Ornamentals

The role of rejuvenated and adult forms of English oak *(Quercus robur)* in *in vitro* cultures.

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Summary: In vitro plant material of clones *(Q. robur)* NL 100 A (adult) and NL 100 R (rejuvenated) received from Germany *(A. Meier-Dinkel,* 1995) were used in these experiments. WPM medium was used for the multiplication phase. Plantlets were subcultured monthly. Differences in quality and colour of the adult and rejuvenated cultures induced us to follow and compare the changes of mineral- and chlorophyll content and dry weight during the propagation phase. Mineral and chlorophyll content as well as dry weight were measured weekly on three samples during the subculture period.

In the case of propagation rates we stated, they were similar around the year, but both clones had a high peak in April. Examining the cation-content, we detected that, the plantlets had a highest quantity of several elements during the 2nd and 3rd week of subculture. The iron content was the highest in the 1st week and after that it decreased continuously. It is supposed, that the content of iron is not enough in the media. The chlorophyll content of the rejuvenated clone was higher than that of the adult one.

In the rooting experiments it was stated that, after one-week cold treatment the rooting ability was the best.

Introduction

The English oak (*Quercus robur*) is a very important tree in Europe, but as *Beloh & Kirch* (1984) detected, more than 50 % of European forest is dying. In the case of *Q. robur* it takes 43 %. The English oak is also very important as ornamental plant. The forestry and the horticulture needs healthy, true to variety plants. But the true to type vegetative propagation of oak is not easy.

The micropropagation is the rapid way, but the age of the mother plant has a great role at the micropropagated plantlets. *Bonga* (1982) wrote, that the adult form of oak was very difficult to propagate. Therefore he suggests the rejuvenation (*Bonga*, 1982; *Farnum et al.* 1983). *Bonga* (1982) reported, about the best results of propagation of the rejuvenated plants. He found that, juvenile explants grew and proliferated easily (*Chalupa* 1983, *Chalupa* 1985). For this reason the rejuvenation is necessary (*Franclet*, 1981). *Meier-Dinkel* (1995) found there was a tremendous difference between adult and juvenile forms in case of proliferation (50%). In case of rooting and acclimatisation the difference between the two forms was also great.

Gruselle Nicaise (1995) measured, the kind of Fe - chelate produced a different effect on the propagations rate. Fe/EDDHA is advised to use.

In our experiment, connecting to Cost 822, we try to characterise oak plantlets under in vitro conditions.

Material and methods

Quercus robur clones arrived from *Andreas Meier-Dinkel* (1995) (Niedersachsische Forstliche Versuchsanstalt, Abteilung Forstpflanzen-züchtung, 3513 Staufenberg-Escherode, German Federal Republic.) at the end of 1994 (NL 100 A, adult form) and in January of 1995 (NL 100 R, rejuvenated form).

Offer of *A. Meier-Dinkel* (1995) was used during the propagation phase: WPM medium (*Lloyd & McCown*, 1980) supplemented with 0.2 BAP and 20 g/l sucrose and solidified with 6 g/l agar Bacto Difco. The pH was adjusted to 5.7 before autoclaving with 0.1 Mol KOH.

Plant material was cultivated in 220 ml glass jars closed with semipermeable plastic foil, under a photoperiod of 16/8 and 1500 lux light intensity in a culture room of 22 degree centigrade. Plantlets were subcultured monthly.

Dry weight content was measured after a drying period at 105 degree centigrade.

Determination of cations occurred from dried, homogenised and fractured plant material with an ICP-AES equipment, in mg/g. Chlorophyll content was obtained from fresh, homogenised and acetone extracted plant leaves. Extinctions were measured by SPECOL-K equipment on 663 nm chlorophyll A and on 645 nm chlorophyll B.

For rooting experiments 20–25 mm long shoots were used, and the treatments were:

- continuous effect of 0.5, 1.0, 2.0, 3.0 mg/l IBA in a half strength MS medium
- cultures were kept in total darkness for a week before root induction
- one week long cold treatment at 4 degree centigrade preceded root induction
- root induction on 50 mg/l IBA medium for one week and rooting on hormonfree half strength MS medium

The rooted plantlets were transplanted into "Jiffy 7" tablets and acclimated under high relative humidity in glasshouse.

The measures were made with 10 plantlets-sample, in 3 parallel measure.

The statistical analysis were made with Mquattro and Statgraf program on PC.

Results and discussion

There are significant differences between multiplications rate and plant quality of adult and rejuvenated *Quercus robur* clones in our experimental conditions.

Trends of the propagation rates –connect with *Chalupa* (1983, 1985) statement– are similar around the year *(Tab. 1)*. Both clones have a really high pick during the spring time propagation, and only very few new shoots grow wintertime.

Although both clones were transplanted and the newly growing shoots were isolated from the mother shoot monthly and the same medium was used the quality of the cultures were different: new leaves of NL 100 A clone were green at the beginning of growth, but some days later the expanded large leaves become yellowish and the shoot tip stopped to grow. The newly grown shoots were hard, too.

Shoots of NL 100 R keep their soft substances till the next transplantation. The leaves of the rejuvenated clones were much smaller then the adult ones and kept their green colour during the whole propagation phase.

By examining the change of some cation contents (like Mg, K, Ca, Mn, Zn and Cu) during propagation phase it should be mentioned that for 2–3 weeks the culture achieved the maximum cation content *(Tab. 2)* as well as dry weight pro unit and decreased during the 4th week, except iron and phosphorus. In our opinion the reason for this detection is the following: the plantlets take up the elements from the medium. On the 3rd week the cation content in the medium is rather poor. The plantlets can not take up more from the medium, and after that, the cations concentration in the plantlets decreases.

Tab. 2 Changing of cation content during the propagation phase of NL 100 A and NL 100 R (mg/g)

	Cation content (mg/g)							
week	1.		2.		3.		4.	
	NL A	NL R	NL A	NL R	NL A	NL R	NL A	NL R
K	25622	22816	25428	26016	23404	34043	26130	27595
Ca	4890	5785	6139	7365	4685	6669	4785	5344
Mg	2202	2113	2013	2092	1943	2299	1641	1903
Mn	276	450	291	402	361	494	279	319
Zn	102	111	93	127	92	95	62	107
Cu	9	9	7	7	6	10	6	7

The iron content continuously decreases during the multiplications time in the case of both clones *Tab. 3*. It should be supposed, that the iron content of WPM medium is not enough for the growing *Quercus robur* shoots.

Tab. 3 Changing of iron content during the propagation phase of NL 100 A and NL 100 R (mg/g)

	Iron content (mg/g)				
Week	0.	1.	2.	3.	4.
NL 100 A	82	158	134	110	83
NL 100 R	104	132	148	110	100

Tab. 1 Changes of propagation rate in case of NL 100 A and NL 100 R around the year

	Propagation rate												
	I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	S. diff.
NL-R	3.3	2.4	4.8	5.2	3.6	3.3	3.2	3.3	3.0	3.2	3.4	2.7	0.35
NL-A	1.8	1.6	3.2	3.4	2.8	2.7	2.7	2.5	2.6	2.2	1.6	1.8	0.26

The phosphorus content followed a 3-week long mostly equally level period grows for the 4th week, caused the ageing of the culture *(tab. 4).*

Tab. 4 Changing of phosphorus content during the propagation phase of Quercus robur, NL 100 A and NL 100 R (mg/g)

	Phosphorus content (mg/g)			
Week	1.	2.	3.	4.
NL 100 A	3328	3542	2882	3257
NL 100 R	2650	3012	2764	3635

The chlorophyll content of the rejuvenated clone is higher than that of the adult ones, on the first week of subculture, the chlorophyll content reached a higher content. But after that continuously decreased during the propagation phase *tab. 5.*

Tab. 5 Changing of chlorophyll content of NL 100 A and NL 100 R during the propagation phase (mg/g).

	Chlorophyll content (mg/g)									
week	0.		1.		2.		3.		4.	
	NLA	NLR	NLA	NLR	NLA	NLR	NLA	NLR	NLA	NLR
A	1.81	2.01	2.65	2.29	1.80	2.26	1.76	2.08	1.77	1.97
B	0.47	0.23	0.65	0.59	0.45	0.62	0.40	0.54	0.44	0.24
Total	2.18	2.22	3.29	2.88	2.25	2.88	2.16	2.62	2.21	2.21

If we see the iron and chlorophyll content together, it shows a correlation, there is a iron deficiency in the plants. As well as *Végvári* (1995) detected in the medium, depending on illumination the uptakeable iron content decreases. We are sure, this is the basic of *Gruselle Nicaise* (1995) detection. So, after our experiment we suggest also using higher Fe content, or choosing stationary form as Fe-EDTA. We don't used Fe-EDDHA during our experiment, but it will be very important to measure with ICP-AES the change of iron content in the case of using this later compound, during the time of subculture.

All of these details suggest, that working 3-week long transplantation period to induce a better culture quality. We found that under our laboratory conditions there were big differences between rooting ability of the two *Quercus robur* clones. Results of rooting experiment is summarised in *fig.6.* No rooting was observed during continuous effect of 0,5 and 3,0 mg/l IBA. The dark treatment caused shoot tip necroses in most of the adult plantlets and in some cases of the rejuvenated ones. Effect of a one week long cold period was stimulating for rooting. Root induction on 50 mg/l IBA medium and rooting on hormonfree half strength MS medium without any pre-treatment gave poor results with A clone and a little bit better with R clone. Shoot tip necroses could be observed in both cases.

Shoots transplanted under glasshouse conditions after rooting period without roots did not root during the acclimation phase. The in vitro rooted plantlets survived the acclimation under high relative humidity.

Tab. 6 Rooting of NL 100 A and NL 100 R after different treatment (%)

IBA	Continuous (mg/l)				Induction 50 mg/l		
	0.5	1.0	2.0	3.0	dark	Cold	Pretreatment
NL A	0	6	9	0	8	21	12
NL R	0	8	18	0	27	40	32

(100 shoots were used for each treatment.)

By reason of our experiments, we are inclined to think, that the better propagations results of rejuvenated plants (*Bonga* 1982; *Farnum et al.* 1983, *Chalupa* 1983, *Chalupa* 1985) is based on the different physiological state.

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