of combinations of block within site by clone (*bsc*) for trait 1, i.e. only present in the model for trait 1.

t = matrix of the uncorrelated random effects for block by clone interactions, and *t* is MVN~(0, T). T is a block-diagonal square matrix and has dimensions equal to the total number of blocks by clone combinations (*bc*) for traits 2 (n2) and 3 (n3), *i.e.* only present in the model for traits 2 and 3. The only non-zero elements for T are along the diagonal. Elements t_{11} through $t_{n2,n2}$ are σ^2_{bc2} and elements $t_{n2+1,n2+1}$ through $t_{n2+n3,n2+n3}$ are σ^2_{bc3}.

e = vector of random residual effects for traits *i* = 1, 2, and 3 and *e* is MVN~(0, R). R is a square matrix and has dimensions equal to the sum of the numbers of observations for traits 1 (n1), 2 (n3) and 3 (n3). R is block diagonal with the only non-zero elements occurring on the diagonal (no covariances among the error terms since the traits were not measured on the same observational units). The first n1 observations along the diagonal equal to σ^2_{e1}, the next n2 observations equal to σ^2_{e2}, and the final n3 observations equal to σ^2_{e3}.

The variance for observations (**V**) is:

$$\mathrm{Var}\begin{bmatrix} y_1 \\ y_2 \\ y_3 \end{bmatrix} = \mathbf{ZCZ' + NDN' + QKQ' + WTW' + R = V} \qquad (12)$$

where:

X, Z, N, Q and **W** are incidence matrices relating records to the fixed, clonal and uncorrelated random effects, described in sub-matrix form in the Appendix, Equation 1. **X, Z, N, Q** and **W** have dimensions equivalent to (*nxp*) where *n* = the total number of observations corresponding to traits 1, 2 and 3 and *p* is the number of levels for the modeled effects.

Performance of Chinese-fir (*Cunninghamia lanceolata* (Lamb.) Hook.) Plantlets from Upper-crown and Basal Origins as Modified by Grafting and Development as Buried Ramets before Explant Harvest

By Li Minghe and Huang Faxin

Central China Agricultural University, Wuhan, 430070, P. R. China

(Received 5th April 2000)

Abstract

Trees that had sprouts at their root collar were selected from a 28-year-old plantation of Chinese-fir. Upper branches, which probably had achieved a less-juvenile maturation state, were taken in the forest. One-year-old seedlings were dug out from a nursery and root systems were harvested below the cotyledon trace. The branches and root systems were grafted together and the grafts were planted as partly-buried donors for explant production. Basal sprouts of the same trees were rooted and similarly planted as explant donors. In addition, upper branches and basal sprouts were taken directly from a 20-year-old plantation as explants for culture with the above explants.

It was found that the average numbers of buds and shoots per plantlet, the average shoot length, the rooting percentage and average root length of the upper-branch-origin plantlets taken directly as explants were lower than those of the grafted and basal-sprout- origin explants. However, there were no consistent or significant differences in these characteristics among either set of basal-sprout-origin and the graft-origin plantlets. The results further indicate that grafting and scion burial, and perhaps same of the tissue-culture protocol, have some functions in rejuvenating or at leat reinvigoration tissues of Chinese-fir.

Key words: vegetative propagation, clonal forestry, rejuvenation, maturation state, in vitro.

Introduction

Clonal propagation of trees old enough to have expressed their desirable characteristics is an effective means of rapidly obtaining improved planting stock (Ahuja and Libby, 1993). Fortunately, the sprouting ability of the stumps and the rooting ability of the sprouts of Chinese-fir (*Cunninghamia lanceolata* (Lamb. Hook.)) are strong.

Afforestation and reforestation of Chinese-fir by planting unrooted or rooted stump-cuttings directly in the field has been used for at least 800 years (Li, 1995; Li and Ritchie, 1999a). In the past two decades, these traditional methods have been improved (Li and Shen, 1990, 1998; Li, 1998;) and methods for rooting cuttings of seedling-origin in quantity have been developed (Li, Yang et al., 1990; Li, Shen et al., 1999; Li and Ritchie, 1999b). However, cloning of mature trees of Chinese-fir using cuttings from upper branches is difficult, and such rooted cuttings typically exhibit sustained plagiotropic growth.

In 1990, a special type of cutting-orchard was developed, in which the rooting ability of sprouts of the donors originating from upper branches from the orchard was improved (for details see below and Li and Li et al., 1990; Li and Shen, 1998). Furthermore, the stecklings that developed showed normal seedling-like appearances. There were no signs of advanced maturation state exhibited; for example, no male or female flowers and no apparent visual differences between them and adjacent seedling trees during their first 6 years (Li and Ritchie, 1999b). A comparison between juvenile stecklings of young seedling origin and these apparently rejuvenated stecklings, from a mixture of clones, was made; no significant or consistent differences in rooting percentage, height, root-collar diameter, or biomass were found between them in the

nursery bed nor in a one-year-old plantation (MA and LI, 1996). The objective of this study was to compare maturation status between juvenile, mature and apparently rejuvenated explants of the same genotype, and to test whether donor culture prior to explant harvest and/or tissue culture may function in their reinvigoration and/or rejuvenation.

Materials and Methods

Origin of the explants

Trees that already had sprouts at their root collars were selected for this experiment. There were two groups of trees. The first group contained 12 28-year-old trees, and these were treated for rejuvenation. The second group contained 10 20-year-old trees, and these were not so treated. Trees in second group were 8 years younger than trees in the first group. The reason for this is that the experiment was moved to Wuhan where no trees were older than 20 years.

For the first group, which provided "treated" explants, upper branches of the trees were taken in the forest and one-year-old seedlings were dug out from a nursery, both located in Tongdao County, Hunan Province. Small shoots have often developed from cotyledon and leaf traces at and above the root collar inside here, and such shoots might later be confused with grafted scions. Therefore, root systems of the seedlings were harvested below the cotyledon traces to ensure that all potentially sprouting buds had been removed. This purpose is easy to achieve because there are great difference between root and stem (see *Fig. 1*). The branches and root systems were grafted together and tied with plant fiber (see *Fig. 2*). When planting, the lower parts of the scions were buried in soil with an oblique angle. The grafts were lifted for checking accidental shoot development from rootstock the next year. Most grafts had developed shoots from the scions (see *Fig. 3*); no shoots from rootstocks were found. The grafts were then moved to Wuhan City, Hubei Province, and planted as cutting donors. After sprouts appeared from the soil from the buried parts of the scions, they were removed beneath the soil surface. When one sprout was removed more sprouts appeared. This hedging allows the tiny stumps in earth to develop new shoots and their own roots (*Fig. 4*). This process of removing sprouts beneath the soil surface may take several times (LI and RITCHIE, 1999b).

Figure 1. – Root system of the seedling was harvested below the cotyledon traces to ensure that all potentially sprouting buds have been removed.

At the same time, sprouts from the root collars of the same mature ortets were harvested, rooted in Tongdao County, Hunan Province and then moved to Wuhan City, Hubei Province the next year as control donors.

Figure 2. – The senior author (LI MINGHE) was grafting a root system onto an upper branch and tied them with plant fiber.

Figure 3. – The graft was lifted for checking accidental shoot development from rootstock the next year. Most grafts had developed shoots from the scions, and no shoots from rootstocks were found.

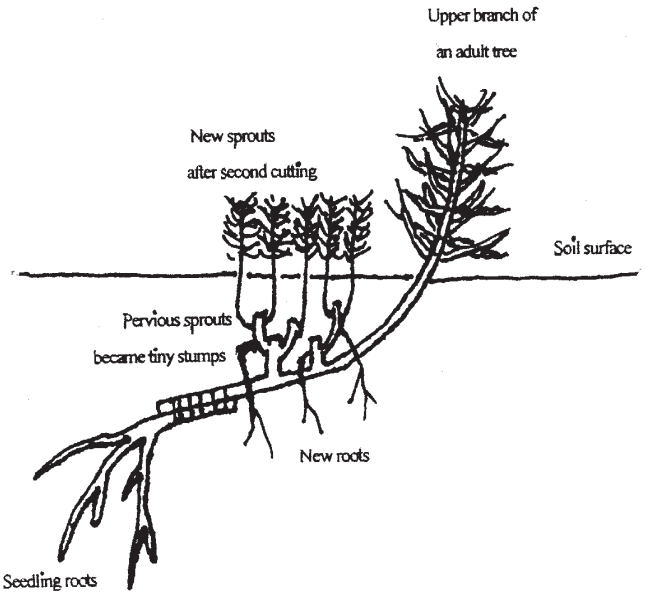

Figure 4. – The development of a grafted donor of upper-branch origin and its sprouts.

The two primary-ramet types of each of 12 ortet trees were paired planted as sets of adjacent pairs in the nursery bed (*Fig. 5*). Two years later, small shoots of less than 10 cm in length and 0.3 cm in diameter were dug out of the soil as explants and cultured in vitro from both types of primary-ramet, i.e. those from grafts and from root-collar sprouts.

Figure 5. – Sprouts developing from two ramets of clone T25, in the second year after planting. The ramet on the left is of upper-branch origin. The leftmost horizontal stem is the original upper branch, which was grafted onto a root system of a one-year-old seedling. Three sprouts, one large and two smaller from the large one (indicated by the knife and point of the scissors) have developed. The large one grows vigorously and has developed its own roots. The ramet on the right is a rooted sprout from the root-collar of the same ortet. Several sprouts have developed from this still-juvenile ramet, and some of them have their own roots.

For the second group, which provided "untreated" explants, upper-branch and root-collar-sprout cuttings were harvested from each of 10 trees in a 20-year-old Chinese-fir plantation in Wuhan, Hubei Province as explants. They were immediately cultured in vitro, with the cultures initiated at the same time as cultures from the two types of primary ramets in first group.

Thus, the "treatment" is burial of primary ramets in soil for 4 years, coded as "bs4", contrasted to taking explants directly from the ortets, coded as "dt0". Upper-branches ("up") and basal sprouts ("low") were harvested to produce both the primary ramets and the explants taken directly from the ortet trees (*Table 1*). The chronological ages of the "treated" and "untreated" explants at the date of culture initiation were 32 and 20 years, respectively.

Table 1. – Abbreviations and origins of the 4 types of explants.

Type of Branch	History	Abb. of explant
12 clones, primary ramets buried in soil 4 years		
Upper branch	Graft	bs4-up
Basal sprout	Cutting	bs4-low
10 clones, with no treatment		
Upper branch	Direct explant	dt0-up
Basal sprout	Direct explant	dt0-low

Culture conditions

Several researchers have successfully cultured Chinese-fir in-vitro (QUE, 1980, 1989; BIGOT, 1987). Based on their experiments, we used MURASHIGE and SKOOG'S medium. The tissue-culture process was divided into three stages, (a) bud differentiation, (b) shoot elongation and (c) shoot rooting. These stages contained 4, 3 and 2 subcultures, respectively. The difference in medium composition at each stage may be found in *table 2*.

Table 2. – Medium composition and number of subcultures at the 3 stages of tissue-culture.

Phase of culture	Number of subcultures	Medium composition
Bud differentiation	4	1/2MS +Agar 0.7% + sucrose 0.3% + BA 2.0 mg/L + IBA 1.0 mg/L
Shoot elongation	3	1/2MS + Agar 0.7% + sucrose 0.3% + IBA 3.0 mg/L + activated charcoal 3g/L
Shoot rooting	2	1/2MS + Agar 0.7% + sucrose 0.3% + IAA 1.0 mg/L + IBA 1.0 mg/L

The pH of the medium at the various stages was adjusted to be in the range of 5.6 to 6.0. The medium for each stage was poured into jars or test-tubes and autoclaved for 20 min at 120 °C.

When the ortet basal shoots were dug out of the soil, they had no needles. After the upper branches were taken, the needles were removed immediately. At this time, neither the basal shoots nor the stripped upper branches had apparent buds. They were washed in (ordinary) running water, disinfected for 5 seconds in 75% alcohol, then for 5 minutes in 0.1% $HgCl_2$, then given three 5-min rinses in sterile distilled water, and then cut into 1 cm sections. The stem sections were placed horizontally on the medium in jars or test-tubes. Each jar or test-tube contained only one stem-section during the bud differentiation phase, and only one elongating shoot during the shoot elongation phase. The jars and tubes were incubated in a growth-chamber at 25 °C with 1000 Lux to 2000 Lux light for 10 hours to 12 hours a day, and were in the dark at 25 °C for the rest of the day.

There were 22 clones and thus 44 sets of sampled explants. During the 4 bud-differentiation and 3 shoot-elongation subcultures, 3 containers were maintained for each of these 44 sets, and each subculture lasted for 30 days. At the end of the third shoot-elongation subculture, all shoots that were longer than 2.5 cm were excised and transferred into rooting medium. During the rooting phase, each subculture was initiated with only one shoot per jar or test-tube. Each rooting subculture lasted for 45 days. When transferring onto the next subculture, the number of buds, the number of new shoots and length of each shoot, and the number and average length of all roots of each plantlet, were counted or measured and recorded.

The Microsoft computer SAS System for Windows was used for statistical analysis. Paired-sample T-test analyses were used to compare the differences between bs4-up (buried-grafted-upper-branch-explants) and bs4-low (buried-lower-sprout-explants) in the "treated" group, and between dt0-up (upper-branch-explants) and dt0-low (root-collar-sprout-explants) in

the "untreated" group. Independent-sample T-test analyses were used to compare the differences between bs4 and dt0 explants.

Result and Analyses

Ortet position differences of "untreated" explants taken directly from adult trees

Upper-branch and basal-sprout explants had been taken directly from each of 10 20-year-old adult trees. Their data are presented in *table 3* as average numbers per clone and overall.

With only one exception (clone W4 shoot length), bud and shoot number per explant, average shoot length, rooting percentage and average root length of the explants originating from upper branches were consistently lower than those of root-collar-sprout explants of the same trees, and all overall differences were highly significant (*table 3*). This suggests that explants taken directly from upper branches were at advanced maturation states, while explants taken directly from root-collar sprouts were apparently more juvenile. Please note that the mean value of the dt0-low rooting percentage, 31.4%, is much lower than the usual 90% or so in nursery practice using root-collar sprouts. The reason for this is that the explant data were recorded at the 45th day. Some of the test-tubes were kept in the growth room for six or more months, and nearly all shoots from both dt0-up and dt0-low rooted at various times during this longer period.

Ortet-position differences of "treated" explants taken from partly buried primary ramets

Primary ramets from 12 ortet trees had been developed as grafted upper-branch shoots and rooted basal sprouts. These were then "treated" by burying in soil for 4 years before donating explants. Data presented in *table 4* were taken as for *table 3*, on the same days and at the same points during the tissue-culture protocols.

In *table 4*, there were neither consistent nor significant differences in any of the 5 traits evaluated (*Table 4*) between upper-crown and basal-sprout-origin explants from donors that had been "treated" by 4-year burial preceded by rooting or grafting. An example is presented in *figure 6.* These results suggest that explants originating from upper branches that had been grafted onto seedling roots and then buried, performed similarly to basal sprouts that had been rooted and then buried. Please note that these reported rooting percentages would be higher if the rooting culture time were longer, and

Table 3. – Comparisons between upper-branch (dt0-up) and root-collar shoot (dt0-low) explants taken directly from 10 20-year-old trees.

Clone name	Bud number per explant 4th subculture		Shoot number per explant 3th subculture		Average shoot length(cm) 3th subculture		Rooting (%) 45th day		Average root length(cm) 45th day	
	dt0-up	dt0-low	dt0-up	dt0-low	dt0-up	dt0-low	dt0-up	dt0-low	dt0-up	dt0-low
W1	1.7	3.7	2.7	9.3	1.4	2.6	11.5	33.0	1.0	1.8
W2	1.7	5.0	3.0	8.3	1.6	2.5	5.0	29.0	0.4	1.9
W3	2.0	5.0	2.7	8.0	1.8	2.4	20.0	33.5	1.3	1.9
W4	1.3	4.7	2.7	7.7	2.5	2.3	14.0	33.5	0.9	1.5
W5	1.5	5.0	3.3	7.0	2.0	2.7	5.0	28.5	0.5	1.6
W6	2.0	5.3	4.3	8.3	2.2	2.9	11.0	30.0	1.0	1.8
W7	2.3	6.3	3.0	8.7	1.9	3.0	20.0	25.0	1.5	1.7
W8	2.3	5.7	3.7	7.7	2.0	2.5	9.0	38.5	0.4	1.6
W9	2.0	5.0	3.3	8.3	2.3	2.7	15.0	35.0	0.8	2.1
W10	1.7	4.0	3.7	8.5	2.3	3.2	18.5	28.0	1.2	2.1
Mean	1.9	5.0**	3.2	8.2**	2.0	2.7**	12.9	31.4**	0.9	1.8**

** Average paired difference, significant at 0.01 level

Table 4. – Comparisons between buried-graft upper branches (bs4-up) and root-collar shoots (bs4-low) explants of 12 28-year-old trees.

Clone name	Bud number per explant 4th subculture		Shoot number per explant 3th subculture		Average shoot length(cm) 3th subculture		Rooting (%) 45th day		Average root length(cm) 45th day	
	bs4-up	bs4-low	bs4-up	bs4-low	bs4-up	bs4-low	bs4-up	bs4-low	bs4-up	bs4-low
T2	5.0	4.5	9.5	8.5	2.8	2.9	35.0	33.5	1.7	1.7
T3	5.3	5.3	8.3	8.0	2.2	3.0	24.0	28.5	1.7	1.9
T4	3.7	5.0	10.0	10.0	2.3	2.6	33.0	32.0	1.8	1.9
T7	4.0	3.7	9.0	8.5	2.7	2.3	23.5	26.0	1.3	1.8
T8	4.3	5.0	7.5	8.0	2.8	2.8	26.0	32.5	2.3	1.4
T9	4.7	4.7	8.0	8.0	2.7	2.5	33.0	35.0	1.4	1.5
T10	5.0	4.3	8.0	7.5	2.5	2.6	31.0	33.5	1.7	2.0
T11	4.7	5.3	7.0	7.7	2.6	3.0	30.0	30.0	1.9	2.2
T14	5.7	6.0	11.0	9.0	3.0	2.9	45.0	33.0	2.0	2.3
T17	6.3	7.0	9.3	8.5	2.2	2.8	44.5	37.0	2.9	2.5
T25	4.5	6.0	8.3	10.3	3.1	2.6	42.5	41.5	1.7	2.0
T30	5.0	4.7	6.5	8.0	2.8	2.8	31.5	31.0	1.5	1.6
Mean	4.9	5.0	8.5	8.5	2.6	2.7	33.3	32.8	1.8	1.9

that the rooting percentages of clone T14, T17 and T25 were higher than those of the other 9 clones 45 days after being transferred to rooting media.

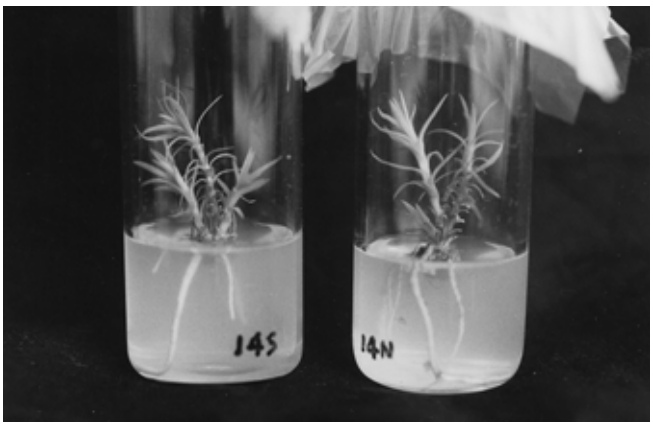

Figure 6. – Low-sprout (14S, a bs4-low) and upper-branch-grafted-origin (14N, a bs4-up) shoots of the same clone (T14). Both rooted at about the same time.

Comparisons among explants from two positions on the ortet taken from primary ramets and taken directly from mature trees

Comparisons of performance of all 4 kinds of explants at the completion of each stage of tissue-culture are presented in *figure 7*. It is clear that explants directly from upper branches, dt0-up, had poorer performance than explants of the other three origin-histories. The paired-sample and independent-sample T-test analyses indicate that the performances between dt0-up and those of the other three origins are significantly different at the 0.01 level in all five traits compared, and that no statistically significant differences occurred among the performances of bs4-up, bs4-low and dt0-low explants.

These results suggest that the treatment enhanced the performance of upper-branch explants, such that they became similar to both the treated and untreated basal-sprout-origin explants, which did not differs from each other.

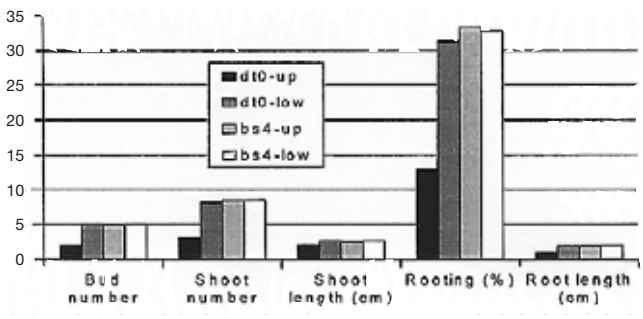

Figure 7. – Comparisons of performances of the 4 kinds of explants. See text for explanation of the explant-kind codes.

Function of the tissue culture

When each of the 9 subcultures were analyzed, a trend of possible future research interest become apparent. The performances of explants in the 9 successive subcultures are presented in *tables 5* and *6*. *Table 5* suggested that the performance of the explants originating directly from upper branches of the adult trees began and remained consistently and significantly lower than these of explants directly from basal sprouts throughout the 9 successive subcultures. This was not surprising. However, *table 6* suggested that organogenesis of the treated upper-crown-origin explants was initially lower than that of the sprout-origin explants, and remained so in the first three subcultures. However, as of the fourth subculture, the difference between the two explant types became small, and statistically non-significant. Considering the results in *table 5,* this result indicated that both the grafting and burial treatment somehow interacted with the tissue culture environment to equalize subsequent performances with that of basal-sprout-origin explants.

Discussion and Conclusions

Afforestation and reforestation of Chinese-fir by planting stump cuttings (with no roots) directly in the field has been used as a routine practice for several centuries; however, upper branches of mature trees are know to be difficult to root, and those cuttings that do root often exhibit sustained plagiotropic (branch like) growth. The in-vitro experiment reported above suggests that explants of upper-branch-origin tissue are in a much more mature state than are explants from root-collar sprouts of the same trees, even though the ortet trees were only 20 years old. An alternative explanation is that there were large differences in vigor between upper-branches and basal sprouts, and the upper-branch ramets from the 28-year-old trees were "reinvigorated" by the grafting/burial/tissue-culture processes. Furthermore, because two different plantations served as sprout and scion donors, the possibility cannot be eliminated in these experiments that there were tree-position differences in vigor in the 20-year-old plantation, but the 28-year-old plantation did not have such differences in the vigor of its upper crown and basal sprouts (although *Table 6* calls this into question). Thus, whether these experiments demonstrated a rejuvenation or a reinvigoration event is not clear, but the general literature leads one to expect differences in maturation state and their performance manifestations between the upper-crown and basal sprouts as source material for propagation (AHUJA and LIBBY, 1993).

Grafting upper branches onto seedling root-systems, burying part of the scion, and the repeated cutting, sprouting and rooting processes apparently changed something that may have been the maturation states of the sprouts that then developed from these scions. The in-vitro plantlets taken as explants from such buried-graft sprouts showed juvenile characteristics similar to those of the plantlets that developed from explants of low-sprout origins of the same trees.

It is important at this point to note that we carefully noted the origins of sprouts used in these studies. It was our observation that the apparently rejuvenated (or perhaps reinvigorated) sprouts originated from the grafted mature scions and not from the juvenile rootstocks. Some have suggested that changes in maturation state may be influenced by the distance between the tissue and root system (reviewed in BONGA and ADERKAS, 1993). In our system (LI and RITCHIE, 1999b), roots develop at

Table 5. – Comparisons of the performance of explants taken directly from the ortets in 9 successive subcultures.

Sub-culture	Bud number per explant		Shoot number per explant		Average shoot length(cm)		Rooting (%)		Average root length(cm)	
	dt0-up	dt0-low	dt0-up	dt0-low	dt0-up	dt0-low	dt0-up	dt0-low	dt0-up	dt0-low
1	0.23	1.13**								
2	0.67	2.20**								
3	1.29	3.27**								
4	1.85	4.97**								
5			0.74	3.44**	0.75	1.41**				
6			1.96	5.87**	1.71	2.16**				
7			3.24	8.18**	2.00	2.73**				
8							11.00	30.5**	0.61	1.65**
9							14.70	32.2**	1.14	1.90**

** Significant at 0.01 level

Table 6. – Comparisons of explants taken from buried primary ramets in 9 successive subcultures.

Sub-culture	Bud number per explant		Shoot number per explant		Average shoot length(cm)		Rooting (%)		Average root length(cm)	
	bs4-up	bs4-low	bs4-up	bs4-low	bs4-up	bs4-low	bs4-up	bs4-low	bs4-up	bs4-low
1	0.91	1.54**								
2	1.63	2.46**								
3	3.18	3.48**								
4	4.85	5.04 NS								
5			3.53	3.82 NS	1.41	1.45 NS				
6			5.96	6.18 NS	2.26	2.29 NS				
7			8.53	8.50 NS	2.64	2.73 NS				
8							34.25	34.00 NS	1.60	1.75 NS
9							32.25	31.58 NS	2.01	1.98 NS

** Significant at 0.01 level; NS not significant

the bases of new shoots produced by the grafted scions. The buried graft produces a clone of tiny (less than 15 cm tall and one-year old) trees (see *Figures 4* and *5*), which were used as donors of explant tissues. The distance between the new shoots and the new roots are very small. This apparent rejuvenation or reinvigoration presumably results from some combination of the effects of grafting onto seedling rootstocks, repeated hedging (once a year in 3 year's time) beneath the surface of the soil, subsequent sprouting from scion tissue, and rooting of the sprouts, rooting of the ramets, harvesting and preparation of explant section, and transplants through the tissue-culture environment through 3 or more subcultures.

Rooting percentage of the plantlets varied among clones. Some clones, for example T14, T17 and T25, quickly developed many roots at the bases of their sprouts, while sprouts of other clones developed fewer roots, or rooted more slowly. This may indicate that there are differences in rooting ability among clones, and the observation that both the bs4-up and bs4-low sublines of these 3 clones rooted above that treatment's average leads some support to this. Similarly, clone T3, T7 and T8 rooted below the treatment average in both sublines (*Table 4*). But it may also be that this protocol, lasting only 4 years, is too short for the rejuvenation (or reinvigoration) process to be complete, or that other elements of this protocol might be improved. One suggested line of further research is to conduct several cycles of graft-sprout-steckling propagation, to see whether several cycles of such serial propagation reduce the symptoms of an advanced maturation state.

From the examples above, it seems that maturation state is not as serious a problem in Chinese-fir as in other conifer species. Many researchers predict that in-vitro mass-propagation of trees of high quality and rapid growth probably will become one of the main tools to improve forest productivity. However, we suggest that in-vitro propagation of Chinese-fir will not become a main tool, because rooting cuttings in quantity is simple, economical and reliable (LI, YANG et al., 1990; LI and RITCHIE, 1999b). However, it is proving to be a useful research tool, as in this study.

Acknowledgements

This project was financed by Hubei Provincial Scientific Foundation. We gratefully acknowledge Dr. W. J. LIBBY at the University of California, Berkeley, USA, for his critical review of this paper and valuable comments.

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Buchbesprechung

Methods for Risk Assessment. III. Ecological Risks and Prospects of Transgenic Plants. By K. AMMANN, Y. JACOT, V. SIMONSEN and G. KJELLSSON (Eds.). 1999. Birkhäuser Verlag AG, Basel, Berlin and Boston. ISBN 3-7643-5917-X. 272 pages. Hardcover DM 148,–/sFr. 128,–/öSch 1081,–.

Leading scientists on risk assessment research with transgenic crops met in Berne/Switzerland and discussed the status quo of the release of genetically modified organisms. The aim was to bring together regulary makers and member of the biotech industry to make progress in the field of risk assessment in times where the global spread of transgenes in agrosystems are expected. During this meeting oral and poster presentations were held revealing the progress made so far in risk assessment, however, also showing some open questions of risk assessment research. The oral presentations were divided in eight sessions. Session 1 described ecological effects of transgenes, session 2 introduced in models of risk assessment, session 3 highlightened short-term and long term effects as well as standardization of limits, session 4 showed some monitoring methods, and session 5 dealed with population genetics. The remaining three sessions comprised decision procedures and harmonization (session 6), methodological lacunas (session 7), and conclusion, strategies and the question where to go from here (session 8). In summary, the present book is a collection of papers on the field of risk assessment, however, as obvious from many other meetings and workshops on this field of scientific research, the heterogeneity of the presentations is hindering the original idea. Some presentations may be of interest for those entering the field of transgenic crop and asking for possible ecological consequences.

M. FLADUNG (Grosshansdorf)

Herausgeberin: Bundesforschungsanstalt für Forst- und Holzwirtschaft: Schriftleitung: Institut für Forstgenetik und Forstpflanzenzüchtung, Siekerlandstrasse 2, D-22927 Grosshansdorf — Verlag: J. D. Sauerländer's Verlag, Finkenhofstrasse 21, D-60322 Frankfurt a. M. — Anzeigenverwaltung: J. D. Sauerländer's Verlag, Frankfurt am Main. — Satz und Druck: Graphische Kunstanstalt Wilhelm Herr, D-35390 Giessen
Printed in Germany.

ISSN 0037-5349