Propagation of Japanese Quince (*Chaenomeles japonica*) Plants

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SUMMARY

In this paper, research on vegetative propagation of Japanese quince (*Chaenomeles japonica*) plants is reported and summarised. An efficient system for propagation of Japanese quince based on softwood cuttings was developed. For this purpose, 20 cm long top cuttings, with the base of the cuttings soaked in 30 mg/l IBA for 18 hours, were used. Although hardwood cuttings seemed less useful for propagation of chaenomeles plants, a high rooting percentage was obtained using 20 cm long base cuttings, soaked in 100 mg/l IBA for 24 hours. Procedures for micropropagation were improved and micropropagation was shown to be a very efficient method for propagation of Japanese quince, with an average proliferation rate of around 3–4 and a rooting percentage of over 90%. For the proliferation phase a medium consisting of complete MS medium with macronutrients reduced to 3/4, iron added as NaFeEDTA, 1.0 g/l BA as growth regulator and 35 g/l sucrose as a source of carbohydrates, was useful in large scale commercial propagation. For rooting of microshoots, a shoot elongation phase of two weeks and a soaking of microshoots in 250 mg/l IBA before planting seemed important. It was shown that the ability to form roots was genotype dependent. Rooting potential should therefore be evaluated during plant breeding and selection.

INTRODUCTION

Japanese quince (*Chaenomeles japonica*) is currently being developed as a potential fruit crop for Northern Europe. Rapid and cheap propagation methods are indispensable for the successful introduction of the new crop. Generative propagation of Japanese quince is easy, but chaenomeles plants do not breed true through seeds. Varieties must thus be vegetatively propagated to maintain their characteristics (Rumpunen 2002). Japanese quince can be propagated by softwood cuttings (Kviklys 1996, 1998; Kviklys & Rumpunen 1996), whereas propagation by hardwood cuttings seems more difficult (Albrecht 1980, Ratomskyte 1990, Wells 1961). Propagation by root pieces is also possible (Eley 1970), but is not considered to be economically viable on a large scale using existing methods. Studies on micropropagation of Japanese quince indicate that rooting of microcuttings can be difficult (Panavas 1994), and that proliferation rate *in vitro* may be low compared to proliferation rates of other species within the Rosaceae (Norton & Boe 1982). In this paper, attempts to further develop and improve propagation methods for Japanese quince, using softwood, hardwood and microcuttings, are summarised.

PROPAGATION BY SOFTWOOD CUTTINGS

Materials and methods

Softwood cuttings were prepared from shoots of Japanese quince collected from plants in the field during a period of intensive growth in June-July. Only semi-mature shoots were used, in which the middle part was sufficiently firm and the base had started to lignify. The shoots were kept in water to maintain turgor until preparation of cuttings. All leaves were removed from the lower part of the cutting (4–5 cm). A premixed peat/perlite substrate (50/50% by volume) was spread in trays with cylindrical wells, (40 mm in diameter and 85 mm deep), and watered. Cuttings were inserted approximately 4 cm into the substrate. In the greenhouse, cuttings were automatically misted. After four weeks the number of rooted cuttings, number of roots, root length and position of roots were evaluated. A cutting was considered rooted when primary roots were more than 1 mm long. Thus, cuttings with just callus were not considered rooted. On each cutting, the length of the longest root was measured. Root position was scored using the numbers 1 to 4. A cutting with roots on the base was given a score of 1. When roots were more on the base than on the stem, the cutting was given a score of 2. When roots were more on the stem than on the base, the cutting was given a score of 3. Finally, when roots were on the stem the cutting was given a score of 4.

In the first experiment (Kviklys 1998) the effects on rooting of cutting origin and of the growth regulator applied were studied. Long shoots were cut into three parts: base, middle and top. The very soft and herbaceous apical part of the shoot was always discarded. Cuttings of 12–15 cm length were soaked for 18 hours in water (control), 10 mg/l IBA or 30 mg/l IBA, respectively. In the second experiment (Kviklys 1998), the effect on rooting of the length of the cutting was studied. Cuttings were cut into segments of 5–6 cm, 10–12 cm and 20–22 cm length and soaked for 18 hours in 30 mg/l IBA. For each treatment, 50 cuttings were evaluated.

Results

The rooting percentage obtained was 37%, 45% and 70%, for base, middle and top cuttings, respectively, when estimates were averaged over treatments (Kviklys 1998). Top cuttings formed 6.2 roots per cutting. This was three times higher than the number of roots formed on base cuttings. Roots on top cuttings were longer than roots on base and middle cuttings. Cuttings treated with 30 mg/l IBA rooted best. Even without growth regulators, softwood top cuttings rooted better than base and middle cuttings soaked in 10 mg/l IBA.

Rooting strongly depended on the length of cuttings (Figure 1). On average 25%, 42% and 78% rooting was obtained for 5 cm, 10–12 cm and 20–22 cm long cuttings, respectively (Kviklys 1998). In addition, number of roots (1.9, 3.2 and 8.1, respectively) and root length (2.3 cm, 4 cm and 5.6 cm, respectively), increased from short to long cuttings.

Discussion

Top cuttings rooted better than middle and base cuttings. The difference in rooting percentage reflects the difference in maturity of the shoot from which the cuttings were prepared. Furthermore, on top cuttings, roots were evenly distributed around the perimeter and emerged from a wide zone, not only from the base. This type of root development should result in high quality plants with a good root system.

The highest average rooting percentage (78%) was obtained for the longest cuttings (20–22 cm). A large content of carbohydrates in these cuttings may explain the better rooting, the larger number of roots and the longer roots (compared to short cuttings). In addition, the longest cuttings had most leaves, which could supply the cutting with root promoting substances.

Although the rooting potential of Japanese quince plants is in general moderate, there is a large variation among genotypes in rooting ability. Therefore, genotypes that are easy to propagate should be selected during plant breeding, and as long cuttings as possible should be used for propagation. However,

Figure 1. Rooting of Japanese quince (*C. japonica*) softwood cuttings strongly depended on the length of the cutting, and long cuttings (approx. 20 cm) rooted best.

the length of the cuttings is one of the factors that may limit propagation efficiency, since when new varieties are released there is often a shortage of cutting material.

PROPAGATION BY HARDWOOD CUTTINGS

Materials and methods

A completely randomised design with 8 genotypes (representing the taxa *C. japonica*, *C. speciosa* and *C x superba*), 12 treatments and 10 cuttings per treatment and genotype was used to evaluate rooting of hardwood chaenomeles cuttings in 1998 and 1999. Hardwood cuttings, 20 cm long, were prepared from the top and from the base of the annual growth of dormant shoots collected from plants in the field in November. The cutting was either not wounded or wounded at two sides of the base using a sharp knife, and then soaked in water, 100 mg/l IBA or 200 mg/l IBA for 24 hours. Cuttings were inserted into moist vermiculite in a plastic box and transferred to a greenhouse. In the greenhouse, bottom heat was adjusted to 20 °C (measured at the base of the cutting) to promote root development and the air temperature was kept at 4–7 °C. After three weeks, boxes were moved to a cold store (1–2 °C) for two months, to allow efficient breaking of dormancy. Cuttings were then transferred to the greenhouse and inserted into a substrate of peat/perlite (50/50% by volume). Bottom heat was again adjusted to 20 °C, and the air temperature was maintained at 10 °C during the first week and at 15 °C for another three weeks. Rooting, callusing, number of roots and presence of root rot were then evaluated.

Results

It was noticed that: 1) wounding as a sole factor only slightly improved rooting, 2) base cuttings rooted better than top cuttings, 3) IBA promoted rooting, 4) a combination of wounding and IBA made cuttings very sensitive to root rot, 5) base cuttings treated with 100 mg/l or 200 mg/l IBA resulted in the highest percentage of rooted cuttings, 6) there were genotype differences in rooting ability, 7) there was a high incidence of root rot, 7) the rooting percentage was acceptable for the best treatments in 1998 but in general very low when the experiment was repeated in 1999 (Table 1, Figure 2).

Table 1. Rooting of hardwood base- and top-cuttings on different treatments (average of results for 8 genotypes representing different *Chaenomeles* taxa).

Treatment	Rooting (%)			Treatment	Rooting (%)		
	1998	1999	Average		1998	1999	Average
Base, water	6	0	3	Top, water	8	1	4
Base, water, wounded	10	7	8	Top, water, wounded	4	0	4
Base, IBA 100	85	21	53	Top, IBA 100	61	1	31
Base, IBA 100, wounded	30	29	30	Top, IBA 100, wounded	20	1	10
Base, IBA 200	78	20	49	Top, IBA 200	33	4	18
Base, IBA 200, wounded	10	19	15	Top, IBA 200, wounded	11	1	6
Average	36	16	26	Average	23	1	12

Discussion

It can be concluded that it is possible to propagate chaenomeles plants by hardwood cuttings, but it can sometimes be difficult. The best rooting percentage was obtained for base cuttings soaked in IBA 100 mg/l. The high frequency of root rot on wounded cuttings soaked in IBA is a major problem that needs to be further studied. No fungicide was used before root induction and storage. A practical way to reduce root rot without fungicides would perhaps be to allow the cutting to root completely before transfer to the cold storage. There is no obvious explanation for the considerably lower rooting percentages in 1999 compared to 1998. Propagation of chaenomeles plants by hardwood cuttings seems therefore unreliable from a commercial propagation viewpoint unless methods are further improved.

Figure 2. Differences in callus and root formation on hardwood base- and top-cuttings of different *Chaenomeles* taxa. The cuttings were wounded and then soaked in water, IBA 100 mg/l or IBA 200 mg/l for 24 h. Root induction took place in a greenhouse with low air temperature (4–7 °C) and bottom heat (20°C) for three weeks.

MICROPROPAGATION

Materials and methods

Three genotypes (GI=93010 selected in Finland, GII=NV1395 selected in Sweden and GIII=NV16144 selected in Sweden) of Japanese quince were used to initiate *in vitro* cultures in 1998. Explants were obtained from dormant buds or from actively growing shoot tips. Seven modified Murashige & Skoog (1962) media (A–G, Table 2), and seven rooting treatments (1–7, Table 3) were evaluated (Kauppinen 2001b) .

A complete MS medium with macronutrients reduced to 3/4 was modified by varying the concentration of either BA, iron chelate (NaFeEDTA or FeEDDHA), IBA, or macronutrients (Table 2). Sucrose (30 g/l) was added as a source of carbohydrates. In the medium, pH was adjusted to 5.7 after adding Difco Bacto agar (7 g/l), but prior to autoclaving for 20 min at 121 °C. Assimilation light was kept at 80±8 µmol/m^{2}s or 47±7 µmol/ m^{2}s during proliferation. In glass jars, opaque polypropene enclosures decreased the light from 80±8 µmol/m^{2}s to 55±5 µmol/m^{2}s and from 47±7 µmol/m^{2}s to 37±7 µmol/m^{2}s (Kauppinen 2001a).

Shoots grown on the proliferation medium were either transferred to the greenhouse for immediate planting in peat/vermiculite in propagation trays with lids, or pre-treated *in vitro* (Table 3). When immediately planted, shoots were either inserted in peat/vermiculite after dipping for 5 sec in 500 mg/l IBA, or inserted in peat/vermiculite without dipping. The pre-treatment methods involved a two week treatment either in IBA (1.0 mg/l) or in a growth regulator free 3/4 MS medium. The shoots were then put into peat/vermiculite with or without dipping in IBA (2h in 250 mg/l or 5 sec in 500 mg/l) solution (Kauppinen 2001b). Nematodes (*Steinernema feltiae*) were mixed into the substrate to prevent attack by sciarid flies (*Sciaridae*).

The possibility of storing cultures *in vitro* was tested for 13 genotypes. The cultures were kept at +4 °C in the dark or in faint supplemental light (approximately 10 µmol/m^{2}s) for six months. Shoots were transferred to fresh proliferation medium one week before storing in the cold.

Table 2. Media tested to improve rate of proliferation for Japanese quince (*C. japonica*) microshoots.

Medium	MS	BA (mg/l)	IBA (mg/l)	Iron (mg/l)
A	3/4	0.5	0	36.7 NaFeEDTA
B	3/4	1.0	0	36.7 NaFeEDTA
C	3/4	2.0	0	36.7 NaFeEDTA
D	3/4	1.0	0.1	36.7 NaFeEDTA
E	3/4	1.0	0	73.4 NaFeEDTA
F	3/4	1.0	0	41.6 FeEDDHA
G	1	1.0	0	36.7 NaFeEDTA

Table 3. Treatments tested to improve rooting of Japanese quince (*C. japonica*) microshoots.

Treatment
1 immediate planting
2 5 sec dip in 500 mg/l IBA, then planting
3 Two weeks on 3/4 MS 1.0 mg/l IBA, then planting
4 Two weeks on 3/4 MS 1.0 mg/l IBA, then 5 sec dip in 500 mg/l IBA, then planting
5 Two weeks on 3/4 MS hormone free, then 5 sec dip in 500 mg/l IBA, then planting
6 Two weeks on 3/4 MS hormone free, then 2 h soak in 250 mg/l IBA, then planting
7 Two weeks on 3/4 MS 1.0 mg/l IBA, then 2 h soak in 250 mg/l IBA, then planting

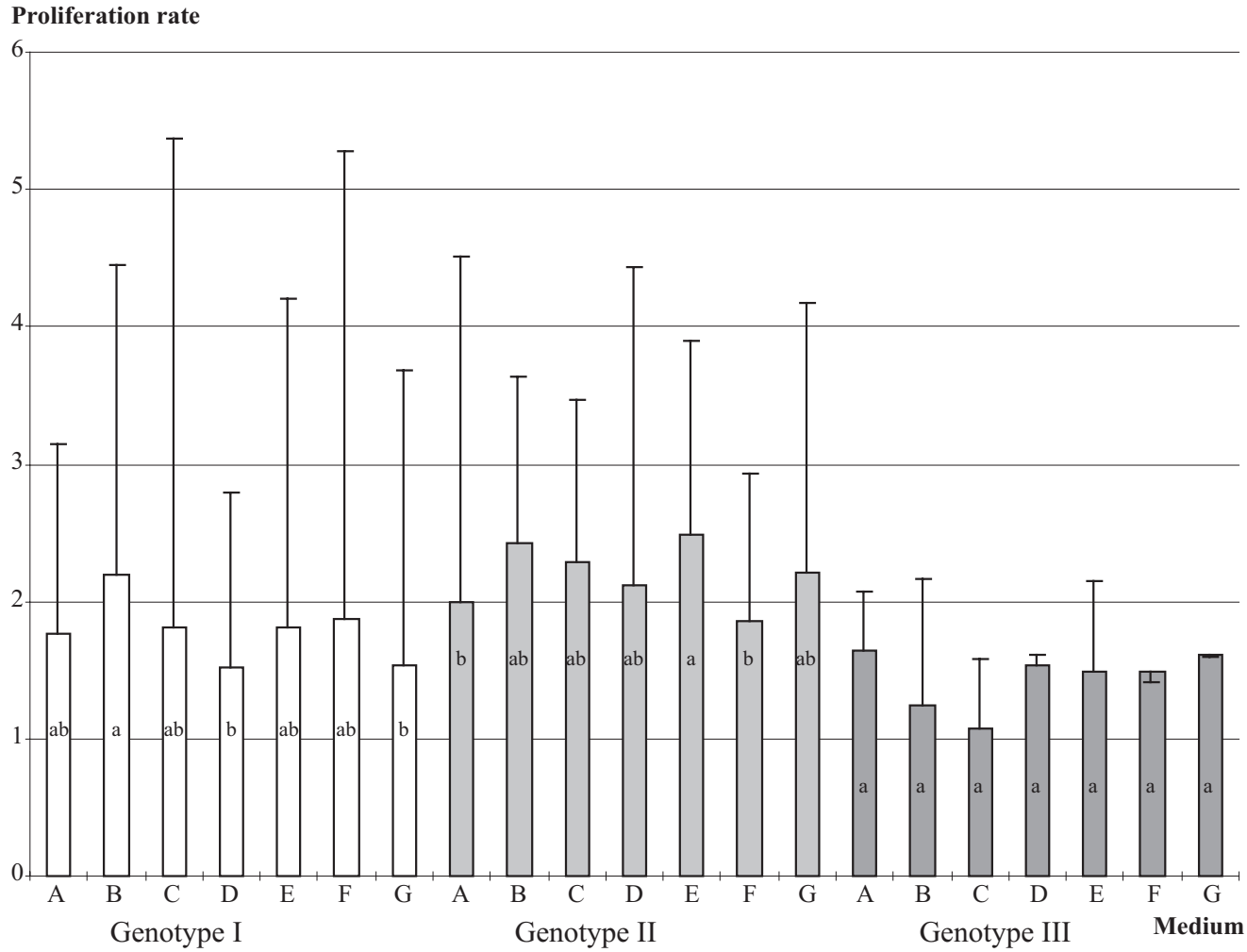

Figure 3. Proliferation rates for each Japanese quince (*C. japonica*) genotype (I–III) in different media (A–G). Columns indicate proliferation rate at 80±8 µmol/m²s whereas columns + bars indicate proliferation rate at 47±7 µmol/m²s. Reduction of light stimulated proliferation markedly. Different letters (a, b) indicate significant differences ($p < 0.05$) between averages (at 80±8 µmol/m²s) for each genotype respectively.

Results

Meristems from dormant buds were more contaminated than shoot tip explants. The growth of meristems more frequently resulted in rosettes compared to the growth of shoot tips. Thus, shoot tips were best for culture initiation.

The response to different proliferation media was strongly dependent on genotype. On average, the proliferation rate was 1.8, 2.2 and 1.4 for GI, GII and GIII, respectively. There were also interactions between genotype and medium (Figure 3). Proliferation rates increased under low irradiation levels to 4.1, 3.9 and 1.8 for GI, GII and GIII, respectively.

Direct planting of shoots resulted in poor rooting (10%) whereas various pretreatments improved rooting (Figure 4) and root length (Kauppinen 2001a). The combination of two weeks on a medium without growth regulators and 2 h soaking in 250 mg/l IBA resulted in the highest rooting for every genotype (GI: 60 %, GII: 88 %, GIII: 90 %).

Shoots stored dark and cold became very etiolated and weak but started to grow when transferred to new proliferation media. However, shoots put in faint supplemental lighting during storage kept their leaves green, remained vital during the storage period and immediately grew very well when transferred to new proliferation media.

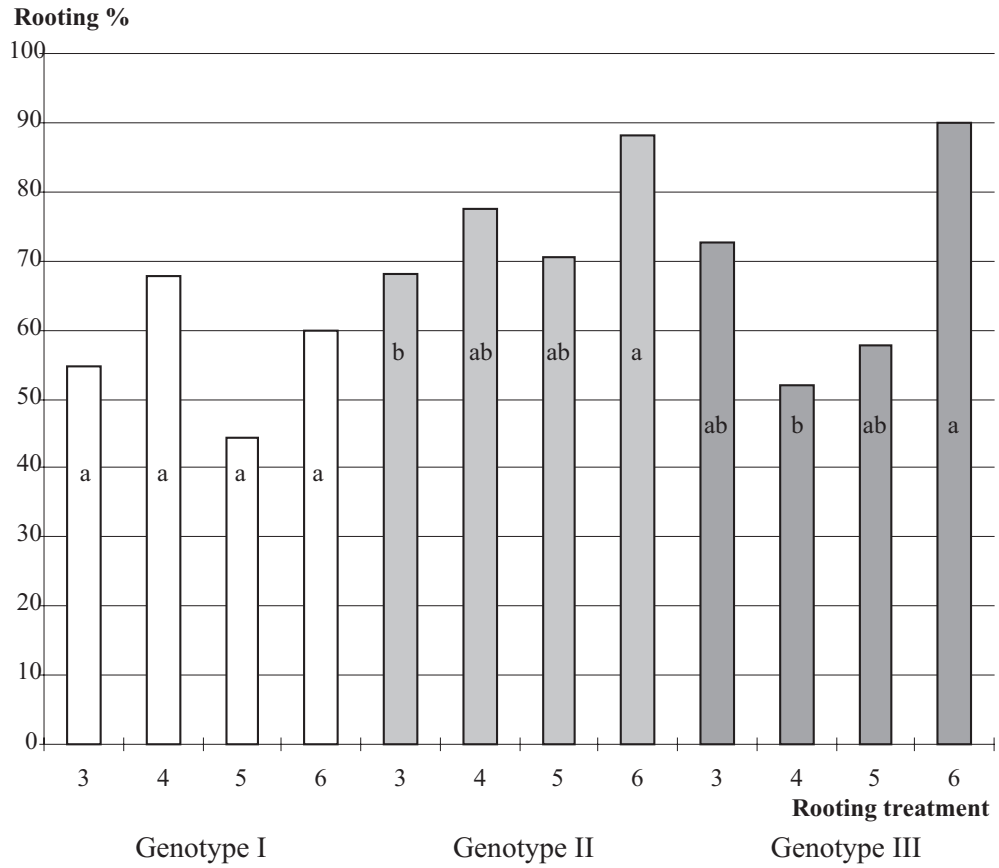

Figure 4. Rooting percentages for rooting treatments 3–6, for each Japanese quince (*C. japonica*) genotype (I–III) respectively. Rooting treatments 1–2 (immediate planting, see Table 3) resulted in less than 10% rooted microcuttings and were excluded from the figure. Different letters (a, b) indicate significant differences ($p < 0.05$) between treatments for each genotype respectively.

Discussion

Shoot tips were better explants for culture initiation than buds, both from a contamination and developmental viewpoint. Difficulties in initiating *in vitro* cultures from dormant buds have previously been noted for other Rosaceaous plants, *e.g.* apple rootstocks M 26 (Welander 1991) and Saskatoon berry (*Amelanchier alnifolia*) (Pruski *et al*. 1990). Actively growing shoot tips should therefore be the preferred explant for culture initiation *in vitro* of Japanese quince.

The level of light seemed to have a large impact on shoot proliferation. High levels suppressed growth in all treatments. When light was reduced from 80 to 47 μmol/m^{2}s, the proliferation rate increased on average from 1.8 to 3.2. For comparison, Norton & Norton (1986) achieved a very high proliferation rate of almost 20 by selecting only the best performing shoots for each subculture. They used a light level of 73 μmol/m^{2}s, although without providing details of how it was measured. Norton & Boe (1982) reported a proliferation rate of 7, using approximately 55 μmol/m^{2}s (4400 lux, converted according to George 1993), also without providing details of how it was measured. The very high proliferation rates reported in the literature compared to our results indicate a potential for further improvement of our procedure. The large differences between genotypes noticed also offer possibilities for improvement through selection of genotypes with high proliferation rates *in vitro*.

Rooting of Japanese quince *in vitro* has previously not been very successful. At most Panavas (1994) obtained 25% rooting when soaking shoots for 2 hours in 250 mg/l IBA and then growing shoots on auxin free medium. When the medium was supplemented with 1.0 mg/l IBA, only 10% of shoots formed roots.

In experiments reported by Norton & Boe (1982) only very high concentrations of IBA (10–20 mg/l) induced roots *in vitro*.

A period of shoot growth on a medium free of growth regulators following the proliferation phase improved rooting (Kauppinen 2001b). A very high rooting percentage (more than 90%) was obtained when shoots were first grown on a medium free of growth regulators and then soaked in 250 mg/l IBA for 2 hours.

During the two weeks of growth on medium without growth regulator, shoots became longer (Kauppinen 2001a). Maene & Debergh (1983) and Stanys (1996) found 1.5–2.5 cm to be an optimal length of microshoots for rooting.

Treatment with auxin and growth in the dark has previously been shown to improve rooting of apples (Welander 1983, Zimmerman 1984), but did not work for Japanese quince according to Norton & Boe (1982) and was therefore not tested in our study.

Shoot clumps of Japanese quince can be stored at +4 °C for several months. Faint supplemental lighting (approximately 10 µmol/m^{2}s) may prolong the storage time by several more months, thus saving costs in the laboratory.

COMMERCIAL MICROPROPAGATION

Materials and methods

Methods for micropropagation of Japanese quince were implemented and improved at the Foundation of the Swedish Horticultural Elite Plant Station for large-scale commercial propagation.

Plant material from advanced selections of Japanese quince was obtained from plant breeders in Sweden, Finland and Latvia.

For culture initiation, Murashige and Skoog (1962) macro- and microelements were reduced to 3/4 concentration according to Panavas (1993), and Fe was added as NaFeEDTA (15 mg/l). The medium was supplemented with myoinositol (100 mg/l), thiamine-HCl (0.1 mg/l), pyridoxine-HCl (0.5 mg/l), 6-benzylaminopurine (BA) (1.0 mg/l), sucrose (30 mg/l) and Difco Bacto Agar (6.5 mg/l). pH was adjusted to 5.8 with NaOH.

Medium for initiation of cultures was dispensed into test tubes with a total volume of 50 ml (10 ml per test tube) and sealed with aluminium foil. Medium for shoot proliferation and rooting was dispensed into jars with a total volume of 330 ml (80 ml per jar) and sealed with plastic caps (Figure 5). All constituents were added before autoclaving for 20 min at 120 °C. Cultures in all stages were incubated at 23±1 °C in 45 µmol/m^{2}s photosynthetic photon flux (PPF) provided by cool white fluorescent tubes, at a photoperiod of 16 hours per day. Cultures were subcultured every 21st day.

Cultures were initiated both from forced twigs and from shoots collected in the field. Twigs with resting buds were forced in the greenhouse (20 °C). Shoots were cut off and surface sterilized in 1.0% sodium hypochlorite solution supplemented with 0.05% Tween 20 for 10 min, and were then rinsed four times in sterile distilled water. Surface sterilization of rosette shoots followed the same procedure except that they were first dipped in ethanol (70%). From elongated shoots, leaves were removed and the shoots were cut into single node explants. From rosette shoots, the outer leaves were peeled off and the buds were used as explants.

For shoot production, the medium was adjusted to improve the overall performance of each genotype. To improve proliferation rate and shoot quality, BA concentration was optimised for each genotype (0.5–2.0 mg/l). To reduce hyperhydration, sucrose concentration was increased to 35 g/l(Panavas 1993) during proliferation. To improve growth of some genotypes, liquid medium (30 ml) was poured on the top of the agar medium.

For root induction, the bases of microshoots were soaked in a semi-liquid solution of 250 mg/l IBA, MS macronutrients and Difco Bacto agar (4 g/l) for 2 h, and the microshoots were then a) directly planted

in the greenhouse; or b) placed on a medium with half concentration MS macro- and microelements, without growth regulators, for 14 days.

For rooting, microshoots were inserted in Jiffy-7-pots (diameter 30 mm) with a volume of 24 cm^3. The substrate consisted of 90% low humified peat and 10% coconut fibre, and was watered with nematodes (*Steinernema feltiae,* $20x10^6/m^3$ substrate) against *Bradysia spp*. The pots were covered with white, non-transparent plastic and the relative humidity was kept at 95%. To prevent spread of fungi, a solution of 0.05% Rovral Aqua (Rhône-Poulenc, Agrochimie) was applied. Greenhouse temperature was kept at 22 °C and additional light was provided by high-pressure sodium lamps at 50 W/m^2, 18 hours per day. After one week, when 50% of the microshoots had rooted, the humidity was gradually reduced for the following 10 days. Twice a week the plants were watered with a weak nutrient solution (conductivity 1.5).

Rooting in the greenhouse was recorded after 4 weeks and the plants were then transplanted to pots (7x7 cm, Figure 5). The soil free substrate consisted of a mixture of 80% fertilized peat and 20% clay with an addition of 3 kg Osmocote per m^3 substrate, and nematodes were added as previously. Temperature was reduced to 16–18 °C, with additional light as during establishment. The daily care consisted of watering and control of pathogens. The plants were sprayed against grey mould, *Botrytis*, with 0.1% Rovral Aqua and the tobacco leaf aphid, *Myzus nicotinae*, was combated with ichneumon flies, *Aphidus colemanii*.

Results and Discussion

When twigs were forced, only a few buds developed into long shoots. Some genotypes did not produce any elongated shoots at all and rosette shoots had to be used. Explants from elongated shoots were established *in vitro* at a high frequency, whereas rosette explants often had to be discarded due to infections. The best sources of explants were new shoots picked from plants in the field. To further improve quality of explants, it would probably be better to use potted plants, if available, for forcing of twigs in the greenhouse.

Of the 31 genotypes studied, 28 were successfully established *in vitro*. Growth of three genotypes declined and the shoots died after several months *in vitro*. The established genotypes showed a large variation in development during the establishment period. Some genotypes obtained a multiplication rate of 2–3 after only 3–4 subcultures, whereas other genotypes still only obtained a multiplication rate of around 1 after 8 subcultures.

Figure 5. Proliferation of Japanese quince (*C. japonica*) microshoots took place in glas jars with a total volume of 330 ml. Following rooting in Jiffy-7 pots (30 mm) plants were grown in 70 x 70 mm pots.

Table 4. Rooting (%) of microshoots of 10 advanced selections of Japanese quince (*C. japonica*). Treatment A: Root induction in a semi-liquid MS medium supplemented with 250 mg/l IBA and 4 g/l agar for two hours, and then immediate planting. Treatment B: Root induction as in treatment A, followed by subsequent growth on 1/2 MS macro- and microelements for 14 days before planting.

Genotype	Origin	Rooting (%) Treatment A	Rooting (%) Treatment B
NV1834	Sweden	71	95
NV18136	Sweden	86	100
NV19100	Sweden	82	98
NV19143	Sweden	81	93
F93012	Finland	79	89
F93014	Finland	68	99
F93016	Finland	67	85
F93018	Finland	49	89
F93019	Finland	43	46
F93021	Finland	88	96

Shoots grown on a hormone free medium *in vitro* following a soak in IBA always rooted better (on average 89%) compared to shoots directly planted after root induction (on average 71%) (Table 4). Although rooting percentage was reduced at direct planting, this method should still be tested in large-scale propagation. Rooting percentage is in general high and propagation cost is reduced when the shoot elongation phase is omitted. In certain plant species, direct planting has been found to have a positive effect root quality and therefore on overall plant quality.

All advanced selections propagated were scored according to ease of establishment *in vitro*, shoot production and rooting, to obtain an indicative measure of their suitability for micropropagation (Table 5). It was concluded that there is a large difference among genotypes in capacity for propagation *in vitro*. Capacity for micropropagation is therefore another important character to consider during final selection and before release of varieties. If a valuable genotype is difficult to propagate by conventional propagation methods, there may still be a possibility to further improve performance *in vitro*.

In total, about 10 000 micropropagated plants have been produced at the Foundation of the Swedish Horticultural Elite Plant Station, of which about 3 000 have been delivered for further evaluation to various field trials in Finland, Latvia, Lithuania and Sweden. The procedure used for commercial micropropagation of Japanese quince is summarised in Table 6.

Table 5. Estimation of performance of 39 advanced selections of Japanese quince (*C. japonica*) obtained from Sweden, Finland and Latvia. Each genotype was scored using the values 0, 1 and 2, where the highest score was given to a genotype that was easy to establish *in vitro*, had a high multiplication rate and was easy to root. It was noted that 24 of 39 propagated genotypes performed very well *in vitro* and was considered easy to micropropagate.

Origin	Score (*n*)[1] 0	1	2	Total (*n*)
Sweden	5	6	10	21
Finland	0	2	11	13
Latvia	1	1	3	5
Total (*n*)	6	9	24	39

[1]*n*=number of genotypes in each group

Table 6. Protocol for commercial micropropagation of Japanese quince. MS=Murashige and Skoog (1962) supplemented with 100 mg/l myoinositol, 0.1 mg/l thiamine-HCl, 0.5 mg/l pyridoxine-HCl and Fe added as 15 mg/l NaFeEDTA, pH 5.8. Temperature: 23±1 °C. Light: 45 µmol/m^{2}s PAR provided by cool white fluorescent tubes, at a photoperiod of 16 hours per day.

Phase	Medium	Period
Culture initiation	3/4 MS, 1.0 mg/l BA, 30 g/l sucrose, 6.5 g/l agar	subculturing every 3 weeks
Shoot proliferation	3/4 MS, 0.5–2.0 mg/l BA, 35 g/l sucrose, 6.5 g/l agar	subculturing every 3 weeks
Root induction	1 MS, 250 mg/l IBA, 4 g/l agar	2 hours
Shoot elongation	1/2 MS, 35 g/l sucrose, 6.5 g/l agar	14 days

CONCLUSION

Chaenomeles plants are rather difficult to propagate vegetatively by conventional methods. However, it may be possible to set up an efficient system for commercial propagation of Japanese quince based on softwood cuttings. For this purpose, the present study showed that long top cuttings should be used and a growth regulator applied. A high rooting percentage was obtained using 20 cm long top cuttings, with the base of the cutting soaked in 30 mg/l IBA for 18 hours.

Propagation by hardwood cuttings was difficult due to a high frequency of root rot and varying rooting percentages between years. Application of a growth regulator was shown to have a positive effect. A high rooting percentage was obtained using 20 cm long base cuttings soaked in 100 mg/l IBA for 24 hours. For both softwood and hardwood cuttings, IBA concentrations should be carefully adjusted to each genotype, to obtain maximum rooting percentage.

Micropropagation proved to be very efficient, with an average proliferation rate of 3–4 and a rooting percentage of over 90%. For shoot proliferation, a medium consisting of complete MS medium with macronutrients reduced to 3/4, iron added as NaFeEDTA, 35 g/l sucrose and 1.0 g/l BA proved most useful. For rooting, two alternative treatments seemed equally successful: The proliferated shoots were grown for two weeks in 3/4 MS medium without growth regulators and then soaked in 250 mg/l IBA for 2 hours before planting. Alternatively, the proliferated shoots were first soaked in 250 mg/l IBA and then grown for two weeks in 1/2 MS medium without growth regulators before planting.

The ability to form roots was genotype dependent and propagation capacity should therefore be evaluated during plant breeding and selection.

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