Genetic Markers in Birch

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Summary

A single gene responsible for purple pigmentation in white birch, *Betula pendula*, has been identified. The gene is completely dominant. It also influences phenotypic traits other than color, but these effects do not interfere with its ornamental value nor its utility as a genetic marker *in vitro*. It could not yet be determined whether the same gene is responsible for the pigmentation of other purple birches of different origin.

The genetic control of some GOT and LAP isozymes has also been investigated. Loose linkage seems to exist between two of those loci. These segregate independently of the pigmentation locus.

Observations on the reproductive behavior of birches carrying genes for early flowering indicated a small number of controlling genes. Experiments must be extended to additional generations of trees in order to analyse the inheritance mode of this trait.

Key words: *Betula pendula* var. *purpurea*, white birch, early flowering, isozymes, linkage.

Zusammenfassung

Ein einzelnes für die purpurrote Pigmentierung des Laubes des Sandbirke, *Betula pendula*, verantwortliches Gen konnte identifiziert werden. Das Gen verhält sich vollständig dominant und ist an der Kontrolle auch anderer phänotypischer Merkmale außer der Laubfarbe beteiligt. Diese Nebeneffekte mindern weder seinen Zierwert noch seine Nützlichkeit als genetischen Marker von *in vitro* kultiviertem Gewebe. Es ließ sich noch nicht feststellen, ob das gleiche Gen die Pigmentierung weiterer Blutbirken verschiedenen Ursprungs verursacht.

Ferner wurde die genetische Kontrolle einiger GOT- und LAP-Isoenzyme untersucht. Zwei der betreffenden Genloci scheinen syntän, aber schwach gekoppelt zu sein. Diese beiden Genloci spalten unabhängig von dem die Pigmentierung kontrollierenden Genlocus.

Beobachtungen über das reproduktive Verhalten solcher Birken, welche Gene für ontogenetisch frühen Eintritt der Blüte tragen, lassen eine geringe Anzahl dieser Gene vermuten. Die betreffenden Versuche müssen allerdings über weitere Generationen fortgesetzt werden, wenn der Vererbungsmodus dieses Merkmals einer Klärung zugeführt werden soll.

Introduction

In the diploid birch species, *Betula pendula* Roth, several traits displaying pronounced variation within populations have been described in the literature (Johnsson, 1974; Stern, 1963). There exist numerous reports on the variation of growth speed and its utilization in breeding. In this paper more detail on some of these traits is presented and an attempt is made to analyse their genetic control. In angiospermous trees such as in the beech family only a few genetic markers have been identified due to the small number of progeny arising from a single pollinated flower. The *Betulaceae* are easier to manipulate, since prolific crosses are fairly easy to conduct.

A breeding experiment (Stern, 1961) provided a large supply of early-flowering birches. These seedlings may set male flower buds as early as after formation of their sixth leaf, i.e. at the age of three weeks. In this instance the terminal buds and many of the lateral buds are male flower buds. Female flowers generally do not develop before the second season. In the extremely early-flowering seedlings mentioned, the shoots die off upon fructification after the second year, and new shoots arise producing the appearance of a little bush 20 cm tall. However, most seedlings do not form male flower buds until the end of their first or subsequent seasons. Then they tend to flower abundantly, and their growth is reduced to about one half. Another feature of the early flowers is a trend towards dioecy: in many of these seedlings, catkins of one sex prevail, and quite a few of these seedlings are unisexual. All of these retain their sexual type, as could be observed in a field plantation during six years.

Material and Methods

Two purple birches (*Betula pendula* var. *purpurea*) of unknown origin were used as pollen donors in various experiments (denoted BG and BM). Their leaves and young twigs are colored dark purple owing to high anthocyanin content. They exhibit upright growth form but somewhat reduced growth speed. In an average spring they both flush and flower about a week after normal green birches. One of these trees (BG) has been producing abundant male flowers but has never been observed to bear any female catkins. The other produces catkins of either sex.

A large number of early flowerers were pollinated using pollen of either early-flowering or normal birches and planted out in a nursery. In addition, pollen of the purple birches was used on both early-flowering and normal birches; the resulting progeny of one of the two pollen donors was planted out in the same nursery and kept there for up to 10 years. At least at that age, flowering occurred regularly in almost all trees. The seedlings were planted in plots; since green-leaved and purple-leaved seedlings belonging to the same family were kept in separate plots, the nursery plantation consisted of mixed clumps of trees with purple and green foliage. However, the vast majority of the trees have normal green foliage. Trees once classified as purple retained this trait expression.

Purple seedlings were scored either right after germination on filter paper, or a few weeks later after transplantation into small pots.

Early flowering of potted seedlings, which had been sown in January, was assessed in May in the greenhouse. The seedlings were then four months old; the seed had been produced from seed collected during September of the previous year. The incidence of flowers was re-assessed once in summer when the potted seedlings were transferred to the nursery; there was little increase in the number of flowers.

Extracts from young leaves were analysed electrophoretically using starch gel and staining for LAP (EC no. 3.4.11.1) and GOT (EC no. 1.4.1.2). The methods used followed the description given by FERET and BERGMANN (1976) and the procedures adapted by LINARES (1984). Other systems such as MDH and ACP did not display variation or were obviously influenced by the environment; GDH appeared to be active only on some of several occasions checked.

Results

1. Progeny of Purple Trees

About one-half of the progeny after crossing green trees with purple trees had the same foliage type as the male parents, indicating that both of the purple birches are heterozygous carriers of a dominant allele, R_2, at one controlling gene locus, R; normal green trees would then have to be assigned the genotype R_1R_1.

Generally a shortage of purple seedlings occurred which was independent of the male partner, as can be inferred from *table 1*: Four out of a total of ten green trees were pollinated by both BG and BM, and there was hardly a difference between pollen parents as regards this excess proportion of green seedlings. These crosses in the upper four lines of *table 1* had been made on potted green female trees in the greenhouse during February. The crosses on six more trees were done later in the spring in the nursery; their progeny were pooled, since the female parents varied.

Similar results were obtained from 10 crosses made in the field among purple and green offspring of BG: There was a proportion of $p = 0.55$ green offspring among a total of 847 in 10 families. This time, the purple trees had been used as the female parents because they shed pollen rather late. A heterogeneity of p was observed among the families, but its cause could not be resolved.

The purple germinants regularly appear after the green ones. This delayed germination indicates reduced vigor, which may be parallelled with the lower germination percentage among carriers of R_2. A sample comprising nine segregating families in the stage of germination was therefore observed twice. *Table 2* shows that also during the first few weeks in the life of the seedlings mortality among the carriers of R_2 may continue to moderately exceed that among the non-carriers.

Table 3. — Segregation within families arising from crosses among heterozygous carriers of an allele responsible for purple foliage.

Cross no.	green	purple	p
1	10	2	.83
2	336	111	.75
3	9	46	.16
4	28	8	.78
5	70	18	.80
6	79	54	.60
7	8	2	.80
8	52	39	.57
9	112	60	.65
10	137	154	.47
11	21	23	.48
12	62	64	.49
13	110	151	.42
14	4	39	.09
15	41	25	.62
16	113	91	.55
17	139	88	.61
18	15	9	.63
total	1346	984	.58

This also has to be kept in mind when viewing the results of crosses among purple trees which all received their R_2-allele from BG. *Table 3* shows considerable and significant heterogeneity of the segregation ratios in the various families ($X^2_{17} = 103.53$*). A gross $p = 0.58$ among the 2330 seedlings is not compatible with a 3:1 ratio, i.e. an expected $p = 0.25$. Since the proportion of green progeny of $R_1R_2 \times R_1R_2$ crosses very closely resembles that of $R_1R_1 \times R_1R_2$ crosses, it has to be inferred that virtually all progeny of genotype R_2R_2 are lethal.

2. Crosses Involving Early Flowerers

The vast majority of the progeny of early flowering birches after pollination by the purple birches showed

Table 1. — Segregation of purple (R_1R_2) and green seedlings (R_1R_1) among the progeny of various green birches pollinated by two purple birches (BG und BM); p in this and the following tables denotes the proportion of green seedlings.

♀ parent	♂ parent					
	BG			*BM*		
	green	purple	p	green	purple	p
(6 × 69).11	257	267	.49	189	107	.64
(6 × 69).16	93	60	.61	326	288	.53
(413 × 204)	567	453	.56	204	167	.55
(414 × 204)	26	25	.51	12	4	.75
6 other ♀ parents	457	182	.72	14	8	.64
total	1400	987	.59	745	574	.56

Table 2. — Repeated observations of segregating families as germinants on filter paper and after potting the seedlings in standard soil.

cross	germinants			transplants		
	green	purple	p	green	purple	p
(413 × 204) × *BG*	206	171	.55	159	96	.62
8 other families	392	284	.58	366	261	.58
total	598	455	.57	525	357	.60

Table 4. — Proportion q of 4-month-old seedlings producing male flower buds. The seedlings were produced by crossing 'normal' progeny of early flowerers. Four more families of less than ten seedlings are not recorded.

family no.	no. of seedlings with flower buds	no. of seedlings without flower buds	proportion q of early flowerers
1	24	507	.05
2	5	42	.11
3	21	26	.45
4	40	33	.55
5	34	193	.15
6	28	131	.18
7	20	98	.17
8	31	40	.44
9	5	37	.12
10	39	89	.30
12	19	120	.14
13	26	83	.24
15	0	60	.00
16	50	132	.27
17	11	73	.13
18	0	12	.00
19	1	30	.03
20	12	12	.50
21	17	12	.59
22	43	74	.37
24	15	19	.44

the normal growth habit of their male parents; a small fraction of the families contained varying proportions of trees with leaning stems and reduced growth or even bushy growth habit. The onset of flowering in some seedlings with reduced growth was recorded at the age of about 4 years.

In families produced by crossing 8-year-old progeny trees showing normal growth and no early flowering, the incidence of bare male flower buds could be observed at the age of a few months. As is shown in *table 4*, the proportion q of these flowering seedlings varied greatly among the families.

Not only the proportion of flowerers but also the intensity of flowering varied. In some seedlings even more than the upper third of the leaf axillary buds turned into male flower buds. In addition, the developmental status of the flowerers varied from very tiny organs to catkins having about the size of those observed in adult trees.

In a small fraction of the seedlings having male flower buds, female flowers also appeared. This phenomenon usually was accompanied by bushy growth form, and this in turn appeared to be due to a terminal bud that was not vegetative but rather a male catkin. If this happened, additional shoots sprouted from the base of the stem; after male catkins appeared in terminal position, a third wave of sprouting was induced.

A small fraction of the female catkins recorded were actually bisexual in that the lower half consisted of male flowers and the distal half was female.

In 8 out of the 21 families recorded in *table 4*, one or both of the parents were purple. Strangely enough, in green seedlings flowering almost always occurred: out of 52 red seedlings derived in several families presented in *table 4*, only one carried few and less developed male flower buds.

3. Crosses Involving Isozyme Phenotypes

One familily, i.e. (413 × 204) ×BG, was used to study the inheritance of isozymes of GOT and LAP together with leaf pigmentation. *Table 5* lists the phenotypes of the two crossing partners. Segregation data for the two presumed enzyme gene loci are compiled in *table 6*.

The segregation at the presumed *GOT* locus is compatible with the hypothesis that a null allele is absent in the female parent and that the triple band of the male parent denotes a heterozygous genotype ($X^2_1 = 0.44$). The segregation at the presumed *LAP* locus is compatible with the hypothesis that the female parent does not carry a null allele and that the double band of the male parent denotes a heterozygous genotype ($X_1^2 = 1.78$). Other zones visible in the gels stained for both GOT and LAP did not vary among the progeny in this family.

Table 5. — Leaf color, enzyme phenotypes and presumable genotypes of two trees used as crossing-parents. The female parent was assumed to be triple homozygote, the male parent was assumed to be triple heterozygote.

parent	trait	expression	presumable genotype
♀	leaf color	green	R_1R_1
	GOT	single band	A_2A_2 or A_2A_0
	LAP	single band	A_3A_3 or A_3A_0
♂	leaf color	purple	R_1R_2
	GOT	triple band	A_1A_2
	LAP	double band	A_2A_3

Table 6. — Simultaneous segregation at the two presumed isozyme loci, *GOT-A* and *LAP-A* among 144 seedlings in family (413 × 204) × BG (cf. *tables 1* and *5*).

	LAP		
GOT	*A2A3*	*A3A3*	totals
A1A2	27	41	68
A2A2	53	23	76
totals	80	64	144

4. Character Associations

On the basis of this balanced segregation at the two individual isozyme loci, their common segregation was tested for independence. A $X^2_1 = 13.44^*$ indicates linkage displayed in the male parent; the corresponding coefficient of linkage between this isolated pair of loci was estimated to be c = 0.65.

It was also tested whether this pair of possibly linked loci may be positioned on the chromosome carrying the locus controlling leaf color. In *table 7* the data on segregation at the *GOT* and *LAP* loci are therefore detailed to the green and purple phenotypes. First of all also the segregation at the *R* locus could be assumed to be balanced in this famliy, the pertinent test statistic derived from *table 7* being $X^2_1 = 1.36$. Segregation at both the *GOT* locus ($X^2_1 = 1.44$) and the *LAP* locus ($X^2_1 = 2.64$) was inferred to be independent of the gene locus *R*.

Discussion

Leaf Pigmentation

The segregation among offspring of several green trees when pollinated by both BG and BM supports the hypothesis that one gene exerts a major though not exclusive influence on the incidence of red pigmentation (cf. *Table 1*). The gross excess of green seedlings may be interpreted as the result of less germinative power of the carriers of R_2. However, the variability of this excess must be ascribed to additional gene loci in producing the red or green phenotype: Among the upper four female parents of *table 1* the X^2_3 indicating the heterogeneity of *p* among the offspring of BG equals 9.23*; the respective test statistic among the BM-families is $X^2_3 = 11.96^*$.

Experimental results on red pigmentation obtained by THOMPSON (1985) in *Corylus*, another member of the *Fagales*, indicate a similar mode of inheritance of this parallel variation in two different plant families. JAHN (1934) reported a 93:96 segregation among the progeny of a purple specimen of *Fagus* surrounded by normal green beeches. He interpreted this in terms of a single gene locus in heterozygous condition but made it clear that this interpretation could be true only if the purple beech was self-sterile. This assumption is not compatible with variable intensity of pigmentation observed by the same author among the seedlings scored as purple.

WILCOX (1982) reported diverse proportions of purple seedlings from various geographic origins of *Eucalyptus fastigiata*, a member of the *Myrtales*. This author found some individuals difficult to classify because of varying anthocyanin coloration. Data from breeding experiments did not exist, but one-locus control with a dominant allele responsible for pigmentation was assumed.

Actually, *table 1* does not present evidence that the dominant alleles carried by BG and BM are homologous or even possess identical function. Many enzymes might be involved in anthocyanin biosynthesis whereby various genes control various steps. *Table 1* only demonstrates the analogous segregation behavior of the dominant alleles carried by the two trees used as pollen donors only.

Whether or not the interpretation of *table 3* is correct depends on the behavior of the homozygotes, which cannot be identified among the purple trees at this age. The purple seedlings in these families do not display a visible variation in intensity of leaf pigmentation. There is thus full dominance of R_2 over R_1. This is in accordance with THOMPSON'S (1985) results in filbert; she could only discriminate between the effects of the presence of presumably different alleles for leaf pigmentation, but not among the dose effects of one or two like pigmentation alleles. The viability of the homozygotes has to be confirmed later when the offspring will have reached maturity. Homozygous carriers of R_2 transmitted by BG do probably not arise. The proportion of empty seed grains was not observed but it may have been of help in explaining deficiences of purple offspring. The variation displayed by the 18 families supports the conclusion that further gene loci are involved in the control of pigmentation.

Since the origin of the purple trees BG and BM used as the original pollen donors is not known, it is not possible to relate results of R_2 to earlier descriptions of purple pigmentation.

The quality of R_2 as a marker in population studies is limited due to its rarity in the field, the genetic impact of complementary loci, and its evident influence on germination.

It has to be asked whether the heterogeneity of segregation among families shown in *table 1* is related to a linked *S*-locus. All carriers of R_2 had received this allele from BG and may have received also a common *S*-allele. Prezygotic incompatibility in *Corylus* was shown by THOMPSON (1979) to be controlled by one gene locus active in the sporophyte. This reference could apply also to *Betula* which belongs to the same family as *Corylus;* in the majority of the angiosperm families one and the same mode of genetic control of prezygotic incompatibility invariably occurs in all of their species. Results obtained by HAGMAN (1971) from experiments in *Betula pubescens* are compatible with either sporophytic or gametophytic control; HAGMAN (1975) inclined to assume gametophytic control in the genus *Betula*. However, results published by STERN (1963b) on *Betula pendula* support the hypothesis of sporophytic control, since a crossing experiment among full sibs gave rise to *three* incompatibility phenotypes in proportions compatible with a 2:1:1 ratio. HAGMAN'S (1971) result that incompatibility exists in tetraploid *Betula pubescens*, may also be interpreted by assuming sporophytic control. It is therefore not possible that close linkage between the *R* and *S* loci was responsible for variable segregation of green and purple seedlings among families.

The utility of R_2 as a marker in population studies is questionable for still another reason. In *table 8* the fre-

Table 7. — Phenotypic segregation of leaf pigmentation, GOT, and LAP isozymes, respectively, among 144 seedlings in family (413 × 204) × BG (cf. *tables 1* and *5*).

	LAP purple			*LAP* green			
GOT	*A2A3*	*A3A3*	sub-totals	*A2A3*	*A3A3*	sub-totals	totals
A1A2	17	17	34	10	24	34	68
A2A2	24	7	31	29	16	45	76
totals	41	24	65	39	40	79	144

Table 8. — Proportions p of green progeny among the bulked offspring of seed trees in a plantation composed of carriers of either phenotype. The 56 trees are grouped according to the composition of their neighborhood.

phenotype of seed parent	no. of seed parents	phenotype prevailing among surrounding trees	phenotypes among progeny green	purple	p
green	16	green	1041	5	> .99
green	15	purple	362	8	.98
purple	11	green	1795	1427	.56
purple	14	purple	3080	2050	.60

quencies of green and purple seedlings among the spontaneous progeny of green and purple seed parents in the nursery plantation are listed. The material used to support the following statements on the mating system in this nursery plantation was collected in two seasons in which dry and sunny weather conditions prevailed during the flowering period. It becomes obvious from *table 8* that green seed parents are predominantly pollinated by green pollen donors, since only a minor percentage of their offspring are carriers of R_2. This fraction amounts to only two per cent even if their nearest neighbors are purple trees; hence only four per cent of the pollen emitted by the immediate heterozygous purple neighbors becomes effective in a green tree growing amidst them. These low percentages have to be attributed to the retarded flowering period observed in the purple trees. If they were to be explained by the smaller frequency of purple trees in the plantation, the difference between lines 1 and 2, and between lines 3 and 4 of *table 8* had to be larger.

Purple trees surrounded by purple neighbors have 60 per cent green offspring. Since all of these neighbors are also heterozygotes, as little as 0.4/0.75 = 0.53 of the fertilizing pollen may have been shed by purple neighbors flowering at the same time; the true fraction may certainly exceed this expectation if one thinks of the deficit of purple cross progeny. Almost half of the effective pollen may then have come from green donors and may have been floating in the air until the stigmas of the purple trees became receptive.

The shortage of carriers of R_2 originally submitted from BG among the progeny of purple trees enclosed in a green neighborhood is very close to that observed in the cross progenies of BG reported in *table 1*. All effective pollen received by an isolated purple tree was then produced by its green neighbors days before. Even the pseudo-self-compatibility measured in controlled pollinations in *B. pendula* by Stern (1963b) amounts to only 6 per cent, so that virtually no purple offspring may arise from self-fertilization of purple trees having mating conctact with green individuals.

Wilcox (1982) discussed the adaptive significance of dark pigmentation and hence changed light utilization of individuals on the forest floor, and emphasized the importance of the position of pigmented cells in the leaf organs. In our birches, the palisade parenchyma is fully pigmented; pigmentation also occurs in one cell layer of the spongy parenchyma. This is in accordance with the appearance of the leaves: Their upper side is pigmented more intensely. The epidermis is free from any anthocyanin. The morphological conditions do not present any information on the changed absorption of radiation by the purple leaves.

This marker could have an interesting potential in populations composed artificially to study the environmental influences on the assortativity of mating brought about by a variation in flowering time. Any changes in the length of the gap between flowering of green and purple trees should be reflected in the genetic structure of the subsequent generation. Unfortunately, its dominance in addition to the other shortcomings discussed represents a serious obstacle to such attempts.

Leaf pigmentation is nevertheless a very useful marker of tissues *in vitro*, such as in culturing the anthers of heterozygous carriers of R_2. Zamir et al. (1981) proposed that the codominant expression of the alleles of enzyme gene loci rendered them efficient markers of regenerants; the absence of the expression of an allele in a regenerant excludes its sporogenous origin if the sporophyte was heterozygous. This may be extended to a gene locus in heterozygous condition with complete dominance of one allele; the expression of the recessive allele in regenerants helps to identify those individuals possessing priority in testing their genetic makeup in a battery of electrophoretic analyses. However, these subsequent tests are required since more chromosomes have to be represented, and the multiplicity of origins of gametophytic tissue has to be proven (Orton and Browers, 1985).

Early Flowering

The variation of the flowering habit in birch as described by Stern (1961) has to be interpreted by the activity of more than one controlling gene. The present results support this by the wide variation in the percentages of flowerers among families. The number of these genes may be low, as is indicated by the fast progress made by Stern (1961, 1963a) in artificial selection for early flowering. Additional progeny of families with extremely different proportions of flowerers was produced. It is hoped that crosses among those will help to provide more insight into the genetic control of this phenomenon.

Isozymes

The study of isozymes in the present material was restricted to one family only. This means that only such gene loci could be identified which happened to vary in this particular family. According to the position of the variable zones in the zymograms, the two loci provisionally identified had to be named *GOT-A* and *LAP-A*. The genetic control of the few isozymes prepared closely corresponds to the findings of Linares (1984) in the genus *Alnus*. A more systematic study of enzyme gene markers in other material is underway.

Linkage

The inference of linkage was possible due to balanced segregation at all of the three loci involved. The significant but loose linkage found between the *LAP-A* and *GOT-A* loci could not be compared with the results of Linares (1984) because other loci sharing in the control

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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Literature Cited

FERET, P. P. and BERGMANN, F.: Gel electrophoresis of proteins and enzymes. P. 49—77 in: MIKSCHE, J. P. (ed.). Modern Methods in Forest Genetics. Berlin, Heidelberg, New York: Springer (1976). —— HAGMAN, M.: On self- and cross-incompatibility shown by *Betula verrucosa* EHRH. and *Betula pubescens* EHRH. Comm. Inst. For. Fenn. **73**, 1—125 (1971). —— HAGMAN, M.: Incompatibility in forest trees. Proc. Roy. Soc. London, B. **188**, 313—326 (1975). —— JAHN, E.: Bemerkenswerte Gehölze im Forstbotanischen Garten der Forstlichen Hochschule in Hann.-Münden. Mitt. Dtsch. Dendrol. Ges. No. **46**, 132—136 (1934). —— JOHNSSON, H.: Genetic characteristics of *Betula verrucosa* EHRH. and *B. pubescens* EHRH. Annales Forestales 6/4, 91—133, 28 figs., Zagreb (1974). —— LINARES BENSIMÓN, C.: Versuche zur Viabilitätsselektion an Enzym-Genloci bei *Alnus glutinosa* (L. GAERTN. Göttingen Res. Notes in Forest Genetics No. 6, II u. 137 p., (1984). —— ORTON, T. J. and BROWERS, M. A.: Segregation of genetic markers among plants regenerated from cultured anthers of brocoli (*Brassica oleracea* var. 'italica'). Theor. Appl. Genet. **69**, 637—643 (1985). —— STERN, K.: Über den Erfolg einer über drei Generationen geführten Auslese auf frühes Blühen bei *Betula verrucosa*. Silvae Genetica **10**, 48—51 (1961). —— STERN, K.: Über die Abhängigkeit des Blühens der Sandbirke von Erbgut und Umwelt. Silvae Genetica **12**, 26—31 (1963a). —— STERN, K.: Versuche über die Selbststerilität bei der Sandbirke. Silvae Genetica **12**, 80—82 (1963b). —— THOMPSON, M. M.: Genetics of incompatibility in *Corylus avellana* L. Theor. Appl. Genet. **54**, 113—116 (1979). —— THOMPSON, M. M.: Linkage of the incompatibility locus and red pigmentation genes in hazelnut. J. Hered. **76**, 119—122 (1985). —— WILCOX, M. D.: Anthocyanin polymorphism in seedlings of *Eucalyptus fastigiata* DEANE and MAID. Austral. J. Bot. **30**, 501—509 (1982). —— ZAMIR, D., TANKSLEY, S. D. and JONES, R. A.: Genetic analysis of the origin of plants regenerated from anther tissues of *Lycopersicon esculentum* MILL. Plant Sci. Lett. **21**, 223—227 (1981).

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