Using In Vitro Propagation to Rejuvenate Difficult-to-Root Woody Plants

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INTRODUCTION

Juvenility of stockplants is one of the most important factors affecting rooting success in cutting propagation. Especially in difficult-to-root woody plant species, the ease of adventitious root formation declines with the age of the stockplant, resulting in a propagation problem. In that context, in vitro propagation has been used to overcome this problem by producing stock plants with juvenile-like characteristics (Hartmann et al., 1990).

At Humboldt University, various studies have been carried out during the last 10 years on in vitro propagation and the further growth and development of woody plants propagated by this technique. Initially the aims included methods and procedures for in vitro propagation, studies of physiological reactions of different genotypes in vitro and the development of a suitable transfer and acclimatisation system. Later, the use of the apparently changed juvenility status of in vitro-propagated plant species to improve cutting propagation has been investigated.

It is well known that most in vitro-propagated woody plants have a better potential for adventitious root formation (Hackett, 1988; Howard et al., 1988; Howard et al., 1989; Franclet, 1991). During in vitro culture the microcuttings obviously go through a rejuvenation process and this could be of great importance in the production of stock plants to provide cuttings for the propagation of difficult-to-root plant species. Cuttings of several deciduous shrubs and trees from in vitro and conventionally propagated stock plants have been compared for their rooting potential in Berlin (Plietzsch, 1996). Further work with these species has shown that in-vitro-propagated plants had some further differences from conventionally propagated plants. The apparent rejuvenation effect caused a more vigorous growth, increased branching at basal parts, and some more varied morphological characteristics during the first seasons after in vitro propagation. To obtain more detailed information about the value of such plants for garden and landscape use, a supplementary long-term evaluation with *Prunus* from different propagation origins was established in 1994.

PROCEDURE FOR ACCLIMATISATION AND TRANSFER OF MICROCUTTINGS

The following difficult-to-root plants have been successfully propagated in vitro. Currently most of them are still propagated by grafting in German nurseries: *Amelanchier ×grandiflora* 'Ballerina' (syn. *A. lamarckii* 'Ballerina'), *Hamamelis ×intermedia* 'Feuerzauber' (syn. 'Magic Fire'), *P. cerasifera* 'Nigra', *P.* 'Kanzan', *P. tenella* 'Fire Hill', *P. triloba*, *Sorbus* hybrids (*S. aria* × *S. aucuparia*) from Hungary, *S. ×thuringiaca* 'Fastigiata', *Syringa vulgaris* cultivars, and *Tilia cordata* cultivars.

The most difficult stages in micropropagation were acclimatisation after in vitro rooting and transfer to horticultural substrates. Thus it was necessary to develop an effective and comprehensive transfer system for weaning of microcuttings. Currently the rooted and unrooted microcuttings are weaned under fog then transferred to open ground beds sheltered by a low polythene tunnel. A period from the end of April to mid June has proved to be the best time for weaning in this way. The most suitable plant size is 4 to 6 cm. Removing the shelter in August, the plants are hardened off under shade and can be overwintered in open ground or harvested for transplanting. During that period the plants grow to very uniform quality—and reach a size of about 80 to 100 cm in *S. vulgaris* cultivars and about 60 to 80 cm in *P.* 'Kanzan' (Jacob et al., 1991). In contrast to the small potted plants leaving some commercial micropropagation laboratories, such a plant quality is most suitable for further field production in the nurseries.

TRIALS ON REJUVENATION EFFECTS IN ADVENTITIOUS ROOTING

Use of plants propagated in vitro as stockplants has often been reported to promote adventitious rooting in cuttings (Hartmann et al., 1990). Similar results were observed for various difficult-to-root species in our trials.

Hardwood Cuttings. Winter hardwood cuttings of *S. vulgaris* cultivars and *P.* 'Kanzan' from stockplants of different propagation origins and with different ages were compared. The best rooting performance was obtained from the youngest in-vitro-propagated stockplants (Table 1).

Table 1. Rooting percent of *Syringa vulgaris* (1993) and *Prunus* 'Kanzan' (1994) using hardwood cuttings from different stockplants.

Species and stock plant source (with propagation date)	Rooting percentage Without hormone	Rooting percentage With 1% IBA
Syringa vulgaris		
Conventional stockplant (1992)	0	10
In vitro stockplant (1990)	5	15
In vitro stockplant (1991)	30	35
In vitro stockplant (1992)	85	80
Prunus 'Kanzan'		
Conventional stockplant (1992)	0	0
In vitro stockplant (1990)	0	2.5
In vitro stockplant (1993)	80	90

In 1996 further *S. vulgaris* cultivars gave similar good results using hardwood cuttings from 1-year-old, in-vitro-propagated stockplants (e.g., 'Katherine Havemeyer', 76%; 'Madame Lemoine', 86%; and 'Madame Stepman', 72%).

From these results it appears that successful rooting of difficult-to-root woody plants by hardwood cuttings may be accomplished using 1-year-old, in-vitro-propagated stockplants but that the usefulness of such plants wears off very quickly. The influence of juvenility proved a much stronger effect than applications of rooting hormones.

Softwood Cuttings. Further investigations covered the rooting performance of softwood cuttings from different stockplants. For this trial, stockplants were propagated in vitro or by grafting and were pruned back annually in winter. Cuttings from in vitro stockplants often showed an increased rooting rate. In addition, significant differences could also be demonstrated in rooting quality (number of roots, root fresh weight) and in subsequent growth compared with cuttings from conventional stock plants (see Tables 2 and 3). Rooting hormones were not used in these trials.

Table 2. Rooting percentage and shoot growth rate of *Syringa vulgaris* 'Katherine Havemeyer' softwood cuttings (1994) from stockplants of different origins.

Stockplant source (with propagation date)	Rooting (%)	Shoot growth rate (%)
Conventional (1989)	83	3
In vitro (1990)	95	0
In vitro (1991)	93	8
In vitro (1992)	98	46
In vitro (1993)	100	88

Table 3. Rooting percent and rooting quality of *Amelanchier* ×*grandiflora* 'Ballerina' softwood cuttings (1997) from stockplants of different origins.

Stockplant source (with propagation date)	Rooting (%)	Roots (number)	Root fresh weight (g)
Conventional (1990)	27.1	2	0.3
In vitro (1996)	93.8	5	1.4

Rooting Potential and Stress. A further trial was designed to provide information about the interaction between stress tolerance and rooting potential of softwood cuttings from stockplants of different propagation origins. Unrooted softwood cuttings of *P.* 'Accolade' were stored in plastic bags at 5C before inserting them in the propagation medium (without using rooting hormone). The duration of storage was varied from 0 to 16 days. Four weeks after sticking the cuttings were evaluated for rooting success.

Rooting quality was strongly correlated with duration of storage (Table 4). Even after 16 days of storage all cuttings showed 100% rooting. But cuttings from in-vitro-propagated stockplants showed a significantly better rooting quality (measured in terms of number of roots) compared with cuttings from conventional stock plants (Plietzsch, 1997).

Table 4. Influence of storage stress, to unrooted cuttings of *Prunus* 'Accolade' (1995) from different sources, on rooting success.

Storage duration	Cuttings from in vitro stockplants (1993)		Cuttings from conventional stockplants (1989)	
(days)	Rooting (%)	Root number	Rooting (%)	Root number
0	100	10	100	8
2	100	9	100	7
4	100	8	100	5
8	100	8	96	5
16	100	6	100	4

Softwood cuttings from juvenile in vitro stockplants produced a better rooting quality (increased number of roots and increased fresh weight of roots — data not shown) and even after the stress of short-time storage they maintained their rooting advantage.

These findings suggest that cuttings from in-vitro-propagated stockplants should mainly be used for difficult-to-root woody species or for less—difficult species likely to be propagated under unfavourable conditions. Further research is dealing with overwintering problems of rooted cuttings in relation to their juvenility status.

LONG-TERM EVALUATION OF IN-VITRO-PROPAGATED WOODY PLANTS

Apart from adventitious rooting the apparent rejuvenation effect also had a clear influence on morphological growth characteristics of in-vitro-propagated plants. In order to study this, a long-term evaluation has been established since 1994 using *P. glandulosa* 'Alba Plena' and *P. nipponica* var. *kurilensis* 'Brillant' from different propagation origins with 40 plants per treatment. First results after a period of 3 years suggest that significant differences exist between plants of the same genotype depending upon propagation method (Tables 5 to 7).

Table 5. Comparison of plant volume growth (m^3) of *Prunus nipponica* var. *kurilensis* 'Brillant' propagated in 1992 by different propagation methods.

Source	1994	1995	1996	1997
In vitro propagated plants	0.18a*	0.69a	1.65a	1.96a
Cuttings from in vitro stockplants	0.04b	0.22b	0.95c	1.12b
Cuttings from conventional stockplants	0.03b	0.33b	1.28b	1.41b
Grafted plants	0.06b	0.24b	0.77c	1.08b

*Within columns, results with the same letter are not significantly different.

Table 6. Number of flowers per plant and shoot length (cm) in 1997 of *Prunus nipponica* var. *kurilensis* 'Brillant' propagated in 1992 from different sources (measured in 1997).

Source	Flowers (number)	Shoot length	Flowers per cm
In-vitro-propagated plants	12,440	3836	3.2
Cuttings from in vitro stockplants	8869	2824	3.1
Cuttings from conventional stockplants	8585	2904	3.0
Grafted plants	6683	2169	3.1

Table 7. Area of shrub base (cm^2) and sucker formation of *Prunus glandulosa* 'Alba Plena' propagated in 1992 from different sources (measured in 1997).

		Sucker formation	
Source	Base area (cm^2)	Plants with (%)	Suckers (number)
In-vitro-propagated plants	693a*	60a	3.5a
Cuttings from in vitro stockplants	446b	20b	1.5b
Cuttings from conventional stockplants	429b	17b	1.4b

*Within columns, results with the same letter are not significantly different.

In vitro-propagated plants showed particularly significant advantages in terms of morphological growth characteristics such as plant size and volume, shoot length, and sucker formation. These plants had a more vigorous growth than conventionally propagated plants, even in comparison with those grown from cuttings obtained from in vitro stockplants. No differences were found in size of flowers or flower density, growing and flowering periods, susceptibility to pests and diseases, and frost hardiness.

CONCLUSIONS

Young in vitro-propagated plants have a changed juvenility status. That effect may be used to improve the results of commercial cutting propagation in certain circumstances. Using in vitro stockplants as the cuttings source improves adventitious rooting in softwood and hardwood cuttings of many difficult-to-root woody species. Combined with pruning and hedging of stockplants, the rejuvenation effect continued in softwood cuttings for several years. For hardwood cuttings of difficult-to-root species the rejuvenation affect is apparent only in cuttings from 1-year-old in vitro stockplants.

Generally, it is assumed that the observed differences between plant propagation origins had been caused by a different juvenility status. However, there is a trend suggesting that early differences will decrease with increasing age of the plants and this will be investigated during future research.

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