Yellow-cedar *in vitro* Clonal Production and Evaluation of Propagules for Reforestation

**INTRODUCTION**

Existing methods of propagation for the operational planting of yellow-cedar include sexual propagation and vegetative clonal propagation. Because the characteristically low rate of cone production and seed germination in yellow-cedar makes sexual propagation inefficient, there is a move towards using asexual methods to meet operational supply needs. The three vegetative methods are rooted cuttings, organogenic micropropagation (*in vitro*) and somatic embryogenesis (*in vitro*). Of these three methods, only rooted cuttings have been used successfully on an operational scale. The *in vitro* methods are still experimental and are undergoing continued research.

This project was part of an ongoing effort to develop micropropagation techniques on an operational scale and to investigate their possible use in producing genetically improved stock for planting.

There were three main objectives:

1. to develop a commercially viable process that uses organogenic micropropagation to produce yellow-cedar stock for operational reforestation;
2. to evaluate these techniques for developing genetically improved clones; and
3. to establish demonstration plots of micropropagules.

**METHODS**

Organogenic micropropagation involves excising tissue from a source plant, subjecting the material to hormonal treatment to induce shoot production, and transplanting and rooting the produced shoots to grow into propagules that can be used for reforestation.

In July 1986, basal juvenile shoots (explants) were collected from hedged 1- to 4-year-old seedlings of yellow-cedar belonging to four different seedlots. The 25 clones used as source stocks were obtained from the Ministry of Forests' Cowichan Lake Research Station.

The collected shoot tips were 5–7 cm long, with juvenile needle morphology. Shoot tips were surface sterilized, trimmed to size, and cultured in modified Murashige and Skoog (MS) media to induce microshoot multiplication. (For details of these procedures, see Ling and Clay [1989]).

A rootable microshoot is an *in vitro* produced shoot that is more than 1.5 cm long and has a green and actively growing tip. The average number of rootable microshoots produced in 3 months per explant inoculated is termed the multiplication rate.

Five thousand microshoots were harvested and rooted in the spring of 1988, in 313A Capilano forest seedling containers. The rooted microshoots were then grown in the greenhouse according to standard procedures recommended by Ministry of Forests' nurseries at Cowichan Lake and Surrey.

**RESULTS AND DISCUSSION**

Of the 5000 microshoots transferred to soil, only about 1000 survived and were successfully planted in September 1989 as 313A 1-0 propagules. The heavy loss was disappointing but not entirely surprising, given the lack of technical information in this still very experimental area. In spite of the poor survival rate, and in some cases because of it, much valuable information on the survival needs of micropropagules was obtained.

Of primary importance to successful micropropagation is the use of explants taken from vigorous, healthy, clean source plants. This can only be achieved in a greenhouse environment with optimal lighting, ventilation and fertilization. Overhead watering of source plants should be avoided and irrigation water must be free of major pathogenic fungi.

Gradual acclimatization of the microshoots as they adjust to their new, aseptic environment is essential. Initial environmental variables such as sunlight intensity, relative humidity and temperature must be adjusted to allow acclimatization to take place over a period of 4–6 weeks.

During the first 2 weeks, a reduction of sunlight intensity to between 2 and 5% is recommended, with the use of layers of shade cloth. High relative humidity is necessary and can be maintained with a mist tent. Ambient temperature must be kept below 30 °C, since exposure to temperatures at or above that value even for a few hours can kill the shoots during the first month after their transfer to a soil medium.

Adequate ventilation inside the mist chamber or tent where microshoots are being rooted is also important. Microshoots should be treated with Benylate®, Captan or Rotar® to control Botrytis. Affected microshoots must be removed and discarded immediately to protect against spread of the disease.

Yellow-cedar microcuttings are also susceptible to fungal infestation. To achieve control, liquid diazinon drench should be applied, followed by a flushing of the soil a few hours later to prevent damage to microshoots.
Improvements in micropropagation techniques have been ongoing as information and results become available. Research is currently focusing on increasing the \textit{in vitro} shoot multiplication rate from the initial 4 achieved in this project, to between 10 and 15 every 3 months. A combined genetic and cultural approach is being taken, directed at both the source plant and the explant level.

Source plants are chosen from a group of individuals that have already been selected for phenotypic superiority in economically important traits. Those chosen will also exhibit a potential for high multiplication rates in culture. From these source plants will be grown the optimal source plants for micropropagation.

At the explant level, research is being directed at improving cultural techniques to maximize shoot multiplication and elongation and minimize tissue browning and early senescence. With this approach, a multiplication rate of 15 has already been achieved in a few selected clones.

**DEMONSTRATION PLOTS**

Three demonstration plots of yellow-cedar micropropagules were established in this project.

**Site 1**

This is a small plot where the first group of 16 micropropagules produced in the project are planted. These propagules were planted with four comparable rooted cuttings at Clay's nursery, H.H. 9, 3666–224th Street, Langley, B.C., V3A 6H5 in the fall of 1987. Two propagules have since died from an abiotic cause, while the others have shown good growth and survival.

**Site 2**

The second demonstration plot is located in CANFOR's Harrison Operation in Mission just off Highway No. 7, 1 km north on the Norrish Creek mainline (lat. 40° 11', longitude 122° 10', elev. 20 m). It consists of 50 one-year-old yellow-cedar micropropagules produced from a single clone (9777-2-3) and 50 seedlings from the same seedlot. Propagules of the two stocktypes were planted in alternate rows of 10, with rows running down the slope on a south-facing aspect of an open plot in the Dewdney lot.

Initial height measurements were taken of all the propagules at the time of planting in October 1988. Height was measured again for each propagule 21 months later in June 1990. Observation at that time found all 100 trees alive and growing well, with no sustained plagiotropism (horizontal lidergrowth). The planting site is within the CWHa2 biogeoclimatic zone.

**Site 3**

The third site, planted in September 1989, consists of 800 313a 1-0 micropropagules, 1100 rooted cuttings, and 120 seedlings planted in adjacent plots at the West Fork of Norrish Creek (latitude 49° 18', longitude 122° 9'). The planting site lies at an elevation of 750 m. within the CWHb2 biogeoclimatic zone. Observations in June 1990 showed almost 100% survival. This demonstration area provides a useful opportunity for comparing the three stocktypes side by side.

**CONCLUSIONS**

The results and observations in this project suggest that organogenic micropropagation may have the following advantages over rooted cuttings:

1. the potential to produce a higher rate of propagules than rooted cuttings; and
2. the production of propagules with a greater number of juvenile growth characteristics. Propagules grown from this method exhibited a basal portion of juvenile growth with mature growth at the top. They were completely free from plagiotropism and had strong root systems and good form.

Disadvantages may include the need for:

1. laborious and expensive aseptic handling;
2. strict acclimatization; and
3. a longer time period to achieve planting size from rooted microshoots.

Further research and development are needed before organogenic micropropagation can be incorporated into operational tree improvement. Nevertheless, this method has the potential to provide an alternative to rooted cuttings.

**REFERENCES**


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