in tissue culture. Shoot multiplication succeeded with 11 of these (21%; Table 1). Severe problems with contamination occurred in establishing axenic cultures, probably due to the collecting month (September) and storage of twigs over an extended time period (up to four weeks). Many twigs were severely contaminated due to the high levels of micro-organisms existing in nature during the collection period and disinfection was difficult.

Caprifoliaceae

The *in vitro* shoots of *Lonicera chamissoi* were long and slender with few nodes. These were multiplied by subculturing the single nodes in fresh WPM medium supplemented with 1 µM BAP or 2.5 µM BAP together with 0.5 µM IBA (Table 1). Rooting of shoots was achieved on $^1/_2$WPM medium with 2.5 µM IBA, with 80% of the rooted shoots surviving the transfer to the peat-sand mixture (Table 2). Plantlets planted out of doors survived without visible damage over the winters and flowered in next summers (Table 3, Fig. 2A).

Fabaceae

There were problems with phenol exudation in micropropagation of *Maackia amurensis*. After subculture the cutting surfaces darkened and a black exudate was released into the medium, likely inhibiting growth and resulting in the death of most explants. Addition of cystein (50 µM) or 1% polyvinylpyrrolidone (PVP) to the nutrient media failed to prevent the browning of the explants. Shoot-producing cultures could be established from only two buds. DKW media supplemented with BAP (2.5 µM) and IBA (0.5 µM) or BAP (1 µM) alone were used as multiplication media (Table 1). The rooting percentage was 21% and only 11% of the rooted shoots survived the transplanting to the peat-sand mixture (Table 2). The plantlets planted outside in the Botanical Garden survived the first winter without visible injuries but suffered serious frost damage in the second winter out of doors (Table 3).

Grossulariaceae

Many buds of of *Ribes japonicum* sprouted on the initiation media but the shoots did not elongate. Shoot elongation was achieved after transfer to Lepoivre's medium (Quoirin & Lepoivre 1977) supplemented with 2 µM BAP, 1 µM IBA and 0.6 µM gibberellic acid (GA_3, Sigma) (Bouza *et al.* 1992). Shoots were then multiplicated on MS medium either with 1 µM BAP alone or with 2.5 µM BAP and 0.5 µM IBA (Table 1). Lower leaves on the shoots had a tendency to turn brown, but this did not inhibit shoot growth. Shoots rooted readily on rooting medium (Table 2). Most of the plantlets grown out of doors suffered from shoot tip injuries after winter (Table 3, Fig. 2B).

Moraceae

MS medium containing 2.5 or 4.4 µM BAP and 3% sucrose was used as an initiation medium for

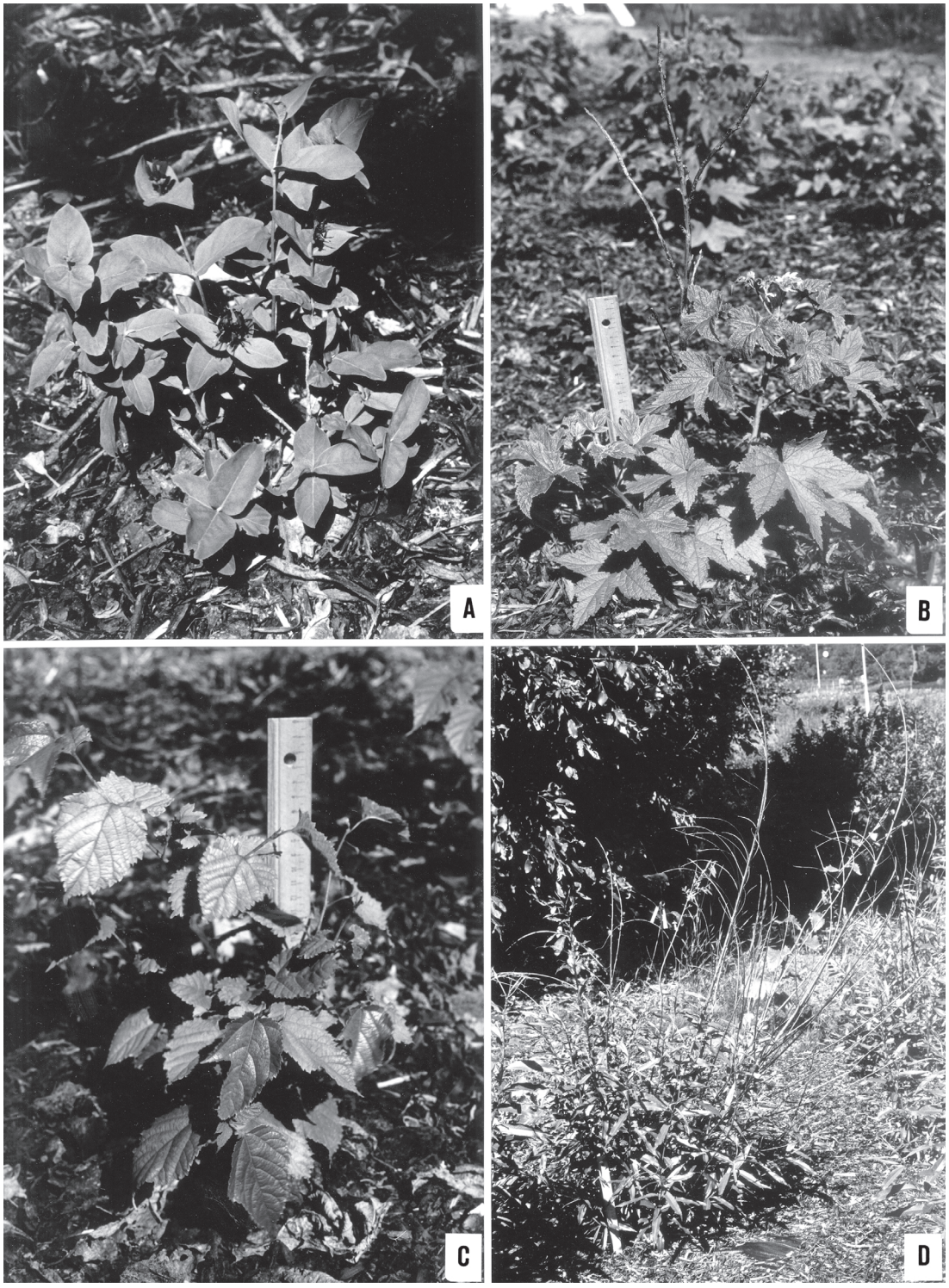

Fig. 2. Wintering of micropropagated plantlets. — A: Flowering plantlet of *Lonicera chamissoi* after one winter out of doors. — B: *Ribes japonicum* suffered from shoot-tip injuries after the hard winter. — C: Well-wintered plantlet of *Morus alba*. — D: *Salix sachalinensis* after the hard winter in the Botanical Garden. The shoots were frost-bitten but show plenty of new growth from the base of the original shoot.

Morus alba (Fig. 1A). For shoot proliferation, explants were transferred to MS medium containing 5 µM BAP (Table 1). Exogenous auxin was unnecessary for root formation: addition of IBA resulted in callus formation at the shoot-base. All shoots formed roots on $^1/_2$WPM or $^1/_2$MS medium without growth regulators (Table 2). They have survived with minor shoot tip damage over two winters (Table 3, Fig. 2C).

Rosaceae

Buds of some rosaceous species dried out during transfer or long-term storage of twigs at +4°C and some difficulties occurred in establishing *in vitro* culture of *Prunus nipponica*. After initial budbreak the buds neither elongated nor multiplicated. After transfer to $^1/_2$MS medium supplemented with 2.2 µM BAP and 2.9 µM indole-3-acetic acid

Table 1. Summary of micropropagation procedures. Only the media succesfully used are reported for culture initiation. Multiplication rates were recorded by determining the number of new microcuttings that had proliferated from the initial single shoots during five successive subcultures (mean ± SD).

	Culture media. Basal media plus growth regulators (µM)		
	Shoot-induction media	Shoot-multiplication media	Number of new shoots ($\bar{x}$ ± SD)
Lonicera chamissoi	WPM BAP 1	WPM a) BAP 1 b) BAP 2.5 IBA 0.5	 a) 0.8 ± 0.5 b) 0.7 ± 0.6
Maackia amurensis	MS, WPM, A3 BAP 4.4 2iP 74	DKW a) BAP 1 b) BAP 2.5 IBA 0.5	 a) 1.3 ± 0.5 b) 3.9 ± 2.6
Ribes japonicum	MS, WPM BAP 2.5, 4.4 IBA 0.5	MS a) BAP 1 b) BAP 2.5 IBA 0.5	 a) 0.8 ± 0.8 b) 0.6 ± 0.6
Morus alba	MS BAP 4.4, 2.5 IBA 0.5	MS BAP 5	 1.7 ± 1.0
Prunus nipponica	MS, WPM BAP 2.5, 4.4 IBA 0.5	$^1/_2$MS a) BAP 2.2 b) BAP 1 IAA 2.9	 a) 1.5 ± 0.5 b) 0.7 ± 0.4
Chosenia arbutifolia 1. 1993-692 2. 1993-694	WPM BAP 1, 4.4	WPM a) BAP 0.44 b) BAP 1	 1. a) 1.6 ± 0.7 b) 2.0 ± 0.7 2. a) 1.9 ± 0.9 b) 2.2 ± 0.7
Populus maximowiczii	WPM BAP 1	MS/2 BAP 0.44	 1.4 ± 0.9
Salix sachalinensis	WPM, MS BAP 1, 4.4	WPM BAP 0.44	 1.0 ± 0.9
Toisusu urbaniana	WPM BAP 1, 4.4	MS BAP 0.44	 1.1 ± 0.8
Callicarpa dichotoma	WPM BAP 1, 4.4	MS BAP 1	 4.3 ± 2.8

$^1/_2$MS = MS medium with half-strength macronutrients and sucrose, MS/2 = MS medium with half-strength NH_4NO_3.

(IAA, Fluka; Ryynänen & Ryynänen 1986), shoot elongation and the outgrowth of axillary shoots was induced. After several subcultures some hyperhydricity occurred, so the amount of BAP was reduced to 1 µM. This reduced the multiplication rate from 1.5 ± 0.5 to 0.7 ± 0.4 (Table 1). Rooting percentage was 88 and 94% for 1 and 2.5 µM IBA, respectively, and 82% of the rooted shoots survived the transfer to soil (Table 2, Fig. 1D). Plantlets planted in the Botanical Garden survived over winter with little shoot tip injuries (Table 3).

Salicaceae

WPM supplemented with a low concentration of BAP (0.44–1 µM) stimulated rapid growth of shoots of *Chosenia* and *Salix* species. On initiation of *Chosenia arbutifolia*, some phenol exudation occurred with buds turning black after a few minutes on nutrient medium. No growth of these buds took place. Multiple adventitious buds differentiated in 6–7 weeks from the bases of explant buds of *C. arbutifolia*, with excessive anthocyanin formation in some shoots at first. WPM medium was also used for multiplication of *Salix sachalinensis* (Table 1). The establishment of *in vitro* culture took several weeks for *Populus maximowiczii*. As use of full-strength MS medium resulted in hyperhydratic cultures, MS medium with reduced concentration of NH_4NO_3 (half-strength) supplemented with 0.44 µM BAP was used for shoot proliferation (Table 1). For *Toisusu urbaniana*, full-strength MS medium with 0.44 µM BAP served as a multiplication medium (Table 1).

Shoots of most Salicaceae species rooted quite readily on $^1/_2$WPM medium supplemented with 1 or 2.5 µM IBA (Table 2). For *Toisusu urbaniana* shoots were chlorotic on $^1/_2$WPM medium the rooting medium composed of $^1/_2$MS medium being superior (Fig. 1C). Survival of rooted shoots after transplanting to peat-sand mixture varied be-

Table 2. Summary of rooting and survival of Japanese species. In rooting experiments 75–300 shoots of each strain were used. Survival percentages are for rooted shoots.

	Medium ($^1/_2$WPM) and growth regulators (µM)	Rooting %	Survival %
Lonicera chamissoi	IBA 2.5	71	80
Maackia amurensis	IBA 1	21	11
Ribes japonicum	IBA a) 1 b) 2.5	a) 90 b) 99	90
Morus alba	–	100	98
Prunus nipponica	IBA a) 1 b) 2.5	a) 88 b) 94	82
Chosenia arbutifolia 1. 1993–692 2. 1993-694	IBA a) 1 b) 2.5	1.a) 32 b) 36 2.a) 44 b) 61	1. 84 2. 72
Populus maximowiczii	IBA 1	97	96
Salix sachalinensis	rooted in the multipl. medium		97
Toisusu urbaniana	IBA a) 1 b) 2.5 *	a) 74 b) 90	28^{PS} 87^{S}
Callicarpa dichotoma	IBA a) 1 b) 2.5	a) 99 b) 100	95

*) $^1/_2$MS medium
^{PS} = in peat-sand mixture, ^{S} = in pure sand.

tween 72 and 97% for all species except for *T. urbaniana* which had a survival percentage of 28% (Table 2). When these plantlets were potted in pure sand the survival percentage was almost 90%. Plantlets of *Chosenia arbutifolia* (clone 1993–694) planted out of doors survived with shoot tips frost-bitten over the winter, but the other clone (1993–692) had serious damage (Table 3). The shoots of *Salix sachalinensis* were also severely frost-bitten, but the roots remained alive: there was vigorous growth of root sprouts in the following summer (Fig. 2D). Shoots of *Populus maximowiczii* and *Toisusu urbaniana* suffered from shoot tip injuries after winter (Table 3).

Verbenaceae

Buds of *Callicarpa dichotoma* sprouted on WPM medium supplemented with BAP (1 or 4.4 µM). The shoots were subcultured on MS medium with various concentrations of BAP which at a concentration of 2.5 µM together with IBA (0.5 µM) significantly increased the number of axillary shoots. However, this resulted in very short shoots and so MS supplemented with 1 µM BAP was chosen as the multiplication medium (Fig. 1B, Table 1). Shoots rooted readily on rooting medium and were successfully transplanted to soil (Table 2). The shoot tips of plantlets planted in the Botanical Garden suffered from frost in the following winters (Table 3).

Table 3. Winter survival of micropropagated plantlets. Evaluation made after two years out of doors.

Lonicera chamissoi	no damage
Maackia amurensis	shoots severely frost-bitten
Ribes japonicum	ST
Morus alba	ST
Prunus nipponica	ST
Chosenia arbutifolia	
1993–692	shoots severely frost-bitten
1993–694	ST, RS
Populus maximowiczii	ST
Salix sachalinensis	shoots severely frost-bitten, RS
Toisusu urbaniana	ST, RS
Callicarpa dichotoma	ST

ST = shoot tips frost-bitten; RS = root sprouting

DISCUSSION

Results show that it is possible to transport buds of woody plants from long distances for micropropagation purposes. The plant material remained viable without drying or decaying in test tubes for some days, or even for some weeks. It may, however, be difficult to free explants from micro-organisms, if the twigs are collected in autumn and stored for a long time. The risk of contamination is season-dependent: dormant buds of adult birch (*Betula pendula*) trees are more difficult to sterilize than buds collected after dormancy has been broken (Welander 1988). In addition, contaminants might have built up during cold storage (Hohtola 1988). Contamination rates are often higher for explants from field-grown trees than for those obtained from greenhouse material.

Several woody species studied here were recalcitrant to micropropagation: there may have been problems with toxic exudation from the explant, lack of shoot elongation and rooting, as well as with hyperhydricity (vitrification). The responses can be manipulated by choice of plant-growth regulators and by the nutrient salts of the medium (Preece 1995). The physiological condition of plant material is crucial to the success of *in vitro* culture. In our experiments, *Chosenia arbutifolia* and *Maackia amurensis* suffered from phenolic exudation and *Prunus nipponica* failed to elongate on the initiation media used.

The timing of collection is often a critical factor in micropropagation. In our experiment the twigs were collected in September when the trees were entering dormancy. For bud cultures of four *Larix* species the response was high in explants from material collected in August and September before bud dormancy or in March after dormancy had been broken (Bonga & Pond 1991). Seasonal changes have also been reported in *Fagus sylvatica* buds in response to plant-growth regulators (Nadel *et al.* 1991). In autumn the hormonal balance of woody plants is more favorable to induce rest than growth. This may explain the problems with lack of shoot elongation of *Prunus nipponica*. For most species tested in our work, dormancy was not, however, developed appreciably, because many buds sprouted and shoots proliferated on nutrient media supplemented with plant-growth regulators.

A nutrient medium containing a reduced salt level has been employed by various workers in *in vitro* culture of *Salix* species (Chalupa 1983, Bergman *et al.* 1984). WPM medium has also been used for micropropagation of *Lonicera periclymenum* (Boonnour *et al.* 1988). The same basal medium was also suitable for *L. chamissoi* (Table 1). This has a growth habit similar to that of *L. periclymenum*, consisting of a non-branching single-stemmed growth style in *in vitro* culture (Boonnour *et al.* 1988). A high-salt MS medium has been successfully used in cultures of *Morus alba* (Oka & Ohyama 1986, Sharma & Thorpe 1990). This species of Japanese origin also multiplied readily on MS medium supplemented with BAP. The sucrose used in our experiments did not prevent shoot elongation as was the experience of Oka and Ohyama (1986), who recommended fructose in shoot elongation medium for this species.

Populus maximowiczii and *Prunus nipponica* suffered hyperhydricity on full-strength MS medium. One reason for hyperhydricity may be the high content of Cl^- and NH_4^+-ions in this nutrient medium (Quoirin & Lepoivre 1977, Vieitez *et al.* 1985) or the use of growth regulators, especially cytokinins, along with the high relative humidity in the culture vessels (Kataeva *et al.* 1991). Hyperhydricity was reduced when shoots of *Populus maximowiczii* were multiplied on a modified MS medium with half strength concentration of NH_4NO_3 (10.3 mM), or when shoots of *Prunus nipponica* were transferred to a medium with reduced concentration of cytokinin (Table 1).

Phenolic exudation is a serious problem in micropropagation of some woody species (Compton & Preece 1986). A wide variety of techniques have been used to overcome this problem and to obtain healthy cultures. All the nutrient media used in this work contained ascorbic acid (28.4 μM) as an antioxidant. Some buds of *Chosenia arbutifolia* turned black when tissue culture was initiated from axillary buds. The exudate may have inhibited growth of the meristems as no growth of buds took place; also after the first subculture there was serious damage in explants of *Maackia amurensis*: most buds died. Addition of cystein or PVP did not prevent the browning of explants. The production of phenolics might also have reduced the rooting and subsequent survival of this species (Table 2). The browning was not detrimental in all cases: lower leaves of *Ribes japonicum* turned brown when in contact with the medium, as was the case also with *R. grossularia* (Welander 1985), but this did not affect the survival or rooting of shoots (Table 2).

In conclusion: 10 different species were successfully micropropagated in this work, and as far as we know, seven of them for the first time. Field trials are now being carried out to discover how the Japanese species survive winter conditions in Finland. The minimum winter temperatures in Hokkaido are comparable to those of south Finland. Opposed to this is the photoperiod, which differs significantly between north Japan (42–43°N) and south Finland (60°N). Photoperiod is the primary environmental factor controlling the growth cessation of many woody species in autumn (Sylvén 1940, Pauley & Perry 1954, Wareing 1956, Vaartaja 1959, Håbjørg 1978, Junttila & Kaurin 1985, Howe *et al.* 1995). In a majority of trees and shrubs there exist clinal variations (ecotypic variation) in critical photoperiods for growth cessation and winter bud formation both with latitude and altitude. The higher the latitude, the longer the critical photoperiod for apical growth cessation (Sylvén 1940, Pauley & Perry 1954, Vaartaja 1959, Håbjørg 1978, Junttila & Kaurin 1985, Howe *et al.* 1995). Also the high elevation populations stop growing at longer photoperiods than do low-elevation ones from the same latitude (Pauley & Perry 1954, Håbjørg 1978). In general, movement from the latitude of the natural habitat northward (i.e., into longer days) prolongs the active period of growth, and movement southward (i.e., into shorter days) shortens it. Such modifications in the length of the active growth period normally result in marked differences in total seasonal increment and frost hardiness. Thus, movement of plants into a longer-day conditions characteristically gives increased height growth accompanied by decreased resistance to early autumnal frosts; whereas movement into a shorter-day regime results in dwarfing, associated with increased frost resistance. However, not all woody plants show this photoperiodic response. Some species of the Rosaceae family as well as some species of *Syringa* and *Fraxinus* have little or no photoperiodic sensitivity (Wareing 1956, Nitsch 1957).

It is apparent that long-day conditions in south

Finland affect the development of dormancy of Japanese woody plants, but some species may survive in Finland. Of 11 Japanese strains planted outside in south Finland, one (*Lonicera chamissoi*) has survived over two winters without visible frost damage, seven strains have had some damage, mainly shoot tip injuries, and three strains have been seriously frost-bitten (Table 3). Long-term experiments in the Forest Research Institute and Arboretum Mustila (Elimäki, 61°N) have shown that many Japanese forest trees, some of them originating from Hokkaido, are hardy in Finland (Heikinheimo 1956, Tigerstedt 1970, Lähde *et al.* 1984).

Acknowledgements: The authors are indebted to Associate Professor H. Takahashi, Ph.D., (Sapporo Bot. Garden, Univ. of Hokkaido) who organized the logistics of the field excursion on Hokkaido; to head gardener K. Niini (Bot. Garden, Univ. of Helsinki) for selecting the material for micropropagation in the field; to the intendent of the same Garden, A. Koponen, Ph.D., for the final identification and nomenclature of the plants; to M. Sc. S. Heikkonen for help in micropropagation and to the gardener P. Lönnqvist (Dept of Biosciences, Univ. of Helsinki), for help in the initial growing of plantlets. Financial support was received (TK) from the Univ. of Helsinki and from the Scandinavia-Japan Sasakawa Foundation.

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