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Original article

Micropropagation and *ex vitro* rooting of several clones of late-flushing *Quercus robur* L *

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Summary — Green acorns from 11 selected late-flushing *Quercus robur* trees were used as initial explants for micropropagation. From 60 acorns, 45 clones which produced shoots of suitable quality for *ex vitro* rooting were obtained. Half-sib clones derived form one mother tree produced an average of 409 microcuttings within 8 months. Half-sib clones of the other 10 trees produced only 11–188 shoots per clone. Microcuttings were rooted *ex vitro* after treatment with rooting powder containing 0.5% indole-3-butyric acid or 1.0% indole acetic acid. Shoots derived form subcultured shoot tips and nodal segments had a low rooting and survival rate (21%) after 4 months. 56% of shoots derived from subcultured basal segments with a callus, rooted and survived.

tissue culture / micropropagation / *in vitro* propagation / *Quercus* / *ex vitro* rooting

Résumé — Micropropagation et enracinement *ex vitro* de plusieurs clones de *Quercus robur* L à débourrement tardif. *Des glands encore verts ont été récoltés sur 11 clones de* Quercus robur *à débourrement tardif et utilisés comme matériel de départ pour la micropropagation. Sur 60 glands, 45 se sont avérés de qualité suffisante pour être enracinés* ex vitro. *Des clones demi-frères d'un seul arbre mère ont produit 409 microboutures en 8 mois. Des clones demi-frères issus des 10 autres arbres n'ont produit que de 11 à 188 pousses par clone. Les microboutures ont été enracinées* ex vitro *après avoir été enduites d'une poudre comprenant 0,5% d'acide indole butyrique et 1,0% d'acide indole acétique. Les pousses issues des cultures ultérieures des parties apicales et des segments de tiges (comprenant un nœud) manifestaient un faible enracinement et taux de survie (21%) après 4 mois; 56% des pousses issues des cultures de segments récoltés à la base des tiges et ayant un cal se sont enracinées et ont survécu.*

***culture* in vitro / *micropropagation / multiplication* in vitro / Quercus / *enracinement* ex vitro**

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INTRODUCTION

In vitro techniques in the genus *Quercus* can or will be used in future for propagation, tree improvement or gene conservation. Methods for the large scale micropropagation of juvenile material are already available (Chalupa, 1984, 1988). Genetically improved seeds from seed orchards or controlled pollination can be micropropagated for reforestation. The micropropagation of selected or tested mature trees is more difficult. In *Quercus robur* and *Quercus petraea*, rooted plantlets (Vieitez *et al*, 1985; Evers *et al*, 1987; San José *et al*, 1988, 1990; Juncker and Favre, 1989) as well as plants in soil (Meier-Dinkel, 1987; Chalupa, 1988) have been produced from adult trees. Shoot cultures can be cold stored without any subculturing or medium replenishment. Samples of clones which are being field trialed are maintained in our lab by repeated cold storage cycles (4 yr at 4°C + 2 normal subcultures at 25°C) until results are available (Meier-Dinkel, unpublished). Moreover, valuable genotypes can be cold stored for medium-term periods in gene conservation programs. For long-term storage, cryopreservation methods are available (Jörgensen, 1990). In this article, we report the micropropagation of late-flushing *Q robur* from acorns of selected trees. To our knowledge, results of *ex vitro* rooting of micropropagated oak are presented here for the first time.

MATERIALS AND METHODS

Experiments were performed with selected trees of *Quercus robur Münsterländer Späteiche*. These oaks flush late in the spring (usually 14 d after normal *Q robur)* and have a very good stem form. Due to the late flushing they escape damage from late frosts and are not attacked by the oak leaf roller (*Tortrix viridana*).

To establish *in vitro* cultures, 4–6 acorns were harvested from 11 grafted trees of 2 stands (7 trees from Viersen (V) and 4 trees from Königsforst (K)) grown in a plastic greenhouse. The acorns were surface-sterilized in 70% ethanol for 1 min and 3% NaOCl for 5 min. The seed coats were removed and the whole embryos were surface sterilized in 0.5% NaOCl for 5 min and then rinsed in sterile water 3 times for 5 min. In each case, half of the embryos of the 11 mother trees were placed on solid Gresshoff and Doy (GD) medium (1972) and woody plant medium (WPM) (Lloyd and McCown, 1980), respectively, supplemented with 0.5 mg/l benzylamino-purine (BAP). The explants were kept in a culture room at 25 ± 1°C under 16 h photoperiod at 1500 Lux (Philips TLD 84).

Developing shoots were cut into shoot tips and nodal segments 4 weeks after culture initiation and were subcultured on the same media (the cotyledons having been removed). After the first subculture, 3 different types of explants were used for further propagation: shoot tips, nodal segments and basal segments with callus. BAP was used at a concentration of 0.5 mg/l until the 3 subculture. For the 4th monthly subculture, the level of BAP was reduced to 0.2 mg/l. *Ex vitro* rooting experiments were carried out in April and May 1991 after the 4th and the 5th subcultures. In April, 925 microcuttings of 24 clones (20 V, 4 K) were treated with 2 different types of commercial rooting powder: Rhizopon AA (0.5% indole-3-butyric acid (IBA) and Rhizopon A (1.0% indole acetic acid (IAA). In May, microcuttings of all 45 clones obtained were treated only with Rhizopon A.

The microcuttings were inserted in a light peat substrate (80%) with 20% perlite and placed under a plastic tunnel with bottom heating, which was located in a larger glasshouse. After 6 weeks, the humidity was gradually reduced by opening the plastic tunnel. In August, the surviving plants were potted into 10-cm Jiffy pots and percent survival was calculated.

RESULTS

From 60 acorns, 52 sterile shoot cultures were established *in vitro*. After 5 subcultures, microcuttings suitable for rooting were obtained from 45 clones. The *in vitro*

shoot productivity depended upon the clone; the number of shoots produced after 8 months varied between 10 and more than 1000 clone. There was also an effect of the mother tree on the *in vitro* productivity. Five clones from mother tree V 9 produced an average of 409 microcuttings/ clone (table I). The remaining clones form the other 10 mother trees produced an average between 11 and 188 microcutting clone (table I).

In the first rooting experiment with 24 clones, no difference was observed between Rhizopon AA and Rhizopon A. However, there was a big difference in survival between the 2 types of microcuttings. Shoots derived from subcultured shoot tips and nodal segments had a low survival rate (21% (119/564)) after 4 months. Shoots derived from subcultured basal segments with a callus had a better success rate 56% (201/361).

In the second rooting experiment with 45 clones, microcuttings from shoot tips and nodal segments were not separated from those grown from basal segments with a callus. The percent survival after 3 months was 35% (1326/3738), which is intermediate between the values obtained in the first experiment. Survival was found to be strongly dependent upon the clone with values ranging between 10 and 80% for the individual clones. The influence of the genetic background of the mother tree is demonstrated by differences found in plant survival

Table I. *Quercus robur Münsterländer Späteiche.* Results of micropropagation of half-sib clones derived from acorns of 11 selected late-flushing mother trees from 2 stands (V = Viersen, K = königsforst). Mean number of microcuttings produced *in vitro* and mean number and percentage of surviving plants/clone.

Mother tree	*No of acorns*	*No of sterile cultures obtained*	*No of clones transferred to the glasshouse*	*Mean no of microcuttings/ clone transferred ± SE*	*Mean no of surviving plants/clone ± SE*	*Mean percentage of surviving plants/clone ± SE*
V 7	6	6	6	188 ± 32	84 ± 18	45 ± 9
V 9	6	5	5	409 ± 194	92 ± 16	43 ± 12
V 12	6	6	6	51 ± 35	30 ± 22	65 ± 9
V 15	6	6	6	48 ± 12	20 ± 8	30 ± 13
V 18	6	3	3	60 ± 23	26 ± 12	40 ± 12
V 23	4	2	2	(11)*	(5)*	
V 25	6	6	5	44 ± 27	11 ± 6	29 ± 6
All V	Σ 40	Σ 34	Σ 33	$\bar{x}$ = 119	$\bar{x}$ = 38	$\bar{x}$ = 42
K 3	6	6	4	24 ± 7	11 ± 7	45 ± 4
K 4	4	3	2	35 ± 22	24 ± 19	58 ± 20
K 6	6	6	3	58 ± 24	34 ± 17	41 ± 21
K 11	4	3	3	46 ± 30	16 ± 7	49 ± 11
All K	Σ 20	Σ 18	Σ 12	$\bar{x}$ = 41	$\bar{x}$ = 21	$\bar{x}$ = 48
Total V + K	Σ 60	Σ 52	Σ 45	$\bar{x}$ = 91	$\bar{x}$ = 32	$\bar{x}$ = 45

* Since there were only 2 clones with only a few microcuttings and surviving plants, ± SE was not calculated for this mother tree.

as shown by the mean number of surviving plants/clone (table I). Depending upon the source tree, 5–92 plants/half-sib clone survived. The mean percentages of surviving plants/clone derived from one mother tree ranged from 29 to 65% (table I).

DISCUSSION

The results presented here were obtained with elite material which is in great demand for planting programs. A large variation was observed between the 52 clones investigated regarding shoot productivity under the same multiplication conditions over a period of 8 months. Juncker and Favre (1989) also found important between-clone differences concerning *in vitro* growth behavior of 16 clones derived from juvenile seedlings. This between-clone heterogeneity can cause problems and will have to be taken into account when many different clones will be propagated commercially on a large scale. Clonal mixtures for woodland planting should contain approximately the same number of plant clone. *Ex vitro* rooting was applied in order to simplify the protocol and to reduce production costs. The advantages are that the rooting step under sterile conditions is eliminated and that rooting and acclimatization take place at the same time. However, *ex vitro* rooting requires *in vitro* shoots of high quality. The best results were obtained with microcuttings grown form subcultured basal segments with a callus. These shoots were stronger and probably in a better physiological condition for root formation. Future research should be directed at the improvement of the physiological status of the shoots regenerated from nodal segments and shoot tips in order to achieve a high rooting potential comparable to that of the shoots from segments with a basal callus. For practical application, micropropagated plants could be used as stock plants for cutting propagation. This would improve the commercial feasibility of vegetative propagation of selected oak material.

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