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# Induced polyploidy in *Betula*

Eliisa Särkilahti & Terho Valanne

*TIIVISTELMÄ: INDUSOITU POLYPLOIDIA BETULA-SUVUSSA*

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Seeds of ten different *Betula* species were treated with colchicine solution during germination, to induce duplication of the chromosome set. The species included in the study were *B.pendula*, *B.pubescens*, *B.papyrifera subcordata*, *B.papyrifera papyrifera*, *B.papyrifera occidentalis*, *B.populifolia*, *B.alleghaniensis*, *B.nigra*, *B.glandulosa* and *B.nana*. The total number of individually labelled, colchicine-treated trees was 1550. Colchicine treatment induced changes in morphological features, especially in the leaves. These features proved to be good indicators of polyploidization. The experiments produced 687 polyploid trees, 287 of which are still alive. The polyploid *Betula* trees offer possibilities of studying the significance of the genome dosage for the growth, breeding, adaptability and evolution of *Betula*.

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Kymmenen eri koivulajin siemeniä käsiteltiin kolkisiiniliuoksella itämisen aikana tavoitteena kromosomiston kertautuminen. Käsitellyt lajit olivat *B.pendula*, *B.pubescens*, *B.papyrifera papyrifera*, *B.papyrifera subcordata*, *B.papyrifera occidentalis*, *B.populifolia*, *B.alleghaniensis*, *B.nigra*, *B.glandulosa* and *B.nana*. Yksilökohtaisesti merkittyjen, kolkisiiniliuoksella käsiteltyjen puiden lukumäärä oli 1550. Kolkisiinikäsittely aiheutti muutoksia morfologisissa ominaisuuksissa, erityisesti lehdissä. Nämä ominaisuudet osoittautuivat hyviksi polyploidisaation osoittajiksi. Kokeet tuottivat 687 polyploidia puuta, joista on vielä elossa 287. Polyploideja koivuja voidaan hyödyntää koivun jalostuksessa sekä tutkittaessa ploidiatason merkitystä kasvuun, sopeutuneisuuteen ja koivujen evoluutioon.

Keywords: polyploidy, colchicine, *Betula*, Finland.
ODC 232.1+165+176.1

Authors' address: University of Turku, Department of Biology, SF-20500 Turku, Finland.

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## 1. Introduction

Natural polyploidy is very frequent in the genus *Betula*, which comprises approximately 40 different species (e.g. Winkler 1904, McCown 1989). The number of ploidy levels differs between the four subsections of the genus. The subsections *Costatae* and *Albae* are the most variable ones: *Costatae* has diploid (2X=28), tetraploid (4X=56) and hexaploid (6X=84) *Betula* spp., and, in addition to these three ploidy levels, *Albae* also has a pentaploid (5X=70) species. *Nanae* has both diploid and tetraploid species, *Acuminatae* only diploid ones.

The great natural variation in the number of chromosome sets in *Betula* and the great

Table 1. *Betula* materials and treatments used in the colchicine experiments in 1962–1967. (C = colchicine solution, W = distilled water)

| Subsection<br>Species | Seed origin numbers | Treatments and their codes[1] | Individual tree numbers | Total number of trees |
|---|---|---|---|---|
| *Albae* | | | | |
| *B. pentula* | 1, 3–4, 8–9, 12–13, 16–18 | 4.–54.–62:* | 98– 853 | 755 |
| | 6 | 2.–63: 2x(1C+1W)+1C+7W 0.06% C | 860– 867 | 8 |
| | 15 | 46.–63: " " | 2037–2048 | 12 |
| | 15 | 1.–64: 10 000r +3x(2C+1W)+2C+3W 0.06% C | 2213–2367 | 155 |
| | 15 | 2.–64: 15 000r + " " | 2368–2397 | 30 |
| | 6 | 3.–64: 2x(2C+1W)+2C+6W 0.06% C | 2398–2410 | 13 |
| *B. pubescens* | 19 | 4.–64: 17 days in 0.1% C | 2411–2458 | 48 |
| | 19 | 4.–64: " 0.05% C | 2459–2512 | 54 |
| | 19 | 5.–64: 5x(1C+1W) 0.1% C | 2514–2550 | 37 |
| | 19 | 6.–64: 17 days in 0.04–0.05% C | 2576–2600 | 24 |
| *B. papyrifera subcordata* | 43 | 13.–64: 5C+8W 0.06% C | 2633–2651 | 19 |
| *B. papyrifera papyrifera* | 42 | 16.–64: 3C+2W+2C+8W 0.06% C | 2835–2988 | 154 |
| *B. papyrifera occidentalis* | 44 | 14.–64: 2C+3W+5C 0.06% C | 2652–2776 | 125 |
| *B. populifolia* | 46 | 19.–65: 10 000r +0.06% C 4 days | 3410–3442 | 33 |
| | 46 | 21.–65: 15 000r +0.06% C 4 days | 3489–3511 | 23 |
| *Costatae* | | | | |
| *B. alleghaniensis* | 41a | 15.–64: 2C+4W+7C 0.06% C | 2777–2834 | 58 |
| *B. nigra* | 47 | 5a.–67: 5C+0W 0.1% C | ** | |
| *Nanae* | | | | |
| *B. glandulosa* | 30 | *** | | |
| *B. nana* | 63 | *** | | |

[1] Seeds were either germinated in colchicine or kept alternately in colchicine solution and distilled water. The explanation to 2x(1C+1W)+1C+7W: seeds were first kept in colchicine solution for one day, then in distilled water for another day. This was repeated. Thereafter the seeds were again kept in colchicine for one day, and finally in distilled water for seven days.

* many treatments (Vaarama and Valanne 1969: 16–17)

** 56 saplings, all died in winters 1969–1970 and 1970–1971

*** no surviving seedlings

very old due to the aging effect of colchicine, the colchiploid trees are convenient explant trees in studying rejuvenation through meristem culture. Developing *in vitro* culture methods for polyploid birches is also studied in this research programme.

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## 2. Materials and methods

The materials of this research represent three of the four subsections into which *Betula* is divided: *Albae*, *Costatae* and *Nanae* (Table 1). The different *Betula* species used, and their seed origin codes are presented in Table 1. A detailed summary of the seed origins was presented by Vaarama and Valanne (1969) and Valanne (1972).

The polyploidization experiments were performed by treating seeds with colchicine during germination in 1962–1967. Seeds were either kept in colchicine solution for the whole germination period or alternately in colchicine solution and distilled water. The different colchicine treatments and their code numbers as well as the numbers of individually labelled seedlings produced and their total numbers are shown in Table 1.

The chromosome determinations were done either on squashed root tips of rooted leaf petioles (Valanne 1978) or on flushing leaves, according to a double-staining method (Hömmö and Särkilahti 1986).

The young colchicine-treated birches were transferred to their final growing sites at the age of 4–5 years in 1965 and 1967–69. The locations of the different colchicine treated trees are on the island of Seili (60°12'N, 21°55'E), at the Experimental Station of the Finnish Forest Research Institute, Punkaharju (61°43'N, 29°25'E), in the Botanical Garden of the University of Turku, Ruissalo (60°26'N, 22°10'E), and at Päilahti by Orivesi (61°37'N, 24°29'E; Figs. 1–2).

## 3. Results

A rough estimate of the number of suspected polyploids was obtained using the colchicine-induced morphological features described by Valanne (1972) as an indication of polyploidization. These features are a robust habit, ramification of the trunk and especially aberrant leaf morphology (Fig. 2).

On the basis of the colchicine-induced changes in leaf morphology, the trees were divided into three different morphological groups: no morphological differences compared with normal trees, slight colchicine morphology (++) and pronounced colchicine morphology (+++).

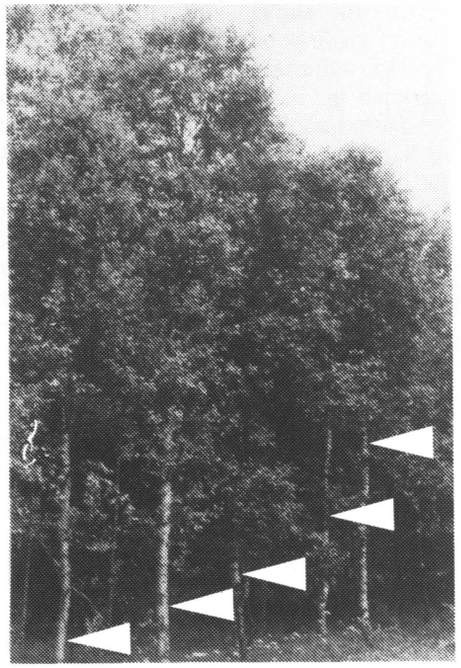

Fig. 1. Polyploid *Betula pendula* and *B.pubescens* materials in Päilahti, Orivesi in 1988. Trees marked with arrowheads from right to left:
*B.pendula* no. 2382 2n=56, irradiation-mutant,
" 862 2n=56,
" 2315 2n=56(60),
*B.pubescens* no. 2440 2n=70-84 and
" 2485 2n=98.

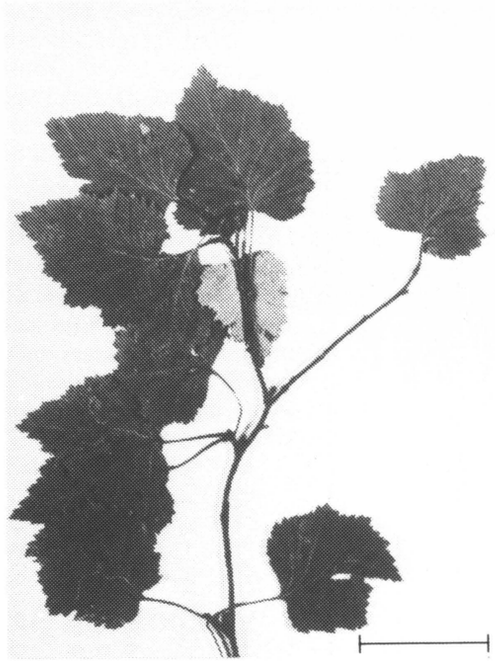

Fig. 2. Leaf specimen of *B.pendula* tree no.2382. Scale 5 cm.

This division was used when a sample of adult *B.pendula Roth* and *B.pubescens Ehrh.* trees was chosen for chromosome determinations in 1988 (Tables 3 and 4, Figs. 1–2).

The numbers of induced polyploid trees of different *Betula* confirmed during the juvenile and adult phases (1969 and 1987) are summarized in Table 2. Polyploids were recorded in all the investigated species except *B.nana* L. and *B.glandulosa* Michx.. The total number of treated and individually labelled trees in the inventory of 1969 was 1550. The total number of polyploids confirmed during the juvenile phase was 687 (Vaarama and Valanne 1969) and approximately half of these are still alive.

Tables 3 and 4 exemplify the great variability in chromosome numbers of the adult colchicine-induced polyploids of both *B.pendula* and *B.pubescens*. The abundance of aneuploids is also conspicuous in both species. In *B.pendula*, colchicine seems to induce autotetraploids and aneuploids in the ratio of 2:1. In *B.pubescens*, however, half of the induced polyploids have the chromosome number 98. Fig.1 shows polyploid *B.pendula* and *B.pubescens* trees in Päilahti, Orivesi in 1988.

In addition to the $C_0$ generation (colchicine-treated trees), our materials include $C_1$ progenies of colchicine-polyploid individuals of three species: *B.papyrifera Marsh.* (tree no.2867.16.-64, 2n=140), *B.pendula* (tree no. 314.29.-62, 2n=56), and *B.pubescens* (tree no. 2574.6.-64, 2n=112). The analysis of these open-pollinated 9–12 years old progenies is in progress (Vataja, unpublished). The $C_0$ *B.papyrifera* tree produced hexaploid seedlings (2n=84), the *B.pendula* triploid (2n=42) and the *B.pubescens* tetraploid and pentaploid (2n=56,70) seedlings (Särkilahti, unpublished).



Table 2. The numbers of polyploid *Betula* trees in 1969 and 1987 at different sites.

| Species | Treatment no. | Numbers of polyploids analyzed in 1969[1] | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Seili[1] | | Punkaharju[1] | | Ruissalo | | Orivesi | |
| | | | 1969 | 1987 | 1969 | 1987 | 1969 | 1987 | 1969 | 1987 |
| *B. pendula* | 4.–54.–62 | 188 | 137 | – | | | 41 | 22 | 10 | 3 |
| | 2.–63 | 6 | | | 1 | 1 | | | 4 | 2 |
| | 46.–63 | 9 | | | 4 | 2 | | | 2 | 1 |
| | 1.–64 | 136 | | | 116 | 72 | 13 | 8 | 2 | 1 |
| | 2.–64 | 30 | | | 26 | 15 | 3 | 3 | 1 | 1 |
| | 3.–64 | 13 | | | 13 | 10 | | | | |
| *B. pubescens* | 4.–64 | 77 | | | 46 | 33 | 5 | 3 | 10 | 5 |
| | 5.–64 | 36 | | | 22 | 18 | 5 | 4 | 9 | 3 |
| | 6.–64 | 22 | | | 10 | 9 | 10 | 5 | | |
| *B. papyrifera subcordata* | 13.–64 | 12 | | | 6 | – | 4 | 4 | 2 | 0 |
| *B. papyrifera papyrifera* | 16.–64 | 86 | | | 49 | – | 27 | 9 | 10 | 6 |
| *B. papyrifera occidentalis* | 14.–64 | 58 | | | 18 | – | 30 | 18 | 10 | 6 |
| *B. populifolia* | 19.–65 | – | | | | | 18 | 6 | 15 | 3 |
| | 21.–65 | – | | | | | 13 | 8 | 10 | 1 |
| *B. alleghaniensis* | 15.–64 | 8 | | | | | 6 | 4 | 2 | 1 |
| *B. nigra* | 5a.–67 | 6 | | | | | 6 | 0 | | |

[1] Dash means lack of observation

# 4. Discussion

Comparison of the leaf morphology with the cell size in different tissues and the chromosome numbers (Pieninkeroinen and Valanne 1989, Särkilahti and Valanne 1990) showed that identification of adult polyploid trees is possible visually on the basis of the leaf morphology in the *B.pendula*, *B.pubescens* and *B.papyrifera* complex. The other species do not show such strong colchicine-induced changes in leaf morphology.

The characteristics that are most indicative of polyploidization are larger leaf blades, thicker leaf petioles and a rougher network structure on the abaxial leaf epidermis than in normal trees. These are all consequences of the enlarged cell size in the leaves (Pieninkeroinen and Valanne 1989, Särkilahti and Valanne 1990).

The $C_1$ progenies are characterized by severe growth retardation in all growth phases. This result is in disagreement with that of Johnsson (1956), whose $C_1$ progenies showed only minor growth retardation. In general, the flowering ability of $C_0$ trees seems to vary greatly within the genotype, as can be concluded from our results and from previous experiments (Johnsson 1956, Eifler 1967).

The most effective method of polyploidization has generally proved to be colchicine treatment during seed germination. Compared with the large number of induced polyploids in several *Betula* species presented in this report, few references to successful polyploidization in *Betula* species can be found in the literature. The previous experiments comprise only three species: *B.pendula*, *B.pubescens* and *B.latifolia* Komar. (Johnsson and Eklundh 1940, Schröck 1951, Eifler 1955, Hyun and Kim 1963). In their experiments, colchicine was given in overdoses: high concentrations (e.g. 0.2%) and long treatment times were used. This resulted in a low number of polyploids, evidently due to the large

Table 3. Chromosome numbers of colchicine-induced polyploids of *B. pendula* trees.

| Treatment no. | Tree no. | Morphological groups[1] | Cromosome numbers (2n) | See figure |
|---|---|---|---|---|
| 48.–62 | 745 | +++ | 56 | |
| 51.–62 | 770 | +++ | 56 | |
| 2.–63 | 862 | +++ | 56 | 1 |
| ” | 865 | +++ | 56 | |
| 46.–63 | 2040 | ++ | 56 | |
| 1.–64 | 2213 | ++ | 56 | |
| ” | 2229 | ++ | 56 | |
| ” | 2245 | ++ | 56 | |
| ” | 2248 | ++ | 56 | |
| ” | 2265 | ++ | 56 | |
| ” | 2266 | ++ | 28, 29, 42, 48, 50 | |
| ” | 2267 | ++ | 56 | |
| ” | 2283 | ++ | 56, ca.60, ca.84, ca.98 | |
| ” | 2293 | ++ | 56 | |
| ” | 2303 | ++ | 56 | |
| ” | 2315 | +++ | 56 (60) | 1 |
| ” | 2347 | ++ | 28, 42, 56, 70, 30, 34, 37 | |
| ” | 2361 | ++ | 56 | |
| 2.–64 | 2372 | ++ | 56 | |
| ” | 2382 | +++ | 42, 56 | 1 and 2 |
| ” | 2386 | ++ | 56 | |
| ” | 2388 | +++ | 56 | |
| ” | 2389 | ++ | 56, ca.60 | |
| 3.–64 | 2401 | +++ | 28, 56 | |

[1] Key: ++ = slight colchicine morphology of leaves
+++ = pronounced colchicine morphology of leaves

proportion of undeveloped seedlings and the high mortality. Johnsson and Eklundh (1940) achieved polyploidization in eight *B.pendula* individuals and two *B.pubescens*. Three of their polyploidized *B.pendula* trees started flowering at the age of 12–13 years (Johnsson 1956). The colchicine experiments performed by Schröck (1951) with *B.pendula* produced only six polyploid individuals (Eifler 1955), which were examined at the age of 19 years, at which time none of the trees had flowered (Eifler 1967). Two polyploid individuals of *B.latifolia* were obtained by Hyun and Kim (1963).

The most effective technique for induction of polyploidization in *Betula* species was developed in the course of the experiments in 1962–1966, as shown by the great number of induced polyploid individuals in several *Betula* species (Tables 2–4). The technique of holding seeds alternately in colchicine solution and distilled water, using a low colchicine concentration (0.06%), resulted in a large number of polyploids. This technique did not arrest the cell mitosis cycle due to a too high polyploidization rate, but affected only one mitosis cycle, resulting in continuing but decelerated mitosis. These results suggest that the genomes of the various *Betula* species are far from conservative and can be manipulated artificially to produce genotypes with higher ploidy levels, unlike those of coniferous tree species, which seem to become inviable beyond their natural ploidy level (e.g. Johnsson 1975, Illies 1976). In general, deciduous tree species are younger from the evolutionary point of view than coniferous



Table 4. Chromosome numbers of colchicine-induced polyploids of *B. pubescens* trees.

| Treatment no. | Tree no. | Morphological groups[1] | Chromosome numbers (2n) | See figure |
|---|---|---|---|---|
| 4.–64 | 2424 | ++ | ca.98 | |
| ” | 2426 | ++ | ca.70–98 | |
| ” | 2428 | ++ | 98 | |
| ” | 2433 | ++ | ca.112 | |
| ” | 2435 | ++ | ca.98 | |
| ” | 2439 | ++ | ca.98 | |
| ” | 2440 | +++ | 70–84–98 | 1 |
| ” | 2452 | ++ | 98, 70, 56 | |
| ” | 2461 | ++ | ca.98 | |
| ” | 2463 | +++ | 98 | |
| ” | 2464 | ++ | ca.84—98 | |
| ” | 2465 | ++ | ca.98 | |
| ” | 2466 | ++ | ca.98 | |
| ” | 2468 | ++ | ca.98 | |
| ” | 2473 | +++ | ca.56, ca.70–84 | |
| ” | 2475 | +++ | 84–98 | |
| ” | 2476 | ++ | ca.98 | |
| ” | 2477 | ++ | ca.98 | |
| ” | 2480 | ++ | ca.98 | |
| ” | 2481 | +++ | 80–98 | |
| ” | 2485 | +++ | 98 (112 ?) | 1 |
| ” | 2490 | ++ | ca.84–98 | |
| ” | 2494 | ++ | ca.98 | |
| ” | 2496 | ++ | ca.98, ca.100 | |
| ” | 2497 | ++ | ca.98, 112 | |
| ” | 2498 | ++ | ca.90 | |
| ” | 2504 | ++ | ca.112 | |
| 5.–64 | 2514 | ++ | 90–100 | |
| ” | 2515 | ++ | ca.98 | |
| ” | 2516 | ++ | ca.98 | |
| ” | 2520 | ++ | ca.98 | |
| ” | 2521 | ++ | ca.112 | |
| ” | 2529 | ++ | 98–112 | |
| ” | 2535 | ++ | ca.98 | |
| ” | 2538 | ++ | ca.98, 104 ? | |
| ” | 2542 | +++ | 98 | |
| ” | 2544 | +++ | 98, 112 | |
| ” | 2548 | ++ | ca.98 | |
| 6.–64 | 2571 | ++ | 84–100 | |
| ” | 2579 | ++ | ca.98–101 | |

[1] Key: ++ = slight colchicine morphology of leaves
+++ = pronounced colchicine morphology of leaves

trees. This explains the polyploid nature and the occurrence of various ploidy levels, e.g. in *Betula*. Evolution is still proceeding in *Betula*, as shown by the frequent cases of hybridization and introgression at the northern tree limit in Fennoscandia (Sulkinoja 1981, 1983, Kallio et al. 1983).

The birch materials presented in this paper offer possibilities of studying the significance of the genome dosage for the growth, breeding, adaptability and evolution of *Betula*. A large polyploid series is available, consisting of both natural and induced polyploids. Ploidy levels ranging from diploid to dodecaploid (2X, 3X, 4X, 5X, 6X, 8X, 10X and 12X) are represented in these materials. In the colchicine experiments reported here, polyploids were achieved in all the three sections used, except *Nanae*, which did not produce any viable seedlings from colchicine treatment at all. *Nanae* birches evidently lose their viability at other ploidy levels than their natural one.

Thus, we have large autopolyploid materials of different *Betula*, which can be maintained through micropropagation methods. Some of our colchicine-treated and polyploid individuals have already been cultured as sterile shoots, which have also produced micropropagated plantlets (Särkilahti 1988, 1989). Clonal materials allow testing of genetic identity both between the parent tree and its clonal progeny, and within a clonal progeny (e.g. McCown 1989). A significant question in the production of micropropagated forest trees is whether micropropagation results in true rejuvenation (Hackett 1985, Franclet et al. 1987) or merely an epigenetic change (Varga et al. 1988, Karp 1989). The effects of topophysis and cyclophysis in the meristems of adult trees (Evers 1987) may not be truly overcome by *in vitro* culture, but only delayed due to environmental factors in the culture procedures. This would produce rejuvenated plantlets and juvenile trees which would switch to the adult phase much earlier than trees of the same age originating from seed. The results on micropropagation of an adult irradiation-mutant and colchitetraploid of *B.pendula* suggest that there is no true rejuvenation induced during the culture of the meristems (E.Särkilahti, unpublished).

# References


Eifler, I. 1955. Künstliche Polyploidie-Erzeugung bei Picea abies und Betula verrucosa. Zeitschrift für Forstgenetik und Forstpflanzenzüchtung 4:162–166.

— 1967. Anwendungsmöglichkeiten der Polyploidiezüchtung in der Forstwirtschaft. Archiv für Forstwesen 16:515–528.

Evers, P.W. 1987. Correlations within a tree. In: Bonga, J.M. & Durzan, D.J. (eds.). Cell and tissue culture in forestry. Vol. 2. Specific principles and methods: growth and developments. Martinus Nijhoff Publishers, Dordrecht. p. 218–228. ISBN 90-247-3431-2.

Franclet, A., Boulay, M., Bekkaoui, F., Fouret, Y., Verschoore-Martouzet, B. & Walker, N. 1987. Rejuvenation. In: Bonga, J.M. & Durzan, D.J. (eds.). Cell and tissue culture in forestry. Vol. 1. General principles and biotechnology. Martinus Nijhoff Publishers, Dordrecht. p. 232–248. ISBN 90-247-3430-4.

Hackett, W.P. 1985. Juvenility, maturation and rejuvenation in woody plants. Hortic.Rev. 7:109–155.

Hyun, S.K. & Kim, C.S. 1963. Some characteristics of autotetraploids of tree species induced by colchicine treatment. FAO World Consult, Forest Genetics, Stockholm, Sweden. No. 1/3. 16 p.

Hömmö, L.M. & Särkilahti, E.M. 1986. A method of counting chromosomes of hardwood trees using root tips and young leaves. Can.J.For.Res. 16:401–403.

Illies, Z.M. 1976. Experiences in doubling and reducing chromosome numbers in woody species for further breeding programmes. Proc. XV IUFRO World Congr. Oslo 1976. 5 p.

Johnsson, H. 1956. Auto- and allotriploid Betula families, derived from colchicine treatment. Zeitschrift für Forstgenetik und Forstpflanzenzüchtung 5:65–70.

— 1967. Olika vägar för rasmässig förbättring av vårt skogsodlingsmaterial. Silva Fennica 3(3):29–56.

— 1975. Observations on induced polyploidy in some conifers. Silvae Genetica 24:62–68.

— & Eklundh, C. 1940. Colchicinbehandling som metod vid växtförädling av lövträd. Svensk Papperstidning 43:355–360, 373–377.

Kallio, P., Niemi, S., Sulkinoja, M. & Valanne, T. 1983. The Fennoscandian birch and its evolution in the marginal forest zone. Nordicana 47:101–110.

Karp, A. 1989. Can genetic instability be controlled in plant tissue cultures? IAPTC Newsletter 58:2–11.

McCown, B.H. 1989. Birch (Betula spp.). In: Bajaj, Y.P.S. (ed.). Biotechnology in agriculture and forestry. Vol. 5. Trees II. Springer-Verlag, Berlin Heidelberg. p. 324–341.

Pieninkeroinen, K. & Valanne, T. 1989. Old colchicine-induced polyploid materials of Betula pendula Roth and Betula pubescens Ehrh.. Ann.Sci.For. (Supplement) 46:264–266.

Schröck, O. 1951. Stimulierende Wirkung des Colchicins bei der Keimung und dem Wachstum der Sämlinge. Züchter 21:142–149.

Sulkinoja, M. 1981. Lapin koivulajien muuntelusta ja risteytymisestä. Lapin tutkimusseura. Vuosikirja 22:22–30.

— 1983. Lapin koivut. Oulun yliopisto. Pohjois-Suomen Tutkimuslaitos. C 47:40–46.

Särkilahti, E. 1988. Micropropagation of a mature colchicine-polyploid and irradiation-mutant of Betula pendula Roth. Tree Physiology 4:173–179.

— 1989. In vitro propagation of several Betula species representing various ploidy levels. Ann.Sci.For. (Supplement) 46:152–154.

— & Valanne, T. 1990. Colchicine-induced increase of cell size and polyploidy in Betula papyrifera Marsh.. Manuscript (submitted).

Vaarama, A. & Valanne, T. 1969. Induced mutations and polyploidy in birch, Betula spp. Final Report Part III. Experiments with colchicine-induced polyploidy. Project no. E8-FS-47, Grant no. FG-Fi-133. On file at Department of Botany, University of Turku. 95 p. + 14 plates.

Valanne, T. 1972. Colchicine effects and colchicine-induced polyploidy in Betula. Ann.Acad.Sci. fenn. A, IV Biologica 191:1–28.

— 1978. Soft-wood rooting experiments with Betula. Rep. Kevo Subarctic Res.Stat. 14:72–75.

Varga, A., Thoma, L.H. & Bruinsma, J. 1988. Effects of auxins and cytokinins on epigenetic instability of callus-propagated Kalanchoe blossfeldiana Poelln. Plant Cell, Tissue and Organ Culture 15:223–231.

Winkler, H. 1904. Betulaceae. In: Engler, A. (ed.). Das Pflanzenreich. Regni vegetabilis conspectus. IV.61. Verlag von H.R. Engelmann. Weinhaim. 149 p.


*Total of 27 references*