

Table 2. — Results of analysis of variance. Calculated values of F: ** - significant at 0,01 level, * - significant at 0,05 level.

Source of variance	Df	% of shoots flowering male	% of shoots flowering female	No. of female flowers per 100 shoots
Total	59			
Treatment periods /T/	3	4,69 **	3,41 **	1,75
May - June	1	12,88 **	0,00	0,06
July - August	1	1,19	10,14 **	4,64 **
May - August	1	0,00	0,08	0,54
Clones /C/	4	22,05 **	2,05	2,68 **
T x C	12	1,54	0,77	0,70
Residual	40			

differential effect of GA4/7 on the sex of flowers is probably connected with the stage of development of Scots pine buds during the growing season. The pattern of seasonal development of Scots pine buds in grafts is well known owing to detailed studies done by HEJNOWICZ (1982).

The shoot formed in the previous year begins elongation at the turn of March and April and the process is continued during May and June. Early May initials of the new embryonic shoot are observed and the initiation of dwarf shoot cataphylls begins at the end of that month. In late June in the axils of cataphylls the initiation of dwarf shoot primordia begins which are homologous with male strobili initials in the generative bud.

The newly formed embryonic shoot slowly ends its development at the turn of August and September. In late July the new cataphylls of long shoot buds start to initiate. The phase of cataphylls initiation takes almost three weeks. In mid August the initiation of lateral bud primordia begins which are homologous with female strobili initials in the generative bud.

It is possible that the application of GA4/7 during May and June or during July and August modifies the development of newly formed primordia and promotes their sexual differentiation, male from dwarf shoots and female from lateral buds.

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Short Note: A Commercially Feasible Micropropagation Method for 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 Grossshansdorf,
 Federal Republic of Germany

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Abstract

A relatively simple two-step method for the rapid clonal propagation of mature elite aspen clones (*Populus tremula*, *Populus tremuloides* and their hybrids) is described. Meristematic explants from buds were cultured on a modified Woody Plant Medium, here designated Aspen Culture Medium (ACM), supplemented with low levels of a cytokinin and an auxin. Following shoot differentiation/proliferation on the bud explants of the responsive aspen clones, the microshoots are rooted in soil-free potting mixture. A few thousand plantlets from a large number of mature selected aspen clones have been regenerated by this two-step micropropagation method.

Kew words: Aspen (*Populus*), micropropagation, bud meristems culture, microshoots, plantlets.

Zusammenfassung

Es wird eine verhältnismäßig einfache Zweischnittmethode für eine schnelle Vermehrung von Aspenklonen (*Populus tremula*, *Populus tremuloides* und deren Hybriden), ausgehend von adulten Elitebäumen, beschrieben. Dabei werden Meristemexplantate von Knospen auf einem modifizierten "Woody Plant Medium" kultiviert, das hier als "Aspen Culture Medium (ACM)" bezeichnet wird, welches mit geringen Konzentrationen eines Cytokinins und eines Auxins ergänzt wurde. Nachdem sich Mikrotriebe auf den Knospenexplantaten der reagierenden Klone differenziert und gestreckt haben, werden diese in einem Torf-Perlite Substrat bewurzelt. Einige tausend solcher Pflanzen aus einer großen Zahl von selektierten adulten Aspenklonen konnten mit dieser Zweischnittmethode vermehrt werden.

Introduction

European aspen (*Populus tremula*), quaking aspen (*Populus tremuloides*) and their hybrids are short rotation, fast-growing tree species, which exhibit good growth on marginal sites. In addition to their utility in the paper, plywood, and matchstick industries, aspens appear to be potentially valuable for biomass and energy production. Before establishing aspen plantations on a large scale, it is necessary to ascertain if the conventional methods of reproduction, that is, through seed, root suckers, and bud grafts (HERRMANN and SEUTHE 1982) can adequately meet the market demands. The green shoots can also be used for vegetative propagation (see BEHRENS and MELCHIOR 1978; however, the woody cuttings from aspen are extremely difficult to root) (BRAUN and SCHLENKER 1964). Partial market demands may be met by establishing aspen forests from seedlings obtained from control pollinated plus trees. However, vegetative propagation has potential for selection and maintenance of both additive and non-additive genetic effects in the clones derived from a hybridization program. Clonal propagation of selected aspen tree through root suckers, green shoots, and bud grafts have been practiced, but to a limited extent.

Therefore, for mass scale propagation of selected aspen clones, alternative method(s) had to be explored. We have exploited the potential of tissue culture technology for clonal propagation of a large number of selected mature aspen clones (AHUJA and MUHS 1982; AHUJA 1982, 1983). The present paper reports on a commercially feasible method for large scale rapid clonal propagation of aspen.

Materials and Methods

Dormant buds (apical or axillary) from *Populus tremula*, *Populus tremuloides*, and their hybrids (ranging in age from 15 to 40 years) were washed in water containing a dash of a detergent (R4) for about 30 minutes. The buds were then transferred to a 5 percent sodium hypochlorite

solution containing a few drops of Tween 80, for 15 minutes. After that the bud scales were removed and bud meristems with a few juvenile leaves were once again sterilized in 1 percent sodium hypochlorite for about 10 minutes. After washing bud explants 3 times in autoclaved distilled water, essentially the bud meristems were cultured in a modified Woody Plant Medium (LLOYD and McCOWN 1981), previously designated Aspen Culture Medium (ACM; AHUJA 1983). The ACM was fortified with various amounts of growth promoting substances: ACM-1 contained 20 mg/l adenine sulphate, and 0.5 mg/l 6 benzylaminopurine (BAP); ACM-2 contained additionally, 0.02 mg/l naphthaleneacetic acid (NAA); and ACM-3 contained 0.5 mg/l indolbutyric acid (IBA) and 0.01 mg/l NAA but lacked in myoinositol and lysin. Following culture, bud explants were maintained at 25° C, 70 percent relative humidity, and under 16 hour photoperiod (2000—3000 lux). The microshoots derived from bud explants were rooted in ACM-3 or directly in once autoclaved soil-free potting mixture. The microshoots in the potting mixture were maintained in controlled chambers (temperature 22—24° C; relative humidity 70 to 80 percent; photoperiod of 16 hours at about 3500 lux) in plastic trays (33 × 50 cm) with transparent plastic covers. These covers were gradually removed after the microshoots had established a root system. Tissue culture plantlets were then transferred to greenhouse before planting them out under field conditions.

Results and Discussion

In our initial experiments with bud explants, we used ACM-1 for "bud break". Thereafter, the explants were transferred, after 2—4 weeks, to ACM-2 for shoot growth and proliferation. The microshoots were then rooted in ACM-3. Subsequently, tissue culture derived plantlets were transferred to pots, and following hardening period in controlled chambers and greenhouse, they were finally planted under field conditions (see AHUJA 1983). We have simplified

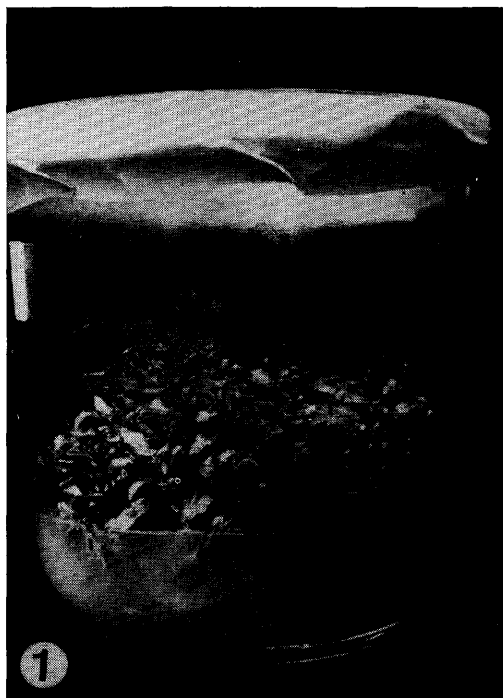


Figure 1. — Numerous microshoots derived by culture of bud meristems of a hybrid aspen clone on ACM-2 in a half liter Marmalade jar.



Figure 2. — Hybrid aspen plantlets derived by rooting microshoots in greenhouse soil mix in plastic trays (33 × 50 cm).

this procedure so as to reduce the number of steps and make the micropropagation method cost-effective. From the four-step method outlined above, we first reduced it to a three-step method, by eliminating ACM-1 from the scheme. The bud explants were directly cultured on ACM-2 where both "bud break" and shoot differentiation was accomplished. The microshoots were then rooted in ACM-3, and the plantlets were subsequently transferred to pots for hardening before transplantation.

We have now worked out a two-step method for micropropagation of aspen, thereby eliminating one more step. We culture the bud meristems on ACM-2 for "bud break" and shoot proliferation (Figure 1), and then root the microshoots (Figure 2) directly in once autoclaved soil-free potting mixture, thus eliminating the need for the rooting medium ACM-3. The microshoots root in the potting mixture in 8–10 days, the same time period required for rooting them in ACM-3. By employing this two-step method, we have regenerated a few thousand plantlets from more than thirty selected mature aspen clones. Initially we had employed buds from nursery grown plants that were established from selected trees through root suckers in order to obtain juvenile material for clonal propagation. Presently, we have even eliminated that step. We harvest dormant buds from the mature trees in the late fall, and directly culture their bud meristems for shoot differentiation.

We have randomly checked the chromosome numbers of some of our tissue culture derived plants. Aspens have a diploid chromosome number of 38 (see SEITZ 1951) and the chromosomes are extremely small. Our chromosome analysis revealed that the tested aspen plants had a genetic constitution in the diploid range.

There was interclonal variation with respect to growth rate and rootability. Some clones exhibited shoot differentiation on ACM-2 after 8 weeks, while others took as much as 20 weeks, or even longer. The percent rootability

varied from 70 to 90 percent between clones. There were also differences in the growth rates of microshoots from different clones after they were transferred to soil for rooting. About 50 percent of the aspen clones (37 out of 70) tested in 1982–83 exhibited the differentiation of shoots on ACM-2. Regeneration studies on the non-responsive clones are in progress. Details on clonal variation, and further regeneration studies on aspen clones will form the topic of another publication.

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Pressemitteilung

Forstliche Hochschulwoche 1985 in Göttingen

Die Veranstaltungen der Forstlichen Hochschulwoche des Forstwissenschaftlichen Fachbereiches der Georg-August-

Universität, Göttingen, finden in der Zeit vom 21.–25. Oktober 1985 in D-3400 Göttingen-Weende, Büsgenweg 2, statt.

Buchbesprechungen

Pollination Mechanism, Reproduction and Plant Breeding. Von R. FRANKEL and E. FALUN. Monographs on Theoretical and Appl. Genetics 2, 281 pp. Springer-Verlag Berlin, Heidelberg, New York 1977. DM 69,—.

Plant breeding and crop production depend in a decisive manner on pollination mechanism and reproduction. Therefore goal of the presented book is "to furnish an integrated botanical, genetical and breeding methodological treatment of the reproductive biology of . . . mainly angiosperms". The author's effort has resulted in a didactic, clear, well organised and with regard to the contents well founded, concise and comprehensive book.

The authors evaluate an overall picture of the importance of the different pollination mechanism for plant breeding and crop production, describe the modes of reproduction in higher plants

and discuss ecology and dynamics of pollination. A following chapter reserved to the crops characterised by selfpollination and their breeding procedures is based on solid informations. At the end the sexual reproduction especially sex expression, incompatibility and male sterility are treated thoroughly. Some 30 pages of references and some 20 of a subject index complete the very usefull book.

The book is addressed to students at a graduate level of Biology and Agriculture, to plant breeders, botanists, geneticists, agriculturists, and foresters to acquire a broader knowledge of reproductive biology of higher plants and to improve intra- and interdisciplinary communication between them. These groups are addressed by a very profound, sound and up-to-date presentation of the knowledge till the time of the books publication.

G. H. MELCHIOR

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