of GOT and LAP isozymes were dealt with by that author. However, the findings of LINARES (1984) in *Alnus* suggest certain crossing experiments in order to study similarities of chromosome structure in two genera belonging to the same family.

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Clonal Propagation of Juvenile and Adult Trees of Sessile Oak by Tissue Culture Techniques

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Abstract

Quercus petraea has been propagated in vitro from juvenile and mature explants. Suitable nutrient medium and culture conditions were first determined for explants from 2 to 5 months old seedling; mature explants responded similarly. The best nutrient medium was GRESSHOFF and DOY's medium supplemented with 0.2 mg/l BA. The nature of the explant (apex, base, node or axillary shoot) had no great influence on multiplication rates; the proliferation coefficient was increased by repeatedly culturing mature mother shoots placed horizontally on the medium. Roots formed on shoots placed in half-strength GRESSHOFF and DOY medium after their bases had been dipped in 0.5 g/l IBA for 6 min or 1 g/l IBA for 2 min. Growth in culture and rooting rates were both markedly affected by the individual plant used as the source of explants.

Key words: *Quercus petraea*, sessile oak, micropropagation, tissue culture.

Introduction

There is increasing interest in the use of tissue culture for clonal propagation of woody plants. The success of clonal reforestation programs is limited by the efficiency with which selected trees can be reproduced vegetatively. Woody species in the juvenile phase are generally easy to clone by conventional techniques; the ease with which many trees are propagated tends to disminish, however, as they approach a size that is sufficient to allow reliable evaluation of their crop potential. This is true of those plants whose cuttings are hard to root, particularly when they are taken from mature trees. If tissue cultured plantlets could be produced, they would be extremely useful for testing clones and/or for direct field planting. The advantages of clonal propagation of forest trees have been reviewed elsewhere (LIBBY, 1974; SCHREINER, 1966).

Sessile oak (*Quercus petraea* (MATT.) LIEBL.) is a case in point. This species is one of the more valuable European hardwoods, but cuttings from mother plants older than 3 to 5 years no longer root (KLEINSCHMIT *et al.*, 1975). Basing our work on our experience of tissue culture of other Fagaceae (specifically, *Quercus robur* and *Castanea sativa*), we have now established suitable culture conditions for *in vitro* initiation, multiplication and rooting of various clones of sessile oak. In this article we report our results with both juvenile and mature explants.

Material and Methods

1. Initiation

Juvenile material

Two-or four-cm stem tips were taken from active 2 to 5 month old stock plants of 6 clones grown in growth chambers (collection in March to June) or a greenhouse (collection in June to July). After removal of leaves, shoots were sterilized by immersion in 70% ethanol for 30 seconds followed by 4% to 7% calcium hypochlorite for 5 to 7 min. After 3 rinses in sterile distilled water, apical and nodal explants (0.5 cm long) were excised and placed in 20 mm × 150 mm test tubes containing 15 ml of culture medium consisting of: macronutrients and vitamins prescribed by GRESSHOFF and DOY (1972), micronutrients and

Fe-EDTA prescribed by MURASHIGE and SKOOG (1962), plus 30 g/l sucrose and 6 g/l agar (basal medium), plus 1 mg/l benzylaminopurine (BA) (pH 5.5).

Adult material

The *in vitro* establishment of the adult clone (Clone 1) from stump sprouts of a 55-year-old tree has been described elsewhere (MEIER-DINKEL, 1987).

2. Shoot Multiplication

After one year in culture, the material previously established was used for different multiplication experiments.

Effect of mineral medium

The following macronutrient formulas were tested for culture the juvenile Clone 85: GRESSHOFF and DOY's (GD); HELLER's (1953) medium at concentrations 1.25 and 1.5 times that originally prescribed and with 1 mM $(NH_4)_2SO_4$ added (Hx1.25 and Hx1.5); SCHENK and HILDEBRANDT's (1972) medium (SH); MCCOWN and LLOYD's (1981) Woody Plant Medium (WPM); CHALUPA's (1981) medium (Ch); GUPTA and DURZAN's (1985) (GuD) and STRULLU *et al.,'s* (1986) (St). These formulas were used in media whose other components were the basal medium and 0.2 mg/l BA. After determination of the multiplication rate, one shoot tip from each culture tube was transferred to a tube with a fresh lot of the same medium and the multiplication rate of these subcultures was determined six weeks later.

Effect of segment type

Multiplication rates were determined for cultures of different explants isolated from shoot multiplication cultures on both juvenile (Clone 17.04.6) and adult origin (Clone 1) placed on basal medium + 0.2 mg/l BA. Details are described under Results.

Effect of reculturing

Explants of the adult Clone 1 were recultured thrice in succession. Shoots 1.5 to 2.5 cm long were excised from shoot multiplication cultures and placed horizontally on the culture medium, four to a jar, after removal of 2 mm of the tip. Eigth jars with 50 ml of medium per jar were used. After 4 weeks, the shoots of at least 5 mm in length that had been produced were excised for rooting or multiplication and the mother shoots were recultured, either by transfer to fresh medium or by replenishing the original medium by addition of 15 ml of a solution of the same medium.

3. Rooting

Juvenile material

The basal 1 cm to 2 cm of shoots from the juvenile clones 85 and 17.04.6 were dipped for 6 min in 0.5 g/l indole-3-butyric acid (IBA) and placed in basal medium with half-strength macronutrients (BM/2).

Adult material

Rooting was induced in Clone 1 shoots either by adding 1 mg/l IBA to the BM/2 medium for 4 days, or by dipping the base of the shoots in 1 g/l IBA for 2 min.

Unless otherwise stated, all cultures were grown in test-tubes containing 15 ml of medium under a 16 h photoperiod (30 $\mu E.m^{-2}.s^{-1}$ from cool-white fluorescent lamps) with day and night temperatures of 24° C and 18° C respectively. Each treatment was applied to 12 explants and each experiment was repeated at least twice.

Results

1. Initiation

Juvenile material

In general, both nodal and apical explants responded satisfactorily to culturing. In the case of chamber-grown mother plants, the best results were obtained collecting in May from 5-month plants. The best results from greenhouse grown mother plants (a 79.2% survival rate) were obtained collecting in late June; the survival rate fell to 65.8% when collection took place in early July and was only 25.5% in late July.

2. Shoot Multiplication

Effect of mineral medium

As is reflected in *Table 1*, the worst shoot growth was obtained with SH macronutrients, which produced the

Table 1. — Effect of various mineral media on the multiplication of *Q. petraea* Clone 85 (SH: number of shoots per explant; L: length of longest shoot; %: percentage of explants producing shoots). The experiment was performed thrice, each time with 12 explants per treatment.

Macronutrient	SH	L (mm)	%
	$\bar{x}$ SD	$\bar{x}$ SD	
GD	3.5±2.0	16.9±9.3	100
WPM	3.0±1.2	18.8±8.9	94.3
H x 1.25	3.1±1.5	14.3±7.3	97.2
H x 1.5	2.8±1.6	13.3±7.0	94.3
SH	1.9±1.2	10.7±4.4	77.7
Ch	2.8±1.3	16.9±9.2	91.6
GuD	2.8±1.3	22.5±11.3	79.2
St	2.6±1.3	16.7±8.6	100

SD: standard deviation

Figure 1. — Shoot multiplication in the juvenile Clone 85 of sessile oak.

shoots with least basal callus, fewest leaves and lowest survival rate. Hx1.25 and Hx1.5 afforded good multiplication rates and development, but the shoots produced were yellowish green in colour. GD, WPM, GuD and St all afforded good response with high multiplication rates, the highest rate being that of GD (3.5). All these shoots were healthy-looking, with large leaves and white, nodular basal callus (*Figure 1*). Other clones responded similarly in culture (dates no shown). In view of these results we have adopted GD macronutrients as standard for *Q. petraea* multiplication cultures.

Effect of segment type:

Juvenile material

After one year in culture, the multiplication rates of 6 clones of juvenile origin were determined in two successive 4-week subcultures using shoot tips as explants and GD macronutrients. The best response was obtained from clones 17.04.6 and 85 (*Table 2*), which have multiplication coefficients (m. c.) (defined as the product of the proportion of explants forming axillary shoots and the mean number of new segments per explant) of 3.7 and 3.2 respectively. Since the response of many species depends on the nature of the explants used, the multiplication rates of Clone 17.04.6 was then determined when 0.5 cm shoot tips (a), 0.5 cm callusless basal segments (b) and axillary shoots less than 1 cm in length (x) were used as explants. No great differences were observed, though the m. c. for apical (5.3) and basal segments (5.2) were the highest. Axillary shoots explants produced fewer shoots, which resulted in smaller m.c. (4.5). When the same experiment was repeated on the various kinds of explants obtained from the shoots so produced, track being kept of their origin, the lowest m. c. (3.4) was afforded by ax explants (i.e. axillary shoots obtained from shoot tips) and the highest, 7.4 and 7.1 by ab and xb respectively (*Table 3*). The ready adaption of Clone 17.04.6 to *in vitro* cultures thus allows the use of all parts of the multiplication shoots for subculture, which considerably increases overall multiplication rates.

Adult material

The separate multiplication rates of 7 mm to 8 mm basal, nodal and apical segments were determined in ex-

Table 2. — Influence of genotype on *in vitro* multiplication of sessile oak (SH: number of shoots per explant; SE: number of segments per explant; L: length of longest shoot; %: percentage of explants producing shoots; m.c.: multiplication coefficient (which is the product of the proportion of explants forming axillary shoots and the mean number of new segments per explant). The experiment was performed twice, each time with 12 explants per treatment.

Clone	SH	SE	L (mm)	%	m.c.
	$\bar{x}$ SD	$\bar{x}$ SD	$\bar{x}$ SD		
17.04.6	2.7±1.6	4.1±2.4	18.4±6.1	91.7	3.7
87.04.2	1.5±0.6	1.6±0.7	9.0±1.8	100	1.6
266.06.5	1.5±0.9	1.9±1.0	14.2±3.6	100	1.9
17.06.5	1.5±0.6	2.7±1.5	18.3±5.8	100	2.7
85	2.6±1.6	3.2±2.0	15.1±3.5	100	3.2
167.04.2	1.4±0.8	1.5±0.5	12.7±2.6	91.7	1.3

SD: standard deviation

Table 3. — Multiplication rates obtained in the second subculture using shoot tip (a), basal (b) and axillary shoots (x) explants. SH, SE, L, % and m.c. as in *Table 2*. The experiment was performed twice, each time with 12 explants per treatment.

Origin	Type of explant	SH	SE	L (mm)	%	m.c.
		$\bar{x}$ SD	$\bar{x}$ SD	$\bar{x}$ SD		
	a	3.9±1.2	6.0±1.2	22.3±7.6	100	6.0
a	b	5.8±1.0	7.4±1.1	17.4±3.3	100	7.4
	x	2.4±1.1	3.6±1.4	18.4±6.8	95.8	3.4
	a	3.7±1.4	5.1±1.6	17.7±3.5	100	5.1
b	b	3.8±1.3	5.9±1.6	19.2±3.3	79.2	4.7
	x	4.4±1.7	5.9±2.0	20.1±5.3	91.7	5.4
	a	3.9±1.2	5.9±1.3	19.1±3.3	95.8	5.7
x	b	5.1±1.7	7.1±1.6	20.0±3.7	100	7.1
	x	3.0±1.7	4.5±2.2	20.1±6.0	95.8	4.3

SD:standard deviation

periments in which culture took place in jars containing seven segments on 50 ml of basal medium + 0.2 mg/l BA. The best m.c., 4.3 and 3.4, were afforded by the apical and basal explants, with a mean number of shoots per explant of 4.3 in both cases. Nodal explants not only had a smaller average number of shoots per explant (1.5), but as many as 39% failed to develop shoots at all, which resulted in considerably smaller m.c. (1.4).

Effect of reculturing

Repeated horizontally culturing considerably increased multiplication rates, with a mean number of shoots per explant of 7.9 for the initial subculture and 9.2 for the first reculture and m.c. of 12.4 and 11.7 respectively. Transfer to fresh medium was more effective than the addition of liquid medium, which afforded a mean number of shoots per explant of 8.9 and m.c. of 10.9. In all cases, the percentage of cultures surviving was 100%.

3. Rooting

Differences in response were observed between the two clones of juvenile origin: in Clone 85, rooting rates of up to 100% were achieved, whereas there was only 58.3% rooting in Clone 17.04.6 (*Table 4*). Roots appeared between 9 to 12 days after induction. The best response in the Clone of adult origin, 38%, was obtained when induction was performed by dipping the base of the shoots in 1 g/l IBA solution for 2 min (*Figure 2*). Addition of auxin to the medium achieved only 12.5% rooting (*Table 4*).

Discussion

Although differences exists between the growth of seedlings and mature material in culture, the use of seedling material for determining the proper condition for culture of mature material has certain advantages: ease of growing seedlings in a greenhouse, tolerance to a wider range of nutrient media and growth condition, and large numbers that are available. The mature material, which is usually less plentiful, can be saved for critical determination of media and other culture conditions.

In the present study of the *in vitro* culture of sessile oak, clones of juvenile origin were used for initial screening of macronutrients formulas for shoot multiplication. Of the formulas inducing the best response (GD, WPM, GuD and St), WPM and GD have previously been used by Prevalek-Zozlina and Jelaska (1986) and Meier-Dinkel

Table 4. — *In vitro* rooting response of various *Q. petraea* clones (N: number of roots per rooted shoot; L: length of longest shoot). The experiment was performed twice, each time with 12 explants per treatment.

Treatment	Clone 85			Clone 17.04.6			Clone 1 (mature)		
	N	L (mm)	%	N	L (mm)	%	N	L (mm)	%
	$\bar{x}$ SD	$\bar{x}$ SD		$\bar{x}$ SD	$\bar{x}$ SD		$\bar{x}$ SD	$\bar{x}$ SD	
IBA 0.5 g/l - 6 min	4.6±2.8	23.9±9.9	100	2.9±1.7	22.3±15.1	58.3	-	-	-
IBA 1 g/l - 2 min	-	-	-	-	-	-	2.5±1.4	15.6±6.6	38*
IBA 1 mg/l - 4 days	-	-	-	-	-	-	1.8±0.4	36.3±15.8	12.5

SD: standard deviation

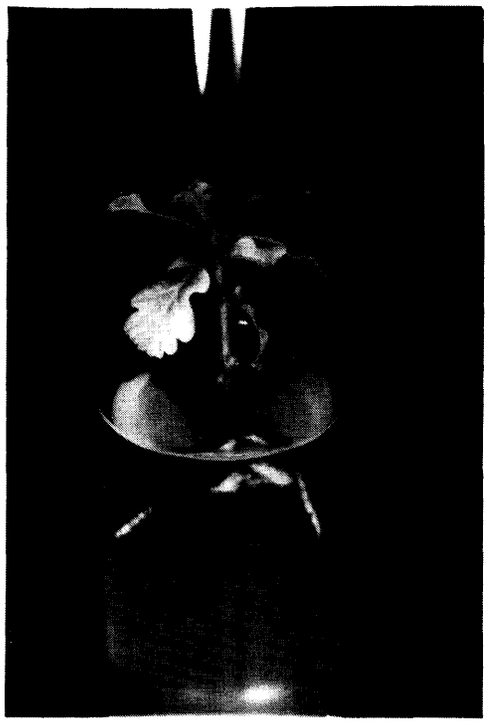

Figure 2. — Plantlet produced from a shoot of Clone 1 (adult) rooted by basal dipping in 1 g/l IBA for 2 min.

(1987) for *in vitro* culture of this species. The GD medium we have adopted for *Q. petraea* also proved to be the best of eight tested for the multiplication of *Q. robur* (Vieitez *et al.*, 1985).

In several of the experiments described here, wide differences among the responses of different clones were observed, lending further support (if any were needed) to the notion that the response to *in vitro* culture depends heavily on the genotype of the stock plant. Differences are apparent not only among different species, but also among different clones of the same species. Differences in the multiplication response of explants derived from different cultivars have been reported by Fordham *et al.* (1982) and Yae *et al.* (1987), and we ourselves have found clonal differences in the *in vitro* behaviour of *Q. robur* (San-José *et al.*, 1988) and chestnut (Vieitez *et al.*, 1983).

Another factor has been identified by several authors as affecting *in vitro* performance in the multiplication stage is the nature of the explant (John and Murray, 1981; Hutchinson, 1984; Shen and Mullins, 1984). In the present study, satisfactorily high multiplication rates were obtained with explants of all kinds in the clones of juvenile origin. In the clone of adult origin, basal, nodal and apical explants exhibited the same relative performance as in the juvenile clones, but the nodal rates were smaller than the basal and apical rates. In our previous work with *Q. robur*, basal and nodal explants performed better than shoot tips, though the differences were not significant (San-José, 1986; San-José *et al.*, 1988).

There have been a number of reports of successful recultured horizontal shoots (Economou and Read, 1986; Yae *et al.*, 1987). We ourselves have increased the multiplication rate of *Q. robur* by this means (San-José, *et al.*, 1988). In the present study, above-average rates were achieved by the reculture of *Q. petraea*, the best reculture method being transfer of the mother shoot to fresh medium rather than the replenishment of their original medium.

In vitro rooting capacity has been shown to be affected by genotype due to the latter's determining the kind and level of endogenous rooting substances and rooting cofactors (Lipecki and Dennis, 1972; Westwood, 1972). These differences mean that the optimal concentrations of applied growth regulators must be determined anew for each genotype. Furthermore, just as cuttings from plants in the juvenile growth phase are known to root more readily than those from adult plants, *in vitro* rooting too has been most successful with material of juvenile origin. Our work with *Q. robur* and *Castanea sativa* (Vieitez *et al.*, 1985, 1986) are cases in point. In this study, the rooting rates of *Q. petraea* clones of juvenile origin varied from 57 to 100%, while the highest rate achieved with the clone of adult origin was only 39%. This latter rate was attained when rooting was induced by dipping in IBA; though Meier-Dinkel (1987) reported high rooting rates in material of adult origin after incorporation of IBA in the medium, we found this method to be inferior to dipping, which has also proved preferable in other species (Abbott and Whiteley, 1976; Collet and Le, 1988).

To sum up, we have determined appropiate conditions for *in vitro* multiplication and rooting of *Q. petraea* clones of both juvenile and adult origin, though further research will be necessary in order to optimize the response of the more recalcitrant clones.

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Family-Site Interaction in Pinus radiata: Implications for Progeny Testing Strategy and Regionalised Breeding in New Zealand

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Summary

A progeny test of 170 open-pollinated families from second-generation plus trees of *Pinus radiata* was established on four sites in New Zealand in 1981. Two test s.tes were on volcanic pumice soils in the Central North Island region and two were on phosphate-retentive clay soils in the Northland region.

Assessments of volume growth, stem straightness, malformation, and branch habit were made at age 4.5 years.

Family × site interaction variance components for stem volume were highly significant ($\alpha = .01$) between pumice and clay sites, and also between the clay sites of differing fertilities, but relatively small between the two pumice sites. When the interactions for stem volume were studied in terms of genetic correlations between sites quite strong interactions were still evident between the regions, but interactions between sites within both regions were very minor, even though the Northland clay sites were of widely different fertility.

Family-site interactions for stem straightness and branch habit scores were less marked overall than for stem volume. For malformation the interactions were marked but only in relation to weakly expressed family differences.

Genetic gains were predicted, using multi-site index selection, for stem volume growth under alternative testing procedures and patterns of regionalisation. On this basis failing to test within a region would lose 50% or more of the potential gain for that region. However, it was possible to select families which performed well in both regions, such that regionalisation would only raise average genetic gain from 22% to 25%.

Key words: Genetic correlation, genetic gain, genotype-environment interaction, plant breeding, *Pinus radiata*, regionalisation, selection index, tree breeding.

Introduction

The question of whether or not it is necessary to select a unique set of parent clones for each region arises in almost every tree breeding programme. Gains will be maximised by regionalising, but the additional cost and effort may not be worth it, unless genotype-environment interactions are very strong. Additional gains from using regional breeds need to be weighed against the additional costs.

Over 50% of the *Pinus radiata* plantations in New Zealand (NZ) are in the Central North Island pumice region. Because of the predominant importance of this region, and the proven effectiveness of these pumice sites for screening genotypes, most progeny testing has been carried out there.

Of the remaining 'regions' throughout NZ, the phosphate-retentive (phosphorus deficient) Northland clays have shown the poorest genetic correlation with the pumice area in respect of growth of *Pinus radiata* (e.g., Burdon, 1971). Therefore the Northland clays would be a prime candidate for setting up a regional breed. If regionalisation does little to improve growth gain in Northland, then regionalisation is unlikely to improve gain much in other regions of NZ. This paper examines the effect of regionalisation on improving growth gains from progeny testing in the Northland clay and pumice regions of NZ.

Materials and Methods

Study Design

One-hundred and seventy 'second-generation' selections were tested in both the pumice and Northland clay regions using open-pollinated progeny tests. The parents were 10-year selections (the '880' series) made within open-