Toro and Nieto (1984) addressed the problem of minimizing genetic drift in a breeding program. They searched for optimal unequal contributions of selected individuals to the next generation under the constraint of a constant selection intensity. They made no analytical derivations but applied quadratic programming to get numeric solutions. Their approach should give the same numerical results as ours in Section 3, as in principle the assumptions are equivalent, though differently expressed. Using simulations, Toro and Nieto found that if different expected contributions are used in a breeding programme, then the long term inbreeding effect will also be smaller than for truncation selection. Evidently, this is an advantage.

Kang and Namkoong (1988) showed that there are situations where, for a given selection intensity, truncation selection generates a smaller inbreeding effective population size than other selective breeding schemes. They did not try to optimize. Somewhat mysteriously, Kang (1989) found by numerical experiments that for breeding values following the normal distribution increasing linear weighting functions did not lead to improved selection. The mystery disappears if his weighting functions are compared to the optimal functions of the form $\beta \cdot (g—a)\ \varphi(g)$, where φ is the standard normal density. These latter functions are decreasing in the right tail.

Groups

We have studied diversity when there is a resemblance within groups of clones. There is no well-established genetic theory for expressing "effective number" or "diversity" when there are clones with different degree of similarity as far as origin is concerned. In spite of that this is a common situation. The theory is better established for the case that there is a single population with a family structure (Falconer, 1981, Chapter 9).

We interpret the similarities as correlations, cf. the beginning of Section 4. Letting the groups be families, we should then have $c_i = 1$ if all the clones within a group are identical, $c_i \approx 0.5$ if they are full sibs, and $c_i \approx 0.25$ if they are half-sibs. Thus the optimization method can easily be implemented in practice when the clones in a seed orchard can be arranged in family groups.

It ought to be possible to assign c-values also to other types of groups than families, like the different origins in the Maglehem case. The c-values above for families indicate that these values should be kept rather low (unless there is coancestry). The c-values could possibly be inferred from measured values of a lot of characteristics (maybe on a molecular level!) that might be relevant from the damage point of view or the inbreeding point of view. At least the first author of this paper, who prefers to think in terms of future damages, means that the within group correlations for the g-values should not be used.

We suggest that optimal thinning (with separate lines for the groups) is practically applied on seed orchards where for some reason the clones are grouped.

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Improvement of Larch Micropropagation by Induced Short Shoot Elongation in Vitro

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Abstract

In vitro shoot propagation in larch using the ability of axillary buds to elongate and form long shoots is mainly limited by occurrence of short shoot formation. To increase the rate of shoot propagation a stem elongating treatment was used on short shoots from hybrid larch clones. For in-

duction the shoots were cultured on media containing different cytokinin/auxin combinations. They were kept at 17° C and exposed to a 16 h white light photoperiod. After 4 weeks they were transferred to hormone-free medium supplemented with 1 mM l-glutamine and cultured under continuous red light at 23° C. Kinetin (0.5 mg l^{-1}) + IAA (0.05 mg l^{-1}) was the best hormone combination in 4 clones tested. Depending on the genotype, up to 64.3% of the short shoots elongated in the course of 8 weeks. Based on newly formed axial buds a continuing propagation scheme is presented. Up to 98.2% of the newly formed long shoots rooted under semi-sterile conditions.

Key words: Elongation of short shoots, *Larix* x *eurolepis*, micropropagation, organogenesis, shoot tip tissue culture, rooting.

Zusammenfassung

Ausgehend von Axillarknospen mit ihrer Fähigkeit zur Langtriebbildung wird die Sproßvermehrung der Lärche in vitro hauptsächlich durch die Bildung von Kurztrieben eingeschränkt. Um die Sproßvermehrungsrate zu erhöhen, wurden Kurztriebe von Hybridlärchen, welche sich zuvor 3 bis 7 Jahre in Kultur befanden einer Behandlung zur Auslösung ihres Sproßstreckungswachstums unterzogen. Dazu wurden die Kurztriebe 4 Wochen auf verschiedenen Cytokinin/Auxin Kombinationen bei 17° C in 16stündigem Weißlicht per Tag kultiviert, um sie danach auf einem hormonfreien, 1 mM Glutamin enthaltenen Medium in kontinuierlichem Rotlicht bei 23° C zur Sproßstreckung zu bringen. Bei 4 Klonen erwies sich die Hormonvariante mit 0,5 mg l^{-1} Kinetin + 0,05 mg l^{-1} IAA als die wirkungsvollste; abhängig vom Genotyp gingen im Verlauf von 8 Wochen bis zu 64,3% der Kurztriebe zum Sproßstreckungswachstum über. Dadurch wurde es möglich, das bereits existierende Verfahren zur Sproßvermehrung erheblich zu verbessern. Die neu gewonnenen Langtriebe erreichten unter semisterilen Kulturbedingungen Bewurzelungsraten bis zu 98,2%.

Introduction

Inter-specific crossings in larch breeding result in genetic improvement in general (PÂQUES, 1989). For some regions, such as middle european mountain sites beneath 1000 m exposed to air pollution, crosses between the European larch and the Japanese larch are suitable in afforestation of damaged spruce forests (HERING et al., 1989).

Large scale cloning of sexually created larch hybrids has been applied successfully (SCHACHLER et al., 1991). Because time is a major constraint in tree improvement programs, in vitro propagation techniques have to be integrated to speed up the process of introducing improved characteristics of trees achieved by selection and breeding (CHELIAK and ROGERS, 1990).

Regarding juvenile larch, reliable methods of adventitious shoot propagation have been developed (DINER et al., 1986; MULCAHEY and KARNOSKY, 1986). For adult trees (up to 30 years old) other groups established methods for in vitro propagation (BONGA and ADERKAS, 1988; LALIBERTE and LALONDE, 1988; BONGA and POND, 1991; ZHIHUA et al., 1991).

Recently, techniques have been developed to regenerate larch embryos from diploid (KLIMASZEWSKA, 1988) and haploid tissues (ADERKAS and BONGA, 1988) or both (ADERKAS et al., 1989), however, for mass propagation and product definition it requires much more research. Regeneration of whole plants from isolated protoplasts was achieved for *Larix* x *eurolepis* (KLIMASZEWSKA, 1989).

Only a few papers have reported application of larch micropropagation on a large scale. To achieve economic plant production via in vitro culture, the advantages of different vegetative methods have to be combined (KARNOSKY and DINER, 1986). To reach substantial cost reductions in larch micropropagation it has been recommended to improve the rate of shoot elongation and the frequency of rooting (KARNOSKY and VERVILLE, 1988).

In preliminary experiments it was shown that shoot tips of young larches are appropriate as starting material for micropropagation by using a basal medium supplemented with 1 mM to 5 mM l-glutamine (HÜBL and ZOGLAUER, 1991). Unfortunately the rate of multiplication depended on the long shoot/short shoot ratio. Long shoots were able to elongate and to form new meristems in the leaf axils whereas short shoots failed to do so.

Our study is aimed at increasing clone sizes of larch by supporting the elongation of stems of short shoots by phytohormonal treatment in addition to a varied light and temperature regime. Results of rooting experiments with these shoots will be shown.

Materials and Methods

For all experiments, shoots were chosen from clones derived from controlled crosses made by the Research Centre for Forestry and Forest Products Eberswalde (WEISER, 1992) and the Sächsische Landesanstalt für Forsten, Department of Experimental and Research Work at Graupa (HERING and BRAUN, 1990) between superior trees of *Larix decidua* and *Larix leptolepis (Table 1)*. Clones had been established in vitro from cuttings of young seedlings (HÜBL and ZOGLAUER, 1991) and were subcultured bimonthly as shoots for 3 years to 7 years before they were used in our experiments.

To induce shoot elongation non-growing short shoots, previously excised from the stem base, were exposed to changed light and temperature conditions during that they

Table 1. — Specification of *Larix* x *eurolepis* clones used in the experiments: 657, 220 European larch; W5, 219 Japanese larch.

clone	1	2	3	4
crossing number	657 x W5	657 x W5	PG12	220 x 219
year of crossing	1986	1986	1982	1987
years in culture	5	5	7	3

Table 2. — Percentages of elongated short shoots 8 weeks after different combined treatments; different subscripts indicate significant differences on a $P < 0.025$ level.

treatments	elongated shoots %	± S.D.
(a) light and temperature without growth regulators	5.1^a	± 3.9
(b) light, temperature and 0.3 mg l^{-1} kinetin + 0.2 mg l^{-1} zeatin + 0.05 mg l^{-1} IAA	43.7^b	±20.6
(c) light, temperature and 0.5 mg l^{-1} kinetin + 0.05 mg l^{-1} IAA	63.6^d	± 6.6
(d) light, temperature and 1 mg l^{-1} kinetin + 0.05 mg l^{-1} IAA	52.9^c	± 1.8

were supplied with phytohormones. For this, they were incubated in ERLENMEYER flasks on 25 ml half strength MCM medium (BORNMAN, 1981) lacking urea and containing 1.5% saccharose and combinations of cytokinins and indole-3-acetic acid (IAA) or α-naphthalene acetic acid (NAA). In addition, this medium was supplemented with 100 mg l^{-1} (0.574 mM) l-arginine, 100 mg l^{-1} (0.684 mM) l-glutamine and 100 mg l^{-1} polyvinylpyrrolidone (poly[1-vinyl-2-pyrrolidone 432], SERVA 33422). During so-called induction, the photoperiod was shortened from 24 h to 16 h per day and the light quality was changed from red to white light (Narva tubes LS 40 white, radiation 30 μE m^{-2} s^{-1}) meanwhile the temperature was reduced by 5 K to 17° C. After 4 weeks the explants were transferred to hormone free medium B (BOULAY, 1979) which contained 1 mM l-glutamine and 3% saccharose and stored at 23° C under continuous red light (Narva tubes LS 65 red W 93, radiation 30 μE m^{-2} s^{-1}, 650 nm peak emission). The effect of light quality and temperature during the induction treatment was tested without the shoots being exposed to plant growth regulators. All media used were supplied with 0.5% to 0.6% agar and pH-adjusted to 5.7 to 5.8 before autoclaving.

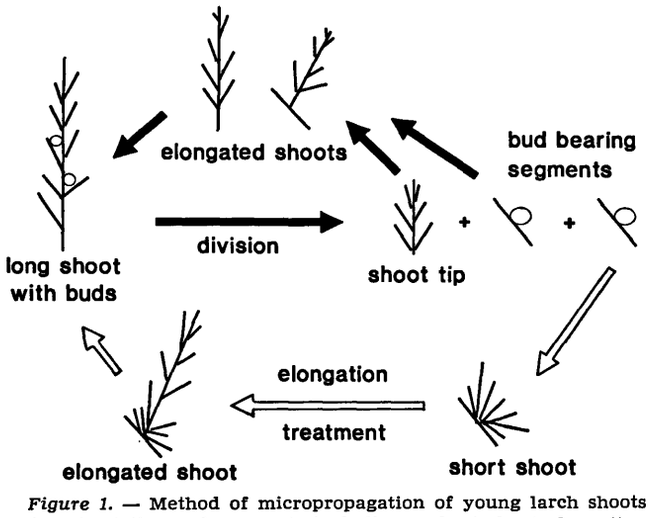

Figure 1. — Method of micropropagation of young larch shoots using shoot tips and segments including a short shoot elongation treatment.

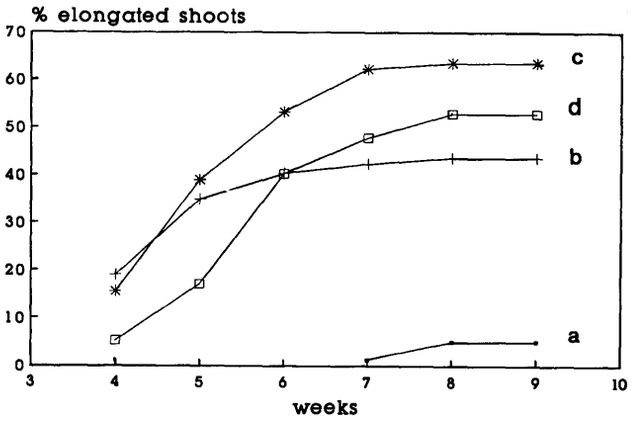

Figure 2. — Cumulative progress of long shoot formation from short shoots of clone 3 after light and temperature treatment without plant growth regulators (a), and the effect of 3 phytohormone treatments in addition to similar light and temperature conditions: 0.3 mg l^{-1} kinetin + 0.2 mg l^{-1} zeatin + 0.05 mg l^{-1} IAA (b), 0.5 mg l^{-1} kinetin + 0.05 mg l^{-1} IAA (c), 1 mg l^{-1} kinetin + 0.5 mg l^{-1} IAA (d).

A statistical analysis was carried out to ensure differences between treatments of long shoot formation in clone 3. Using the Welch test, significances are given in *table 2* based on arcsine transformed percentages of elongated shoots 8 weeks after the end of the treatment. Percentages shown in *figure 2* are means from at least 3 samples representing 50 to 100 short shoot explants each.

In the course of 12 months the propagation rates of clones, produced by the process shown in *figure 1* were ascertained. Shoots were subcultured at 7-week intervals by cutting them into shoot tips and bud bearing segments. Shoot tips and segments were placed on hormone free medium B (*Figure 1*, shaded arrows) whereas short shoots, formed by axial buds in the former culture step, were submitted to elongation treatment (*Figure 1*, unshaded arrows). The rate of propagation was calculated from the number of shoots obtained after 7 weeks, divided by the initial shoot number. Determining the yearly propagation rate the following equation was used:

$$pr_y = pr_m^{(k-1)} \quad (1)$$

where pr_y = yearly propagation rate
pr_m = mean propagation rate per subculture
k = number of subcultures per year.

For rooting, newly formed shoots were given a root-inducing treatment by incubating them on a modified MS-medium (MURASHIGE and SKOOG, 1962) with 2 mg l^{-1} NAA. The macroelements were reduced to one third and the medium was supplied with 0.5% saccharose. During root induction and development the cultures were kept at 17° C. All shoots used had a length of approximately 2 cm and no visible bud primordia.

After 2 weeks the explants were placed into dishes, each containing 5 l of a mixture of equal parts of humus and perlite. The substratum was autoclaved at 134° C for 20 min, then cooled and autoclaved a second time in the same way. Further plantlet development was supported by covering the dishes with plastic hoods and moistening the air under the hoods by spraying of destilled water frequently. After 5 months the plantlets were removed from the humus-perlite mix to determine root development and planted into soil.

Results

Firstly, the stimulation of long shoot development was carried out by treating clone 3 with different cytokinin/IAA combinations under changed light and temperature conditions. The elongation rate varied for different phytohormone treatments, but without plant growth regulators the effect of light and temperature was low (*Figure 2*). Of the 3 phytohormone treatments, 0.5 mg l^{-1} kinetin + 0.05 mg l^{-1} IAA gave the best results because 63.6% of short shoots had elongated their stems after 8 weeks (*Table 2*). Shoot elongation caused by plant growth regulators under changed light and temperature conditions was evidently higher than by changing conditions of light and temperature alone [*Figure 2*: (b), (c), (d) vs. (a)].

Stem elongation in short shoots of different clones were obtained with 0.5 mg l^{-1} kinetin or 0.5 mg l^{-1} BA combined with 0.05 mg l^{-1} IAA or even 0.05 mg l^{-1} NAA under identical light and temperature conditions (*Figure 3*). The highest number of induced long shoots was obtained by the kinetin/IAA combination. For this treatment all clones ranked first. The duration of precultivation (age of clones) was not a critical factor.

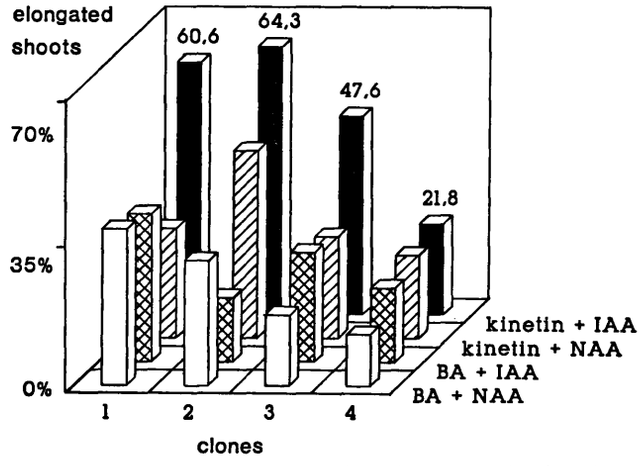

Figure 3. — Influence of different phytohormone treatments on the elongation behaviour of short shoots of 4 larch clones.

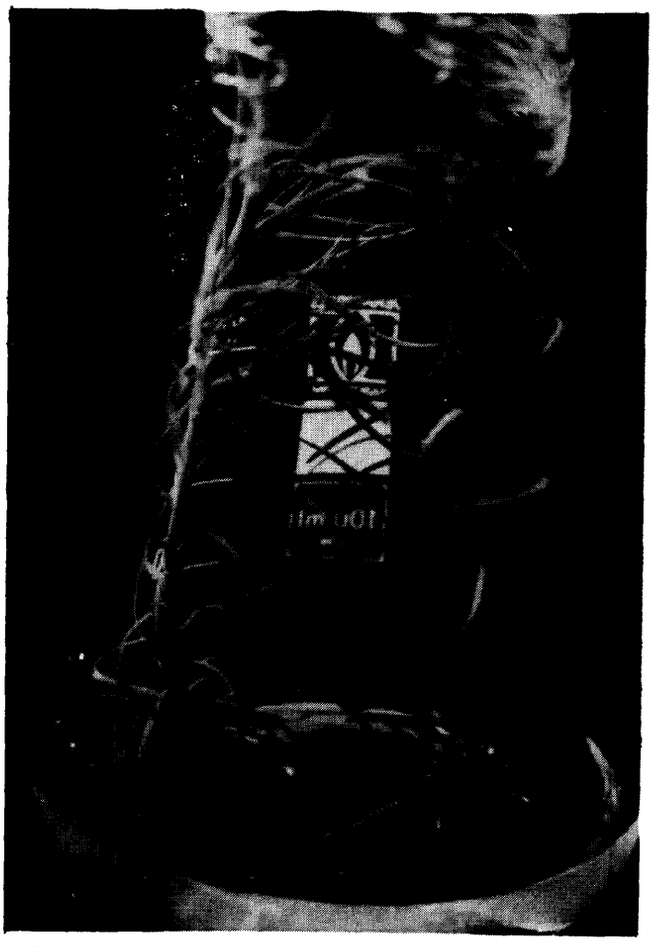

Figure 4. — Elongated shoots of hybrid larch short shoots 3 months after treatment with 0.5 mg l^{-1} kinetin + 0.05 mg l^{-1} IAA.

In all cases the elongated stems grew up to the top of the vessels within a period of 3 months (*Figure 4*). During this time they formed bud primordia which developed into axial, ready to flush buds. The new buds remained closed by green coloured scales until the shoots were cutted in one shoot tip and several segments each to transfer it back into propagation subcultures. After flushing, buds developed either into new long or short shoots, ready for segmentation or for described stem elongation treatment, respectively.

By this, the propagation rates of 4 clones were established in the course of 7 subcultures. As *table 3* shows, the total number of explants per clone was multiplied up to 5.2 fold (clone 2) per 7 weeks, whereas the pr_m extends from 2.4 in clone 3 to 3.0 in clone 2. The pr_y is shown as calculated term according to (1).

Mean values for rooting percent are given in *table 4*. Results comprise well adapted plants because the explants were rooted and hardened simultaneously. The root system was well branched and had numerous hair-covered roots with actively growing red coloured root tips.

Within a period of 5 months all rooted plants elongated whereas unrooted shoots lost their vitality (*Figure 5*). Actively growing plantlets were potted in soil and then

Table 3. — Rates of propagation per subculture (7 weeks) in the course of 7 subcultures (1 year) mean propagation rate per subculture (pr_m) and yearly propagation rate (pr_y) of 4 larch clones: the number of explants per clone varied between 25 and 100 at the beginning of the first subculture.

clone	subculture							pr_m	pr_y
	1	2	3	4	5	6	7		
1	3,2	3,5	1,4	2,7	1,8	2,2	3,2	2,6	308,9
2	2,0	1,7	5,2	2,3	3,0	1,9	4,2	3,0	594,8
3	2,6	1,9	2,7	1,7	3,1	2,0	2,6	2,4	191,1
4	2,9	1,6	3,2	2,0	3,0	1,9	2,8	2,5	244,1

Table 4. — Percentage of shoot tips derived from short shoots that formed roots and grew up after 5 month. Each number is the mean (± S. D.) of 3 replicates per clone; every replicate consists of 50 explants.

clone 1	clone 2	clone 3	clone 4
98,2 ± 6,6	59,6 ± 9,7	73,0 ± 1,9	94,1 ± 10,2

transferred to the greenhouse. During the first growing season the plantlets were staked to overcome the plagiotropic growth habit (*Figure 6*).

Discussion

Cloning of selected individuals in forest tree breeding requires reliable techniques of vegetative propagation with high uniformity and low costs. In larch, in vitro propagation via axial bud elongation is limited by the occurrence of short shoots. On phytohormone free medium as it was used by HÜBL and ZOGLAUER (1991), the stems of short shoots failed to elongate.

Application of cytokinins and auxins to short shoots and a change of light and temperature resulted in stem elongation in 4 clones of hybrid larch. As regard light and temperature regime, a slow response could already be ascertained only by changing from white to red light, a longer photoperiod and an increase in temperature after the induction treatment.

As it stated in numerous reports, light quality and daily photoperiod is considered as decisively in plant morphogenesis. In *Picea*, the elongation of adventitious shoots was stimulated by lengthening the photoperiod (PATEL and THORPE, 1986). KADKADE and WETHERBEE (1983) observed that red light (660 nm peak emission) alone was efficient to stimulate growth of axillary shoots in asparagus tissue culture. Far-red light supported elongation of adventitious shoots in *Picea abies* (BORNMAN, 1983), whereas KADKADE

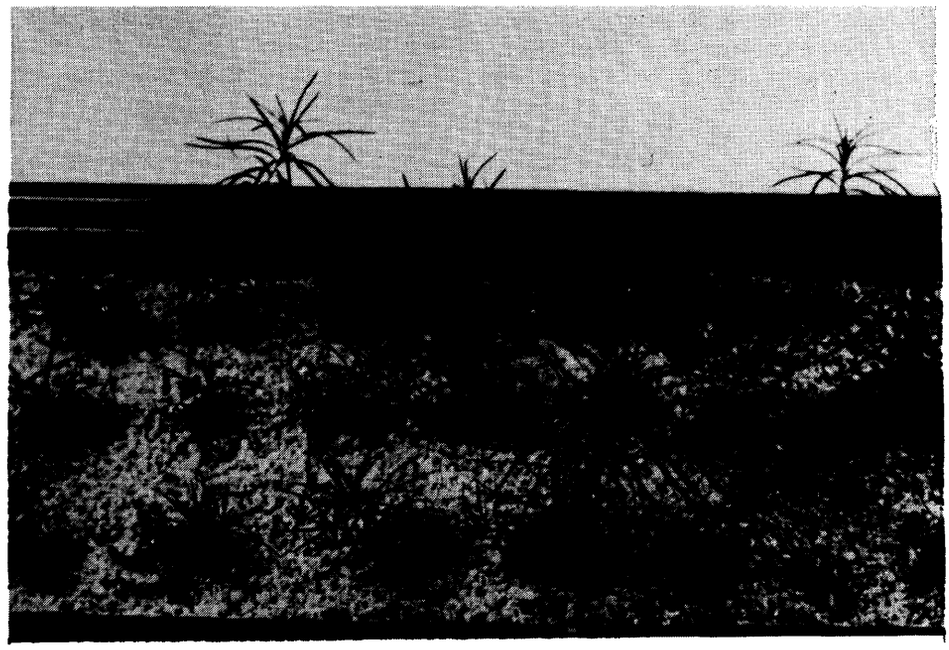

Figure 5. — Hardened and sprouting hybrid larch explants 5 months after root inducing treatment with 2 mg l^{-1} NAA.

Figure 6. — Potted hybrid larch plants derived from short shoots.

and JOPSON (1978) reported about red-light-stimulated formation of adventitious buds in Douglas fir.

We obtained that, except sporadically, a continuous red light culture regime did not provoke stem elongation of short shoots, however, if shoots had undergone a shortened, white light photoperiod for 4 weeks, stem elongation took place 7 weeks after the exposure. It appeared that the transition between the different light conditions was the reason to set a shoot growth signal likewise hormone application can do it on a higher level.

However, light and temperature slightly caused stem elongation of short shoots, but an additional hormonal treatment was more efficient. A kinetin/IAA ratio of 0.5 to 0.05 (mg l^{-1}) was the best growth regulator combination whereas the kinetin/NAA treatment had a little lower effect. LISOVKA (1985) reported that a kinetin/IAA combination was a better tracheid stimulator than kinetin/NAA in spruce, and for application of IAA in *Pinus*-segments, SHERIFF (1983) observed an increase of tracheid lumen and wall thickness. This indicates that the induction of short shoot stem elongation may be closely correlated to the formation and differentiation of tracheids.

Rooting and acclimatisation of micropropagules continues to be a major problem for commercial micropropagation. Application of auxins have been described as usually for root initiation, but vigour and quality of adventitious roots are insufficient if solidified media were used (MOHAMMED and VIDAVER, 1988).

We obtained rooting rates up to 100% and high quality of root systems when we used a semisterile substratum after induction with NAA. For root development controlled environmental conditions are obviously very important. Slightly reduced temperature has been described as beneficial for root initiation and development (POISSONIER et al., 1980; HÜBL and ZOGLAUER, 1991), whereas CARVILLE (1979) rooted dormant conifer cuttings by applying a higher temperature to the bases of the cuttings. We noticed, survival of plantlets during rooting and hardening was high, if the temperature was kept at 17° C to 18° C.

Short shoot stem elongation allows to improve shoot propagation rates because the combined treatment led to incessant shoot growth and formation of new axillary buds. In a repeatable process of culture steps (*Figure 1*) the yearly propagation rate (pr_y) in the best clone was about 600. Thus, described method promises an approach to fit larch micropropagation as a tool of selection if regeneration under the maintenance of known characteristics of superior or rare, juvenile genotypes is desired.

It has to be emphasised, that good results were obtained with explants that have been 3 to 7 years in culture. Clones of different age revealed different growth behaviour: The youngest clone was less competent to respond to elongation treatment than the older ones, whereas the lowest propagation capacity was found both at the oldest and youngest clone. In further studies we intend to use long-time-cultured clones of hybrid larches as a model to enlighten growth patterns under conditions correlated with ageing.

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A System for Repeatable Formation of Elongating Adventitious Buds in Norway Spruce Tissue Cultures

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Abstract

Seedlings of Norway spruce (*Picea abies* L. Karst.) were induced with cytokinin combinations to form adventitious buds. A repeatable induction and formation of elongating buds was obtained via an induction cycle with cytokinins showing low bud-inducing capacity (zeatin, kinetin, 2iP), followed by subculture periods on media without plant growth regulators. Newly formed adventitious buds could be induced again without loosing their ability for elongation. One of the most effective phytohormone combinations for induction of elongating buds was 0.5 zeatin + 0.05 kinetin (mg l^{-1}) for 3 weeks. Zeatin in a concentration range (0.1 mg l^{-1} to 0.2 mg l^{-1}) stimulated the shoot elongation of preexisting terminal meristems in adventitious buds used for repeated cytokinin induction. 2iP promoted shoot formation and elongation in a concentration range from 0.1 mg l^{-1} to 0.5 mg l^{-1} depending on the explant type (adventitious bud/shoot) used.

Key words: Norway spruce, adventitious buds, repeatable formation, shoot elongation, gelling agents.

Zusammenfassung

Sämlinge von Fichte (*Picea abies* L. Karst.) wurden zur Adventivknospenbildung mit verschiedenen Cytokininkonzentrationen induziert. Eine wiederholbare Induktion und Bildung sich streckender Adventivknospen wurde durch den ständigen Wechsel eines Induktionszyklus mit Cytokininen, die eine geringe Induktionswirkung hinsichtlich der Anlage neuer Adventivknospen aufwiesen (Zeatin, Kinetin, 2iP), gefolgt von Subkultivierungsperioden auf phytohormonfreien Nährmedien erreicht. Neugebildete Adventivknospen konnten erneut induziert werden, ohne ihre Fähigkeit zur Sproßstreckung zu verlieren.

Eine der wirksamsten Cytokininkombinationen zur Induktion sich streckender Adventivknospen war 0,5 mg l^{-1} Zeatin + 0,05 mg l^{-1} Kinetin über einen Zeitraum von 3 Wochen. Zeatin im Konzentrationsbereich von 0,1 mg l^{-1} bis 0,2 mg l^{-1} stimulierte die Sproßstreckung bereits existierender Meristeme in den zur wiederholten Adventivinduktion eingesetzten Adventivknospen.

2iP förderte die Sproßbildung und Streckung in einem Konzentrationsbereich von 0,1 mg l^{-1} bis 0,5 mg l^{-1} in Abhängigkeit vom verwendeten Explanattyp (Adventivsproß/-knospe). Abbreviations: BA — 6-benzylaminopurine; PVP — polyvinylpyrrolidone, 2iP — 6-($\gamma\gamma$-dimethylallylamino)purine.

Introduction

The formation of adventitious buds in different spruce species has been described by several authors (Bornman, 1983; von Arnold, 1984, 1985; Rumary and Thorpe, 1984; Patel and Thorpe, 1986). A repeated induction of adventitious buds has also been described by Bornman (1987).

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