Ann. Sci. For. (1989) 46 suppl., 152s-154s
Forest Tree Physiology, E. Dreyer *et al.*, eds.

In vitro propagation of several *Betula* species representing various ploidy levels

E. Särkilahti

Department of Biology, University of Turku, SF-20500 Turku, Finland

Introduction

One of the most important applications of *in vitro* culture is micropropagation, which enables vegetative propagation of adult trees, not usually achieved with traditional methods. Genotypic variability in response to successful *in vitro* propagation within a species has been a difficulty in finding universal propagation methods for some species. This paper introduces the establishment of sterile shoot culture and micropropagation of 4 different mature birch species having tetraploid and pentaploid chromosome sets. The effect of a lanolin layer on relative humidity (RH) in a culture vessel, as well as on shoot growth and multiplication, was studied to improve survival in a greenhouse.

Materials and Methods

In vitro cultures were initiated from dormant apical and axillary buds of 6 birch genotypes representing different species and ploidy levels (Table I).

Cultures were grown on MS medium (Murashige and Skoog, 1962) having a modified macromineral composition (Simola, 1985), supplemented with 2.0 mg/l benzylaminopurine (BAP), 0.01 mg/l α-naphthalene acetic acid (NAA) and casein hydrolysate (CH) under growth conditions described in detail by Särkilahti (1988). The same medium was used for

Table I. *Betula* genotypes cultured *in vitro*.

Species	*Ploidy, 2n*	*Genotype origin*
B. pubescens ssp. *tortuosa*	4*x*, 56	mountain birch
B. pendula	4*x*, 56	colchicine-tetraploid and irradiation-mutant
" "	4*x*, 56	colchicine-tetraploid
B. papyrifera var. *subcordata*	4*x*, 56	colchicine-treated, normal
" " var. *papyrifera*	5*x*, 70	" " "
B. pubescens ssp. *tortuosa*	5*x*, 70	mountain birch hybrid

both induction of adventitious buds and culture of new shoots. Rooting medium had the same composition but with 0.1 mg/l NAA as the sole growth regulator. Rooted plantlets were transferred into a peat/soil mixture (1:1) and acclimatized in a greenhouse. Chromosome counts were determined from root tips of 5 plantlets/genotype to check the ploidy level.

The effects of a lanolin layer and lack of CH on the multiplication rate and growth of the shoots were studied separately. In the lanolin experiment, culture media were covered with a lanolin layer of 1–2 mm. Shoots measuring 5 mm with 2 nodes from sterile shoot cultures of the colchicine-tetraploid and irradiation-mutant *B. pendula* were used to start the experiments. Both the experiments and the controls consisted of 60 shoots in 10 culture vessels. After 4 wk, the number of regenerated shoots/original shoot, their lengths and number of nodes were evaluated.

Results

The medium (Särkilahti, 1988) allowed both the induction of adventitious buds and the development of new shoots from *in vitro* culture of 6 genotypes representing different *Betula* species and different ploidy levels. New buds and shoots were produced in a multiplication cycle of 3–4 wk from the cut ends of shoots transferred into fresh medium (Fig. 1). Shoot cultures of the tetraploid ssp. *tortuosa* and the tetraploid variety *subcordata* were lost because of bacterial contamination.

Adventitious shoot induction and shoot growth were accelerated on the medium lacking CH (Fig. 2). Rooting ability of the genotypes cloned *in vitro* varied from 50 to 100% and the survival rate after transfer into soil was poor, around 50%. Lanolin did not reduce the RH, furthermore, it severely retarded both shoot growth and multiplication. RH was 97.1% with and 96.8% without lanolin at the beginning of the experiment. After 4 wk, RH was 95.1% with and 99.3% without lanolin. The temperature was 26 ± 1°C during the experiment. All the genotypes, except the mountain birch hybrid, had the original ploidy level in the *in vitro* culture. Plantlets of the mountain birch hybrid showed variability in their ploidy level: 2 tetraploids, 2 aneuploids and 1 pentaploid were recorded.

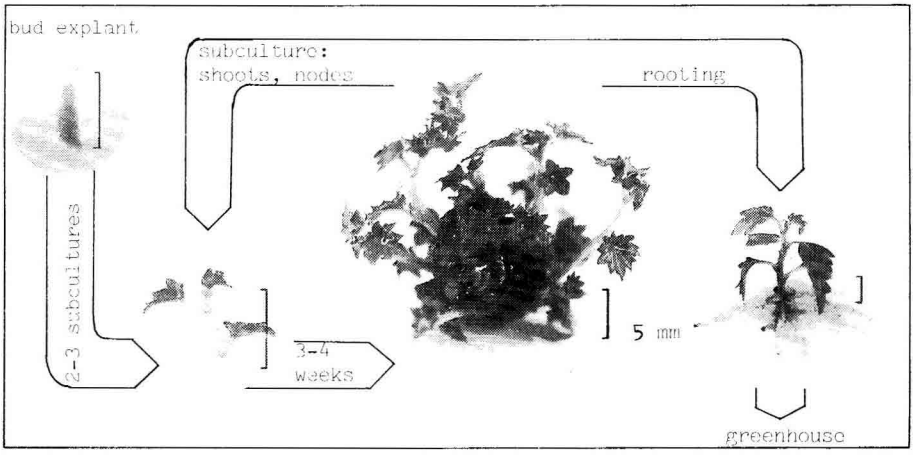

Fig. 1. *In vitro* culture procedure for *Betula* sp.

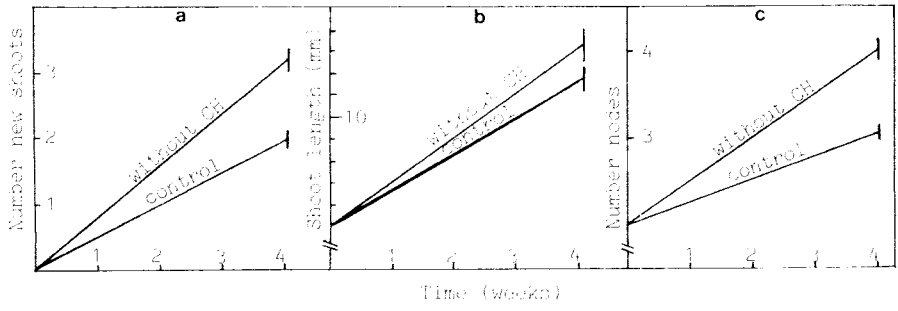

Fig. 2. Effect of CH on regeneration of the average number of new shoots/original shoot **(a),** average shoot length **(b)** and average number of nodes **(c).**

Discussion and Conclusion

Both genetic variation and environmental conditions are known to have an effect on the responses of different genotypes to *in vitro* culture. The present results with positive responses of different *Betula* genotypes in a single culture medium would support the major role of environmental conditions and the minor one of genotype. Thus, it should be possible to develop a universal *in vitro* culture medium for the genus *Betula*. Growth and multiplication can be further improved, as shown by omitting CH from the culture medium. Three genotypes retained their ploidy levels during several subcultures. This suggests that the instability of chromosome number in plantlets of the mountain birch was due to the hybrid nature of the mother tree rather than the culture conditions.

Acknowledgments

The author thanks Dr. Terho Valanne for his advice in preparing the manuscript and B.Sc. Soile Kiilunen for technical assistance. This study was financially supported by the Foundation for Research of Natural Resources in Finland.

References

Murashige T. & Skoog F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15, 473-497

Simola L.K. (1985) Propagation of plantlets from leaf callus of *Betula pendula* f. *purpurea*. *Sci. Hortic.* 26, 77-85

Särkilahti E. (1988) Micropropagation of a mature colchicine-polyploid and irradiation-mutant of *Betula pendula* Roth. *Tree Physiol.* 4, 173-179