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In Vitro Propagation of Salix caprea L. by Single Node Explants

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Summary

Salix caprea was propagated *in vitro* from single node explants of field-grown plants. Two media were used for the induction of shoot growth: SCHENCK-HILDEBRANDT'S and AHUJA'S medium. The newly formed shoots were rooted on 1/50 strength AHUJA medium. Plantlets were acclimatized to soil and greenhouse conditions under waterlogging conditions. In the greenhouse the plants grew to a height of 1 m within 5 months.

Key words: Salix caprea, goat willow, micropropagation, tissue culture.

Introduction

The techniques of micropropagation may promote breeding programs for difficult-to-root forest species by facilitating clonal propagation and plant selection. Several reviews on micropropagation of woody plants are available (BONGA, 1982; BROWN and SOMMER, 1982), however publications on the genus *Salix* are very infrequent (READ et al., 1989; GARTON et al., 1983), possibly because of the ease by which most willow species can be propagated from cuttings. However, the pioneer shrub and excellent pollen donor, *Salix caprea* L., cannot be propagated by hardwood cuttings (NEUMANN, 1981; CHEMLAR and MEUSEL, 1986) and propagation by green softwood cuttings under mist is subject to considerable seasonal variations (LATTKE, 1965). This study was initiated to develop an *in vitro* method for an year-round propagation.

Material and Methods

Plant source

Young shoots ($\leq$ 4 mm diameter) were harvested throughout the year from 9 individual plants of *Salix caprea* L. growing along roadsides in Nußloch/Baden. They were defoliated and cut into segments of $\leq$ 6 cm length. Segments with large catkin buds were discarded.

Shoot formation on explants

Shoot segments were washed with running tap water for 15 min and, generally, surface sterilized for 30 min by stirring in solutions of 1% to 10% NaOCl with some drops of Tween 20 as a surfactant. Subsequently the segments were washed with sterile water (3 x 10 min). Under a laminar flow cabinet shoot segments were cut into single node explants (a node with an internode segment of 4 mm length at its base) and placed in test tubes (2.5 cm diameter) with 20 ml solidified nutrient medium, leaving the buds above the medium. The tubes were closed with Caputs (Bellco R) and incubated under culture conditions: 25° C, 12:12 hours light-dark regime with 2000 Lux fluorescent white light (Sylvania cool white, GTE).

Two media with some variations were used:

1. SH medium (SCHENCK and HILDEBRANDT, 1972) consisting of SH salts and vitamin mixture (Sigma Chemicals, Deisenhofen) supplemented with 20 g/L sucrose, 2.5 g/L gelrite and varying amounts of benzylamino purine (BAP) or kinetin; before autoclaving pH was adjusted to 5.7.

2. ACM medium consisting of the macro- and microelements, vitamins and organic compounds as described by AHUJA (1983) supplemented with 20 g/l sucrose, 2.5 g/l gelrite and varying amounts of BAP or kinetin; before autoclaving pH was adjusted to 5.5 to 5.6.

Rooting

After buds had grown into young shoots *in vitro* for 4 weeks to 5 weeks the shoots were cut off the original explants, transferred into new test tubes (2.5 cm diameter)

whith 20 ml rooting medium and kept under culture conditions as decribed earlier.

For rooting 2 different media were used:

1. ACM/50: the macroelements (without Fe) of ACM were diluted 50-fold and solidified with 8 g/l Difco Bitek^R agar; pH 5.7.
2. ACM/50 as before but supplemented with 0.1 mg/l napthaleneacetic acid (NAA).

Acclimatization

After shoots had produced a single root of about 3 cm length or several shorter roots they were planted into clay pots (7 cm diameter) with autoclaved soil (300 L 'Fruhstorfer' soil, 200 L peat, 100 L sand, supplemented with 500 g fertilizer Hakaphos Green^R). Twenty-four pots each were placed into a plastic tray with water (1 cm to 2 cm deep; water-logging conditions), covered by a transparent plastic hood and incubated in the green-house at an average temperature of 24^0 C to 26^0 C and a long-day regime. During September to April the daily light period was extended to 16 hours by a mixture of fluorescent and incandescent light (Osram HMLS, 500W) in a distance of 110 cm to 150 cm above the plant tops.

After 3 days to 4 days the plastic hood was lifted partially and after a week it was removed completely. After 8 weeks under water logging conditions the plants were transplanted into bigger pots and stored on the greenhouse shelf.

Results

The most serious problem during the micropropagation of *Salix caprea* was the contamination of the explants by microorganisms, mainly fungi. Varying unpredictably with explant source (clone and habitat) and with the season explants treated with a constant concentration of NaOCl (1%, $\geq$ 20 min) were contaminated at 6% to 69%. Increasing the NaOCl concentration up to 10% or addition of a treatment with ethanol (70%; 1 min) did not eliminate this variation or constantly reduce the degree of contamination. In the following paragraphs only explants without contamination during the 4-week culture period of shoot development were considered.

1. Bud growth

Results from all clones used are summarized here. On the hormone-free media SH and ACM an average of 35.6% (SH) or 36.4% (ACM) of the explant buds became activated within 1 week (*Table 1*). During the following 3 weeks a bud grew to a length of 20.1 mm $\pm$ 10.5 mm (SH) or 23.3 mm $\pm$ 13.0 mm (ACM). The production of 2 shoots from 1 explant was a rare exception (1.5% or less).

The composition of the SH medium was varied in different ways: omitting sucrose, omitting sucrose and vitamins or replacing the macroelements with a fertilizer mixture (Mairol^R) resulted in negative effects on shoot production, shoot elongation and subsequent rooting. Since in many micropropagation experiments the shoot yield can be increased by the addition of cytokinins to the media (George and Sherrington, 1984) BAP (0.05 mg/l to 5 mg/l) or kinetin (0.05 mg/l to 20 mg/l) were added to the media SH and ACM. No significant positive effect on shoot formation was observed.

On the basal media SH and ACM the variability of the shoot elongation was related to the use of different clones (proportion of the shoots long enough to be transfered to rooting medium after 4 weeks) (*Table 2*).

2. Rooting

A total of 761 shoots from all clones and media were

Table 1. — Effect of different media on micropropagation.

Medium	No of explants	% shoots ready for rooting	% shoots rooted without NAA	% shoots rooted with NAA	% plants potted
SH	174	35.6	24.7	2.3	14.4
ACM	214	36.4	30.4	0.5	15.9

Results combined from experiments with 9 clones.

Table 2. — Micropropagation of 9 different clones.

Clone No.	No. of Explants	% shoots ready for rooting	% shoots rooted without NAA	% shoots rooted with NAA	% plants acclimatized
1	77	64.9	51.9	13.0	18.2.
2	42	61.9	50.0	11.9	7.1
3	29	34.5	31.0	3.4	17.2
4	44	29.5	18.2	11.4	13.6
5	35	14.3	5.7	8.6	2.9
6	17	0	0	0	0
7	66	42.4	42.4	0	34.8
8	68	30.9	19.1	11.8	10.3
9	21	14.3	14.3	0	4.8

Table 3. — Acclimatization in different light regimes.

Treatment	SD	LD
No. of plants potted	157	358
% plants after 8 weeks	31.8	94.4

Results combined from experiments with 9 clones.
SD = short day, LD = long day.

Table 4. — The optimal micropropagation protocol Clone No. 7 (3% to 5% NaOCL, 30 min) hormone-free SH medium, rooting on AMC/50, acclimatization under a 16-hour light regime (the 4 best experiments).

Expt. No.	No. of explants	% shoots ready for rooting	% shoots rooted without NAA	% shoots rooted with NAA	% plants potted
39	38	92.1	92.1	0	89.5
40	34	91.2	91.2	0	88.2
41	36	80.6	77.8	2.8	80.6
42	38	89.5	73.7	15.8	86.8

transferred to NAA-free rooting medium. Here formation of new roots began spontaneously 2.1 (average value) weeks after transfer. Plants (19.6%) not rooted within 4 weeks were transferred to the NAA-containing medium for another 4 weeks and, here, 32.2% of these plants responded with root formation. Within 4 weeks shoots had produced an average of 3.8 ± 2.5 roots and the average lenght of the longest root of the explants was 35.7 mm ± 16.8 mm. Again the proportion of rooted explants varied with the clone used (*Table 2*). Of 761 shoots transferred to the rooting media 515 (= 67.7%) rooted and grew uncontaminated to be ready for transfer to potting soil.

3. Acclimatization

Eight to 10 weeks after explantation plantlets were transferred to soil and acclimatized to greenhouse conditions. A very critical factor of acclimatization was the light-dark regime. In long-day (at least 14 hours daily light period) the survival of plants after 8 weeks is much improved over plants in natural short-day (*Table 3*). In the greenhouse the potted plants grew well and attained a height of about 1 m within 5 months.

Again the efficiency of this final step of micropropagation is dependent on the plant clone (*Table 2*).

Discussion

Using the procedure shown several clones of *Salix caprea* could be propagated throughout the year with differential success. The most critical step was the surface sterilization of the explants. Even after heavy sterilization treatments bacteria and — more often — fungi survived in the angles between shoot axis and buds and in the cork warts. The extent of this contamination varied with habitat, season and weather. This problem may finally be overcome by using greenhouse plants as stock plants.

As with other willows shown before (GARTON et al., 1983) the potential for *in vitro* propagation differed considerably with the clone used: some clones yielded weak and yellow-green shoots which rooted slowly; others looked vital, dark-green and rooted rapidly. The reasons for this variation remain obscure.

In contrast to micropropagation procedures of many tree species the addition of cytokinins did not increase shoot number or shoot development which is in agreement with a result on the clone L79-10 of *S. caprea* (BERGMAN et al., 1985).

Fortunately the acclimatization of *in vitro* grown plants to greenhouse conditions was accomplished within 1 week without any problems, and even the addition of non-sterilized soil did not cause visible damage to the young plants. Considering the efficiency of the sterilization method, the percentage of explants forming shoots and roots and the acclimatization process the field clone No. 7 was best suited for the *in vitro* propagation procedure proposed here (*Table 4*).

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