

# Short Note: Isolation and Culture of Mega and Normal Protoplasts in Aspen

By M. R. AHUJA

Federal Research Center for Forestry and Forest Products,  
Institute of Forest Genetics and Forest Tree Breeding,  
Sieker Landstraße 2, D-2070 Grosshansdorf,  
Federal Republic of Germany

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## Abstract

Exceptionally large mesophyll protoplasts, here designated Mega protoplasts, were isolated from young leaves of a forest tree species, *Populus tremula* (aspen). The largest mega protoplasts were up to 64 times greater in volume than average normal mesophyll protoplasts. Budding and cell division have been observed in both mega and normal protoplast derived cells. These studies indicate that in spite of their enormous size, the mega protoplasts are developmentally viable. The origin of mega protoplasts is not known.

**Key words:** Aspen protoplasts, mega protoplasts, isolation and culture, cell division.

## Zusammenfassung

Außerordentlich große Protoplasten des Mesophylls, hier bezeichnet als Megaprotoplasten, wurden von jungen Blättern der Waldbaumart *Populus tremula* (Aspe) isoliert. Die größten Megaprotoplasten waren im Volumen bis zu 64 mal größer als durchschnittliche, normale Protoplasten des Mesophylls. Zellsprossungen und Zellteilungen wurden in Zellen beobachtet, die von beiden, den Mega- und normalen Protoplasten stammten. Diese Untersuchungen zeigen, daß trotz ihrer ganz besonderen Größe, die Megaprotoplasten in ihrer Entwicklung lebensfähig sind. Der Ursprung der Megaprotoplasten ist nicht bekannt.

## Introduction

Protoplasts have been isolated from a handful of forest tree species, and cultured in a few (see AHUJA, 1981, 1982). However, substained cell divisions are rare events in the protoplast cultures of forest tree species. Consequently, it has not been possible to regenerate whole plants from forest tree protoplasts. This may be partly because of inadequate knowledge on the *in vitro* growth and differentiation requirements of cells from forest tree species, and partly because the cells of most long-lived tree species may grow at a much slower rate compared to cells from herbaceous species, perhaps requiring frequent replenishing/modification of the medium. Following isolation, cell wall formation and cell division have been reported in *Pinus pinaster* and *Biota orientalis* (DAVID *et al.* 1981), *Picea excelsa* (STRMEN and CIERNA, 1981), and colony/callus formation in *Pseudotsuga menziesii* (KIRBY, 1981). We have been able to isolate exceptionally large protoplasts, here designated Mega protoplasts, from the leaves of a forest tree species, *Populus tremula* (aspen), which are many-fold larger than normal mesophyll protoplasts. Here we report on the developmental potential of mega and normal protoplasts of aspen.

## Materials and Methods

Branches from *Populus tremula* and *P. tremuloides* clones were maintained in the greenhouse in January for a hybridization program, and for a regular supply of

young leaves. First leaves from *P. tremula* were harvested and surface sterilized in 5 % sodium hypochlorite, containing a few drops of Tween 80, for 5 minutes. Following three washings in autoclaved distilled water, the lower epidermis was scraped with a scalpel. Small pieces of leaves were sliced transversely into 1 mm strips and incubated in an enzyme solution consisting of 0.5 % cellulase R-10 (Serva), 0.1 % macerozyme R-10 (Serva), 0.1 % bovine albumin (Sigma), and 0.7 M mannitol in a CPW solution, pH 5.6. The CPW consisted of 100 mg/l calcium chloride, 170 mg/l potassium dihydrogen phosphate, 250 mg/l magnesium sulphate, and 30 mg/l calcium nitrate. Incubation was carried out in the dark, at 25° C, with shaking at 30 rpm, for 20–22 hours. After filtration through a 80 µm stainless steel sieve, protoplasts and debris were pelleted in 0.7 M mannitol + CPW at 100 g for 6 minutes. The pellet was resuspended in 20% sucrose + CPW and centrifuged at 100 g for 15 minutes. Floating protoplasts were washed twice in 10 % mannitol + CPW, and plated in 2 ml of protoplast culture medium at a density of 10<sup>3</sup> to 5 × 10<sup>4</sup>/ml. We have experimented with various protoplast culture media. The protoplast culture medium used in these experiments consisted of a modified low salt medium (LLOYD and McCOWN, 1981), supplemented with 100 mg/l casamino acids, 500 mg/l glutamine, 1 mg/l ascorbic acid, 1 mg/l calcium pantothenate, 0.5 mg/l benzylaminopurine, 0.5 mg/l indoleacetic acid, 0.5 mg/l naphthaleneacetic, 0.02 mg/l dichlorophenoxyacetic acid, 100 g/l mannitol, 18 g/l glucose, and 10 g/l sucrose, pH 5.6. Cultures were maintained in dim light, at 25° C and 70% relative humidity for a week, and then moved to a 16 hours photoperiod (1000–2000 lux). The mannitol and glucose were gradually diluted out of the medium.

## Results and Discussion

There was variability in the size of mesophyll protoplasts from aspen. We grouped them into two broad categories: normal (*Figure 1*) and mega protoplasts (*Figure 2*). The diameters of the mega protoplasts were 2–4 times greater than the average normal mesophyll protoplasts. In terms of total volume, the ratio between normal and mega protoplasts ranged from 1:8 to 1:64. The mega protoplasts occurred at a frequency of 1 to 2 percent. They were much more densely packed with chloroplasts than normal protoplasts. Cell wall regeneration occurred in both normal and mega protoplasts within 48 hours. After 3–5 days in culture, the normal and mega protoplast derived cells showed initiation of budding. Subsequently cell division or constrictions were observed in the normal and mega protoplasts cultures (*Figures 3 and 4*). Studies are continuing on the growth and differentiation of these protoplasts cultures.

These studies have demonstrated the developmental potential of normal and mega protoplasts from *P. tremula*,

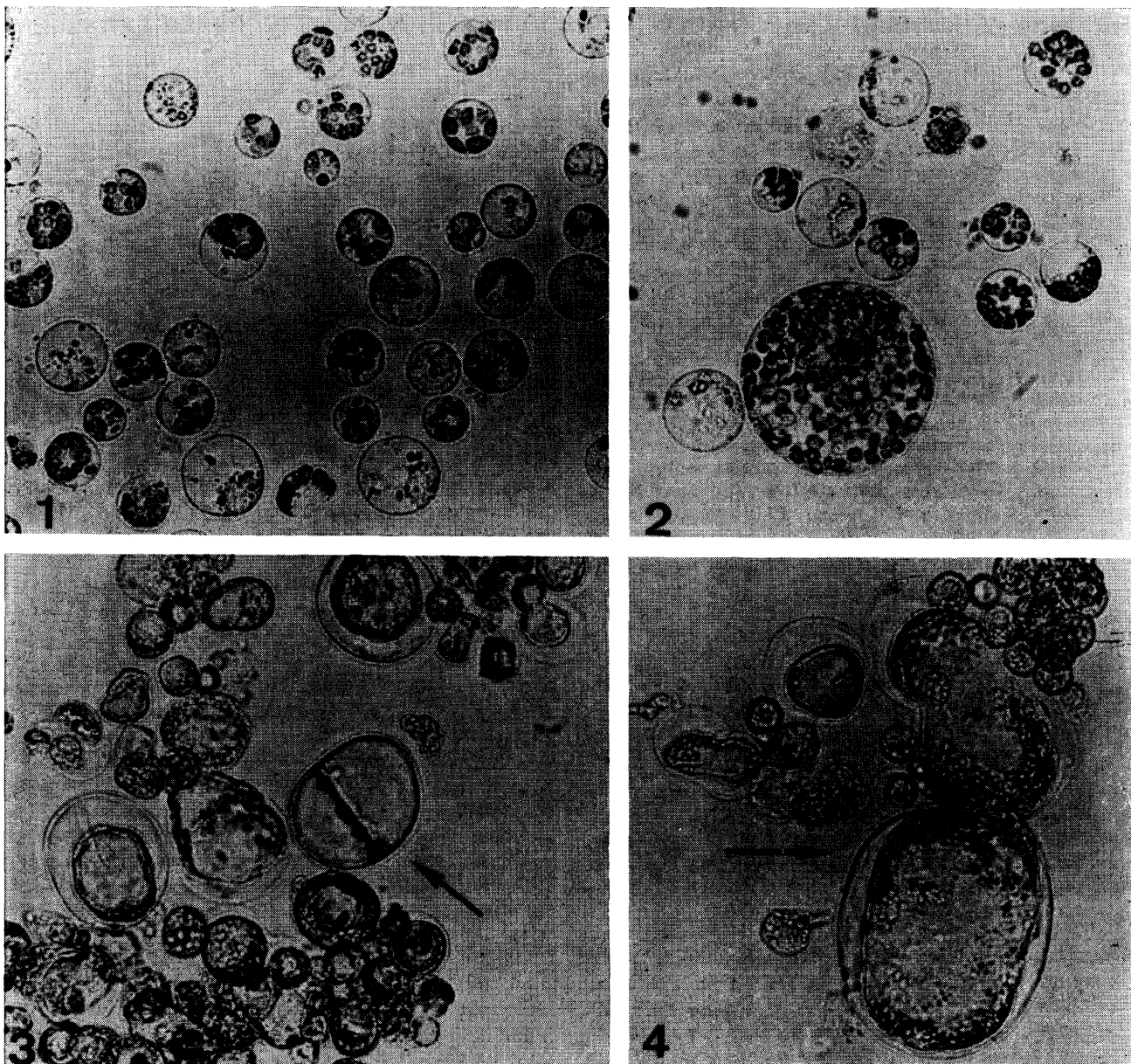


Figure 1. — Freshly isolated mesophyll protoplasts from aspen (*Populus tremula*). Figure 2. — Mega and normal mesophyll protoplasts from aspen. Figure 3. — Protoplast culture showing cell division (arrow) in a normal protoplast derived cell. Figure 4. — Protoplast culture showing cell division (arrow) in a mega protoplast derived cell. (Fig. 1–4  $\times 560$ )

a forest tree species. The initial budding-constriction and cell division show that aspen protoplasts are capable of responding to the *in vitro* conditions. The size difference between mega and normal protoplast derived cells continue to exist in these cultures.

The origin of mega protoplasts is not known. We have observed mega protoplasts in several clones of *P. tremula* and *P. tremuloides*. To what extent mega protoplasts occur in other plant species remains to be investigated. We have considered the possibility that mega protoplasts may be artefacts of the incubation procedure, since they were observed in the enzyme solution before centrifugations, and thus could have arisen following fusions between protoplasts. However, it would require many fusions to yield protoplasts of such dimensions. Alternatively, the mega protoplasts are real entities existing in the leaves

of aspen as mega mesophyll cells. It is conjectured that mega mesophyll cells, loaded with chloroplasts, may develop in the first spring leaves of deciduous tree species, such as *Populus* and perhaps others, possibly involved in a rapid post-winter photosynthetic activity. Leaf anatomy and chromosome constitution of mega protoplasts will shed some light on their origin and ploidy levels. Even if mega protoplasts are highly polyploid, they seem to have a remarkable ability to undergo budding and cell division, just as normal protoplasts.

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## Pressemitteilung

### 6. Hamburger Forst- und Holztagung 1984

Die 6. Hamburger Forst- und Holztagung findet vom 8. bis 10. Mai 1984 in der Bundesforschungsanstalt für Forst- und Holzwirtschaft in 2050 Hamburg 80, Leuschnerstraße 91, statt.

Die Veranstaltungen stehen unter dem Leitthema: Wald - Holz - Umwelt.

Als Rahmenthemen sind vorgesehen:

8. 5. 1984 Erhaltung und Nachzucht unserer Wälder

9. 5. 1984 Nutzung und Verwertung von Schwachholz Holz als Chemierohstoff

10. 5. 1984 Tropische Feuchtwälder - Gefährdung und Nutzung  
(Vortragsveranstaltung der Gesellschaft der Freunde und Förderer der BFH)

Das detaillierte Programm wird zum Jahresende veröffentlicht.

Prof. Dr. W. LIESE

## Buchbesprechungen

**Conifers: morphology and variability.** By M. VIDAČKOVIĆ, Yugoslav Academy of Science and Arts, and Liber University Publisher Publ. Zagreb. 1982. 710 p. (in Croatian).

Prof. VIDAČKOVIĆ has taught dendrology and forest genetics at the Faculty of Forestry, University of Zagreb for more than 25 years. He is well known to the world community for his outstanding achievements in the sciences of tree morphology and genetics. This rare, but very desired combination of the author's expertise has resulted in a new and unique textbook on morphology and genetics of coniferous trees.

The textbook represents in a new approach to dendrology in which the author describes the taxons in a dynamic way. He discusses not only morphology, but also evolution and variability as influenced by genetics and environment, and by man's actions. He also elaborates on the possibilities for selecting and breeding new types of trees.

The book starts with a general, introductory section, in which systems of plant classification, the definition of species, speciation, variation in populations, taxons, morphological characteristics and areas of plant growth are briefly discussed and the terminology given. It continues with a systematic outline of *Gymnospermae* and keys for their identification on the basis of tree morphology and characteristics of the seedlings. The introductory part of the book ends with a brief systematic description of classes, orders, families and genera.

The main part of the textbook is a detailed description of genera in alphabetical order, from *Abies* to *Widdringtonia*. In addition to morphology, the description includes information on their ecology, cytology, genetics and interspecific hybridization. An outline of the systematics of each genus follows with a key for species identification. The description of each species of the genus contains information on morphology, ecology, area of growth, silvics, variability and genetics. Races, varieties, horticultural forms, types and cultivars for each species are also listed and described. A list of references concludes the description of each genus and species.

The concluding portion of the book lists a bibliography pertaining to several genera. It is classified into references for dendrology, monographs, textbooks on plant determination and nomenclatures, phytogeographies, and others. Lists of described genera, species and sub-species taxons (including cultivars) follow. The book concludes with lists of synonyms and vernacular names (in alphabetic order).

The references cited come from a large number of countries and languages. The English-language sources are as well represented as the French, German, Russian, Czechoslovakian, Yugoslav, Hungarian and others. It is indeed a rare opportunity for the reader to find such a vast amount of information reviewed in one volume.

There are 439 illustrations in the book, 170 of these are photos (most of them in colour) and the rest are graphs and drawings. Many are originals, and all are of excellent quality. The publishing, including illustrations, is first class, rarely seen even from well-known publishing houses.

The textbook is intended for students of forestry and natural sciences, as special literature in graduate studies, and as a reference book for scientists and professionals.

The strength of the book is in the systematic and unique outline of material, in the relation of morphology, genetics, ecology and silvics of coniferous tree species, in complete lists of sub-species taxons and cultivars, in extensive reference lists which are well arranged for easy use, and in the high quality of illustrations and publication. The weakness of the book (if there is one) is in the less complete treatment of some species such as *Pinus merkusii* and *P. patula*, and probably others, mainly from the subtropical and tropical range. While this treatment can be explained with possibly less interest of the Yugoslav reader in some species, it is also a fact, that some genera and species are much better known than others and the amount of scientific information available is unequal.

The Croatian language, in which the book is written, creates difficulty to most of the North American and western-European readers. This is a very worthy publication and a revised edition in English would be welcome by many interested persons.

L. ZSUFFA  
Ontario Tree Improvement  
and Forest Biomass Institute  
Ontario Ministry of Natural Resources  
Maple, Ontario LOJ 1E0

**Merkblätter für fremdländische Baumarten:** *Liriodendron tulipifera* 2 S., Juli 1981; *Carya ovata* K. JOCH, 2 S. Januar 1982; *Metasequoia glyptostroboides* HU & CHENG, 2 S. u. Anl., April 1982; *Sequoiadendron giganteum* (LINDL.) BUCHH. 3 S., Okt. 1982. Landesanstalt für Ökologie, Landschaftsentwicklung und Forstplanung Nordrhein-Westfalen.