

Growth and Performance of Micropropagated Hybrid Larch Clones

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Dedicated to Dr. JOCHEN KLEINSCHMIT on the occasion of his 65th birthday

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Abstract

Hybrid larch (*Larix x eurolepis* HENRY) seed from controlled pollinations were micropropagated by a system using nutrient media free of phytohormones. Elongating shoots were multiplied by cutting these shoots into bud-bearing shoot segments which were able to sprout again. The shoots were rooted after an auxin treatment, transferred to the soil and planted in a field trial. The growth and performance (stem form) were recorded five years after planting in the field. Micropropagated clones showed a good growth performance (3.9 m average height and 3.7 cm average diameter at breast height).

The resulting stem form is different between the clones and not related to the propagation system. The origin of the seed (combination) is discussed as a possible reason. No plagiotropic growth could be observed after five years. This is the first time that a field trial with hybrid larch micropropagated via shoot segments was reported after 5 years in the field.

Key words: *Larix decidua* x *Larix kaempferi*, tissue culture, field trial, clones.

Introduction

The outstanding growth of hybrids between European and Japanese larch has been well known for almost one hundred years (HENRY and FLOOD, 1919). Since that time many activities were carried out aimed at breeding of hybrid larch (*Larix x eurolepis* HENRY). Numerous breeding programmes with selected parent trees of both species were initiated and a large number of progeny trials have been established and evaluated (e.g. HERING, 1990; KEIDING, 1980; WEISER, 1992; LANGNER and SCHNECK, 1998). As a result of such field trials trees with a good combining ability were selected and used to establish seed orchards.

These progeny trials often show single trees with an outstanding growth and stem form, far above the average of all the progeny and in some cases with an enhanced resistance of the wood against fungal attacks (LESNINO et al., 1997). The selection and use of such elite trees would lead to a higher genetic gain. True to type reproduction of such superior trees is possible by vegetative propagation exclusively. This paper provides the first results of micropropagated hybrid larch clones. The trial which was established aimed at the investigation of an influence of the chosen tissue culture propagation method on the tree growth in the field. Special attention focused on possible plagiotropic growth because it has been observed in several cases after micropropagation (BOULAY, 1987).

Material and Methods

Plant material

Hybrid larch seed produced by controlled crosses (Tab. 1, column 2) were disinfected and germinated on a sterile nutrient medium as described elsewhere (HÜBL and ZOGLAUER, 1991;

KRETZSCHMAR, 1993; KRETZSCHMAR and EWALD, 1994). The growing shoot axis was excised and cultured on a half-strength medium according to BOULAY (1979) with the addition of glutamine (146 mg l⁻¹) for 7-week subculture cycles. Elongating shoots of fast growing clones (Tab. 1) were divided into shoot tips and bud bearing stem segments which can sprout and form long shoots. When short shoots were formed, different treatments were used to stimulate their elongation (cytokinins, light, temperature, Fig. 1) which have been developed for juvenile larch (KRETZSCHMAR, 1993). Rooting of long shoots was achieved when shoots were induced with 2 mg l⁻¹ naphthalene acetic acid (NAA) on a reduced nutrient medium according to MURASHIGE and SKOOG (1962). After 2 weeks the explants were planted into JIFFY peat pellets and placed in propagation boxes. After three months rooted shoots were potted in containers and transferred to the greenhouse. After five months the plants were transferred to the nursery and planted in a field trial after two and a half years. All plants showed some plagiotropic growth directly after transfer to the soil which disappeared in the following years (EWALD, 2000). Unfortunately no seedlings of hybrid larch were available, thus two-year-old plants derived from a commercial seedlot of European larch (*Larix decidua* MILL.) were planted for comparison together with the clones.

Table 1. – Plant material used for micropropagation and the resulting field trial.

clones	progenies from which the clones originated
HL 3	<i>Larix decidua</i> (Sudeten mountains) x <i>Larix kaempferi</i>
HL 86/2	<i>Larix decidua</i> (Sudeten mountains) x <i>Larix kaempferi</i>
HL 86/6	<i>Larix decidua</i> (Sudeten mountains) x <i>Larix kaempferi</i>
HL 86/11	<i>Larix decidua</i> (Sudeten mountains) x <i>Larix kaempferi</i>
HL 86/16	<i>Larix decidua</i> (Sudeten mountains) x <i>Larix kaempferi</i>
GL 57	<i>Larix decidua</i> (Tatra mountains) x <i>Larix kaempferi</i>
GL 76	<i>Larix decidua</i> (Tatra mountains) x <i>Larix kaempferi</i>
GL 91	<i>Larix decidua</i> (Sudeten mountains) x <i>Larix kaempferi</i>

Field trial

Eight clones of different hybrid larch combinations (Tab. 1) and the seedlings of European larch were planted on a site formerly used for agriculture. Twelve (in some cases 24 trees) per entry were planted in three complete blocks (four or eight trees per plot). Three replicates of 64 trees of clones HL 86/2 and HL 86/6 and the seedlings were planted on the trial area. In the first year it was necessary to irrigate the plants because of a long drought period. Plants which died during the year of planting were replaced the following year.

The first assessment of height, diameter at breast height (dbh) and stem form took place in the field in autumn 1999 five

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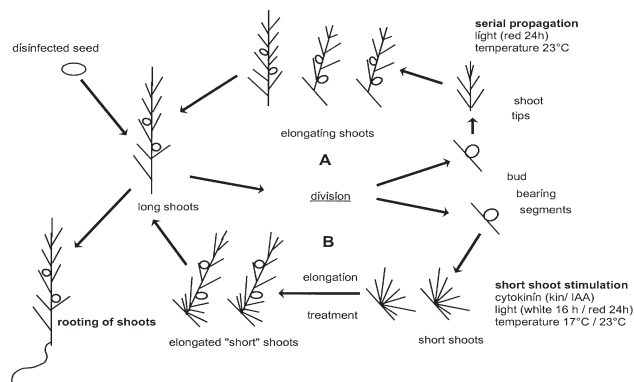


Fig. 1. – Scheme of in vitro propagation of larch seedlings (A – serial propagation of shoot segments, B – stimulation of short shoots).

years after the planting. The stem form was evaluated following a five class scale (1 – straight; 2 – light crookedness but probably useful as sawnwood in future; 3 – light curved, medium crookedness; 4 – medium curved, stronger crookedness; 5 – severe curved and crooked).

Clone effects were tested following an analysis of variance on individual data according to the model:

$$y_{ijk} = \mu + c_i + b_j + e_{ij(k)}$$

with μ = general mean; c_i = effect of clone i ; b_j = effect of block j ; $e_{ij(k)}$ = residual error.

All calculations were done with the help of PROC GLM of the SAS statistic package.

Results

The trial developed well after some problems in the first year. Mean height over all clone plants was 3.90 m five years after planting, the seedlings of European larch reached 3.10 m. Significant differences were observed between clones concerning height, dbh and stem form (Tab. 2). So the best growing clone was 40 % taller than the worst one (Fig. 2 and 3).

The variability for growth traits within the clones did not differ significantly from that of the seedlings. The coefficient of variation for height was between 11% and 32% for clones and 20% for the seedlings.

The results concerning the stem form were more different. Some clones (HL 86/2, HL 86/6, HL 86/11, HL 3) produced only a few or no trees with well formed stems (class 1 and 2; Fig. 2 and 4). The largest number of well formed trees was observed for a hybrid larch clone (GL 57 – Fig. 5). Despite differences in stem form of the different clones, the plagiotropic growth behaviour which was observed shortly after transfer to the soil, completely disappeared after the first few years.

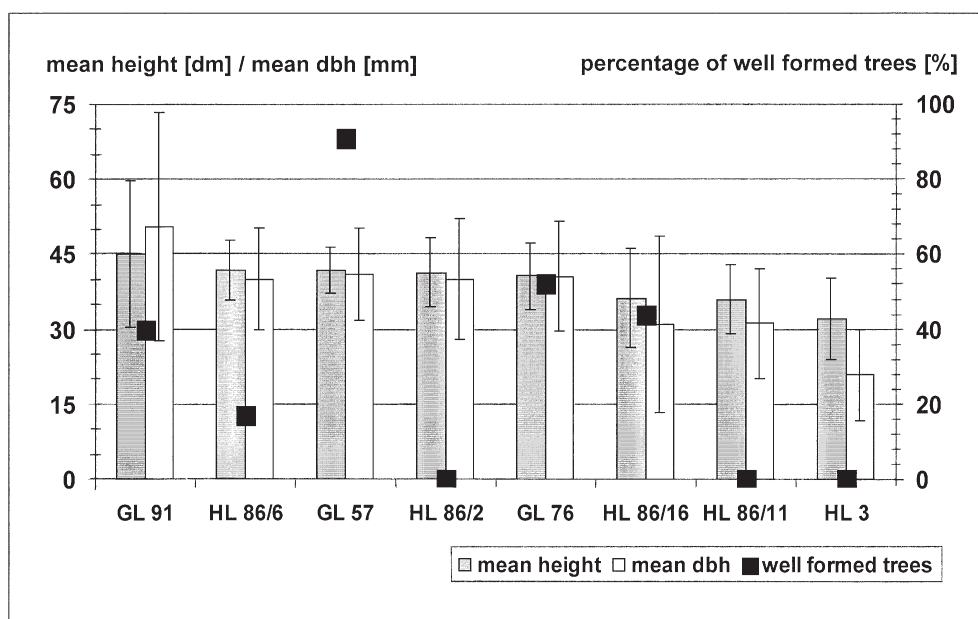


Fig. 2. – Clonal test "Brigittenhof" – height, dbh and stem form at the age of 8 years (error bars show the standard deviation).

Table 2. – Analysis of measured criteria of the clones (tree height, diameter at breast height (dbh) and stem form) at the age of 8 years based on ANOVA.

Source	Height			dbh		Stem form	
	DF	MS	F	MS	F	MS	F
Clone	7	20420.96	3.32 *	871.52	5.35 *	8.18	11.53 *
Block	2	10724.51	1.74 ^{ns}	358.82	2.20 ^{ns}	1.95	2.75 ^{ns}
Error	98	6149.41		162.90		0.71	

* – significantly different at $\alpha = 0.01$

ns – non-significantly different



Fig. 3. – Hybrid larch clone GL 91 with outstanding growth performance (in the centre of figure).



Fig. 4. – Hybrid larch clone HL 86/2 with poor stem form.



Fig. 5. – Hybrid larch clone GL 57 with the largest number of well formed stems.

Discussion

The clones of micropropagated seedlings were selected according to their ability to be micropropagated and thus represented average of the seed material and were not pre-selected according to growth performance or growth behaviour in the nursery.

Nevertheless these first results give evidence that plants of hybrid larch propagated by tissue culture grow well, despite the appearance of a plagiotropic growth after plant regeneration. The reasons for the plagiotropic growth behaviour shortly after transfer to the soil and the recovery after transfer in the field, may result from the micropropagation method and the time required to form a proper root system in comparison with other micropropagation systems (e.g. somatic embryogenesis), which has already been discussed elsewhere (EWALD, 2000). Similar observations concerning plagiotropic growth of a part of the material were described for stecklings of juvenile larch, but the plagiotropic growth was also overcome (EDSON et al., 1996). EDSON et al. (1996) also recorded differences in the branch structure (sparse branching) in micropropagated plants during the greenhouse period and after establishment in the nursery. This growth behaviour became normal after transfer to the field, although no special measurements concerning this trait were carried out. It was observed that the limited root system of plants, especially of those cultured for several years in containers, prevented the resumption of orthotropic growth. Thus the size of containers used and the duration in the container should be selected so that constricted root growth is avoided.

The superiority of most hybrid larch clones compared with the seedlings of European larch is similar to the observations of the growth behaviour of the progenies from which these clones were selected (HERING et al., 1989; WEISER, 1984).

In some cases variation for growth traits within the clones did not differ from that of the seedlings, although a lower variation within clones could be assumed based on their genetic identity. One reason for this variation within the clones may be related to the small number of trees per clone. No difference in variation between clones of hybrid larch and sexually propagated progenies could be detected in field trials of other authors (PÁQUES and CORNU, 1991).

No selection for any trait was possible at the start of the propagation cycle due to use of seedlings as starting material. Therefore the behaviour of stem form of the propagating clones was by chance. The poor stem form of some clones observed in this trial is in accordance with results of other investigations of hybrid larch propagated sexually (HERING et al., 1989; PÁQUES, 1992). The origin of European larch as mother tree is important for good stem form in hybrid larch. Mostly progenies with mother trees from the Alps have better stem forms than those derived from trees originating from the Sudeten mountains (SCHNECK and LANGNER, 2000).

The results of this field trial provided a first evidence for the suitability of tissue culture techniques to reliably propagate juvenile clones of hybrid larch. The absence of plagiotropic growth behaviour in the micropropagated plants after a few years in the field is promising and shows that this propagation method does not have an adverse effect on the later development of the trees.

These results offer the possibility for further investigations concerning the propagation of older well formed trees with high performance or special wood characteristics of hybrid larch which were selected in progeny trials by means of tissue culture or stecklings after rejuvenation (EWALD, 1998).

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References

BOULAY, M.: Propagation „in vitro“ du Douglas par micropropagation de germination aseptique et culture de bourgeon dormants (*Pseudotsuga menziesii* (MIRB.) FRANCO). In: Micropropagation d'arbres forestiers AFOCEL, Nangis, France: 67–75 (1979). — BOULAY, M.: Conifer micropropagation: Applied research and commercial aspects. In: BONGA, J. M.

and DURZAN, D. J. (eds) : Cell and Tissue Culture in Forestry. Vol 3. Case histories: Gymnosperms, Angiosperms and Palms. Martinus Nijhoff Publishers, Dordrecht, 185–206 (1987). — EDSON, J. L., WENNY, D. L., FINS, L. and ROBERTS, L. W.: Growth and form of Western larch stecklings: plagiotropism and reiteration. Can. J. For. Res. **26**, 1273–1283 (1996). — EWALD, D.: Advances in tissue culture of adult larch. In vitro Cell. Dev. Biol.-Plant **34**: 325–330 (1998). — EWALD, D.: Is plagiotropic growth in micropropagated larch a marker for ageing? Proceedings of the international congress „Application of biotechnology to forest genetics“. Sept. 22–25, 1999. Vittoria-Gasteiz, Spain. 479–486 (2000). — HENRY, A. and FLOOD, A.G.: The history of the Dunkeld hybrid larch, *Larix eurolepis*, with notes on other hybrid conifers. Royal Irish Acad. Proc., Vol. XXXV, Sect. B, 55–66 (1919). — HERING, S.: Analysen zur quantitativen Genetik von Lärchenkreuzungsnachkommenschaften sowie Stand und Perspektiven der Kreuzungszüchtung bei der Gattung *Larix* unter besonderer Berücksichtigung ihres Anbaus in den Mittelgebirgen Sachsens. Dissertation, TU Dresden, (1990). — HERING, S., HAASEMANN, W. and BRAUN, H.: Ergebnisse eines 19-jährigen Anbauversuchs mit Hybrid-, Europäer- und Japanerlärchen im Rauchschatzgebiet des oberen Erzgebirges. Beiträge für die Forstwirtschaft **23**, 11–17 (1989). — HÜBL, S. and ZOGLAUER, K.: Entwicklung einer In-vitro-Vermehrungsmethode für züchterisch wertvolle Lärchen. Beitr. Forstwirtschaft **25**: 18–20 (1991). — KEIDING, H.: Hybridlärkens vækst og tilpasningsevne i forhold til de rene arter. Dansk Skovfor. Tidsskrift **65**, 204–234 (1980). — KRETZSCHMAR, U.: Improvement of larch micropropagation by induced short shoot elongation in vitro. Silvae Genetica **42** (4–5): 163–169 (1993). — KRETZSCHMAR, U. and EWALD, D.: Vegetative propagation of 140-year-old *Larix decidua* trees by different in-vitro-techniques. J. Plant Physiol. Vol. **44**, 627–630 (1994). — LANGNER, W. and SCHNECK, V.: Ein Beitrag zur Züchtung von Hybridlärche (*Larix x eurolepis* HENRY). J. D. Sauerländer's Verlag, Frankfurt am Main. pp. 159 (1998). — LESNINO, G., GROSSER, D., BEHM, A., WINDEISEN, E., SCHUMACHER, P. and WEGNER, G.: Biologische, physikalische, mechanische und chemische Holzeigenschaften von Hochzucht-Lärchen. Abschlussbericht zu Forschungsprojekt X27, Institut für Holzforschung der TU München (1997). — MURASHIGE, T. and SKOOG, F.: A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant **15**: 473–497 (1962). — PÁQUES, L. E.: First evaluation of genetic parameters in a factorial mating design with hybrid larch (*Larix decidua* x *Larix kaempferi*). In: WEISGERBER, H. (ed.): Results and future trends in larch breeding on the basis of provenance research. Proc. Meet. of the IUFRO Work. Party S2.02–07, Berlin. pp. 5–12 (1992). — PÁQUES, L. E. and CORNU, D.: Effect of vegetative propagation on field performance up to age 8 of hybrid larch (*Larix x eurolepis*) clones. Ann. Sci. For. **48**, 469–482 (1991). — SCHNECK, V. and LANGNER, W.: A long term breeding program of hybrid larch (*Larix x eurolepis* HENRY). In: DUNGEY, H. S., DIETERS, M. J. and NIKLES, D. G. (compilers): Hybrid breeding and genetics of forest trees. Proc. of QFRI/CRC-SPF Symposium, Apr. 9 to 14, 2000. Noosa, Queensland, Australia. (Dep. of Primary Industries, Brisbane). pp. 75–80. — WEISER, F.: Vorschlag von Hybrid-Hochzuchtsorten der Lärche. F/E-Bericht, A 4, Institut für Forstwissenschaften Eberswalde, 32 pp. (1984). — WEISER, F.: Tree improvement of larch at Waldsiedersdorf: Status and prospects. Silvae Genetica **41**, 181–188 (1992).