and limited number of loci, the best way to investigate genetic consequence of indirect early selection is through computer simulation. The infinite model presented here is only a first approximation for predicting expected genetic gain under several generations of indirect early selection. This expected response can be regarded only as an upper limit of possible genetic gain from several generations of indirect early selection.

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Stomatal Characteristics and Water Relations of *In Vitro* Grown *Quercus robur* NL 100 in Relation to Acclimatization

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Abstract

Experiments were carried out to study stomatal characteristics and the % of moisture loss of *in vitro* grown leaves of *Q. robur* NL 100 in relation to acclimatization. Microscopic observations on the abaxial leaf surfaces of acclimatized and greenhouse grown plants showed elliptical stomata (16 µm to 18 µm) with narrow apertures (3 µm to 4 µm). In comparison, the stomata of leaves from *in vitro* showed spherical stomata (20 µm to 30 µm) with widely opened apertures (6 µm to 8 µm). Leaves from shoot multiplication and the two stages of rooting *(in vitro)* had higher stomatal density and index values as compared to acclimatized and greenhouse-grown plants. The % of moisture loss of *in vitro* grown leaves was considerably greater than that of acclimatized and greenhouse grown plants. This study will help to explain the failure of *in vitro Q. robur* NL 100 plants after transplanting to the greenhouse.

Key words: Fagaceae, *Quercus robur,* stomata, water loss, weaning.

Abbreviations: AC: acclimatization, BA: N^6 benzyladenine, B_5: GAMBORG *et al.* (1968), GD: GRESSHOFF and DOY (1972) medium, GH: greenhouse

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grown, IBA: indole-3-butyric acid, MS: MURASHIGE and SKOOG's (1962) medium, PAR: photosynthetically active radiation, RI: root initiation, RE: root elongation, SD: stomatal density, SI: stomatal index, SM: shoot multiplication.

Introduction

The wide spread use of micropropagation is restricted for several reasons, one of these being that a high percentage of *in vitro* grown plants can be lost or damaged at the time of transfer from culture vessel to soil (FILA *et al.,* 1998). It is known that culture vessel micro-climate is one of the major factors affecting *in vitro* cultured explant physiology and morphology. The main factors considered to be responsible for the specific effects are elevated relative humidity, gas composition inside culture vessels, temperature and, in addition, radiation parameters such as quality, intensity and photoperiod (ZACCHANI *et al.,* 1997). *In vitro* grown plants usually show rapid wilting when transferred to greenhouse or field conditions if care is not taken to maintain a high humidity in their new environment; obviously, the mechanisms that allow the maintenance of their water content are severely impaired (FILA *et al.,* 1998). This has been attributed to poor regulation of leaf transpiration due to a very thin cuticle (SUTTER *et al.,* 1988), lack of leaf epicuticular waxes (AL-AHMAD *et al.,* 1998) and poor regulation of stomata (BRIANERD and FUCHIGAMI, 1982; ROSS-KARSTENS *et al.,* 1988).

To reduce the initial wilting of transplanted *in vitro* grown plants, acclimatization before transfer from culture was recommended (SHAKEL *et al.,* 1990). Many studies used environmental modifications such as decreasing air humidity by forced ventilation (CASSELS and WALSH, 1994), raising CO_2 molar ratio in specially designed culture vessels or increasing light intensities during *in vitro* cultivation (KOZAI *et al.,* 1997). Attempts have also been made to induce stomatal closure by the exogenous application of paclobutrazol (SMITH *et al.,* 1992) or abscisic acid (POSSPISILOVA, 1996) or by decreased osmotic potential of medium using polyethylene glycol (ZAID and HUGHES, 1995). The effectiveness of these methods depends upon ambient conditions as well as the species under consideration. In this research, we followed acclimatization method to transfer micropropagules from culture vessel to greenhouse. The purpose of this research is to study the stomatal characteristics and water relations of *in vitro* grown *Quercus robur* NL 100 in relation to acclimatization.

Materials and Methods

Micropropagation of Quercus robur NL 100

Established *in vitro* cultures of *Quercus robur* NL-100 clone provided by Dr. EVA WILHELM (Austrian Research Centre Seibersdorf, Seibersdorf, Austria) were used. Shoots of 1 to 2 nodes (2 to 3 leaves) and 1.0 cm to 1.5 cm in length were excised from the established cultures. These nodes were placed in 500 ml transparent polystyrol jars (Polarcup, Belgium) containing 75 ml of nutrient medium consisting of GRESHOFF and DOY (1972) mineral salts, B_5 vitamins (GAMBORG *et al.,* 1968), 2 mg/l glycine, 3 % (w/v) sucrose and 0.2 mg/l benzyladenine (BA). The medium was adjusted to pH 5.7 before addition of 0.8 % (w/v) agar (Roland, Belgium) and subsequently autoclaved for 20 min at 120 °C. Proliferating nodal bud cultures were subcultured onto fresh medium every 6 weeks and maintained in a culture room at a temperature of 25 °C with 55 % relative humidity under 16 h light /8 h dark photoperiod with irradiance of 20 µmol m^{-2} sec^{-1} (photosynthetically active radiation, PAR) provided by cool-white fluorescent lamps. Shoots resulting from the shoot multiplication phase were used for rooting. The shoots were approximately 2 cm long and had 4 to 6 leaves, 2 nodes and the shoot tip. Rooting involved a two step procedure: root initiation (RI) and root elongation (RE). Root initiation (RI) was carried out on agar (0.8 %) solidified half-strength MURASHIGE and SKOOG's (1962) medium supplemented with 2 % sucrose and 0.25 mg/l indole-3-butyric acid (IBA) for 3 weeks and root elongation (RE) following transfer to half-strength MS medium devoid of auxin for another 3 weeks. Rooting performed in 500 ml transparent polystyrol jars (Polarcup, Belgium) containing 75 ml of rooting medium upon which 2 shoots were placed. Incubation conditions were the same as in multiplication.

Acclimatization

Rooted shoots were removed from the culture tubes, washed well to remove remnants of agar from roots and transplanted in plastic trays containing autoclaved vermiculite saturated with half-strength MS salt solution. Plants were then covered individually with transparent polystyrol cups to ensure high humidity and kept in the growth chamber (Forma Scientific, USA) at a temperature of 20 °C ± 2 °C with 55 % relative humidity under 16 h light/8 h dark photoperiod with 40 µmol m^{-2} sec^{-1} PAR provided by cool-white fluorescent lamps. Plants were well irrigated twice per week with half-strength MS salt solution and hardened for 3 weeks. Afterwards the plants were repotted in pots filled with a mixture of soil, sand and organic manure (1:1:1) and kept in the greenhouse at a temperature of 20 °C ± 2 °C with 55 % relative humidity under natural light for developing into mature plants.

Detached leaves collected from the shoots/plants of multiplication (SM), root initiation (RI), root elongation (RE), acclimatization (AC) and greenhouse (GH) of micropropagation phases were used to study stomatal characteristics and the % of water loss.

Stomatal characteristics

Stomatal distribution was studied from quick fix leaf imprints (SAMPSON, 1961). A thin layer of quick fix was applied uniformly on the abaxial surface of the leaves. After 5 minutes, the dried membrane was carefully peeled off. The size of stomata and aperture, number of stomata (NS) and epidermal cells (NE) were determined from leaf imprints at 400X magnification by light microscopy (Leitz, Germany). Stomatal density and stomatal index were calculated using the following formula:

Stomatal density (SD) = NS / A

Where NS = number of stomata in the microscopic field, A = area of the microscopic field.

Stomatal index (SI) = NS x 100/ NS + NE

Where NS = number of stomata in the microscopic field, NE = number of epidermal cells in the microscopic field. Three leaf imprints were taken from each leaf and stomatal counts were made at three different locations on each imprint. The data were subjected to statistical analysis using 'standard error of the mean'.

Water loss experiment

Detached leaves were allowed to dry in air of ambient laboratory conditions (approximately at 55 % relative humidity and 21 °C temperature). Leaves from each phase were weighed immediately after excision and every 30 min thereafter for 2 h. The leaves were then oven-dried at 70 °C for 24 h, and reweighed to determine the dry weights. The % of water loss during the different phases of micropropagation was calculated using the following formula:

$WL= (FW_{t0} - DW_t) - (FW_t - DW_t) \times 100/ (FW_{t0} - DW_t)$

Where WL = Percent of water loss (%), FW_{t0} = fresh weight at time zero, FW_t = fresh weight after time, DW_t = dry weight. Each treatment had 12 replicates. The experiment was repeated once and the data subjected to statistical analysis using 'standard error of the mean'.

Results and Discussion

Stomatal characteristics

Microscopic observations on the abaxial leaf surfaces of acclimatized and greenhouse grown plants showed elliptical stomata (16 µm to 18 µm), with the major axis markedly longer than the minor one as stated by BUSSOTTI and GROSSONI (1997). The stomata have distinct guard cells with narrow apertures (3 µm to 4 µm) *(Fig. 1)*. In comparison, the stomata of leaves from *in vitro* (shoot multiplication and the two stages of rooting) showed spherical stomata (20 µm to 30 µm) with widely opened apertures (6 µm to 8 µm) *(Fig. 2)*. The spherical shape is considered to be associated with absent or highly compromised *in vitro* stomata function and the elliptical shape is characteristic of *in vivo* stomata endowed with normal function (ZACCHANI *et al.,* 1997). It has been suggested that high humidity in culture affects the course of development of guard cells as well as the stomatal closing mechanisms during leaf maturation (MARIN and GELLA, 1988).

Leaves from shoot multiplication and the two stages of rooting *(in vitro)* had higher stomatal density values as compared to acclimatized and greenhouse-grown plants *(Fig. 3)*. Findings of the present investigation confirm previous results reported for Apple (BLANKE and BLECHER, 1989), Roses (CAPELLADES *et al.,* 1990) and *Delphinium* (SANTAMARIA *et al.,* 1993). Stomatal

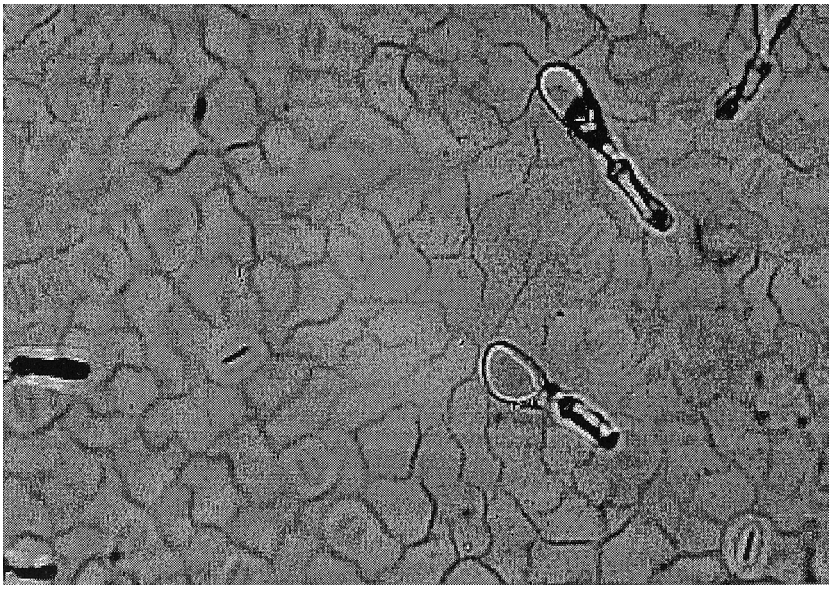

Fig. 1. – Photomicrographs of the abaxial leaf surfaces of greenhouse grown *Q. robur* NL 100 plants at a 400X magnification.

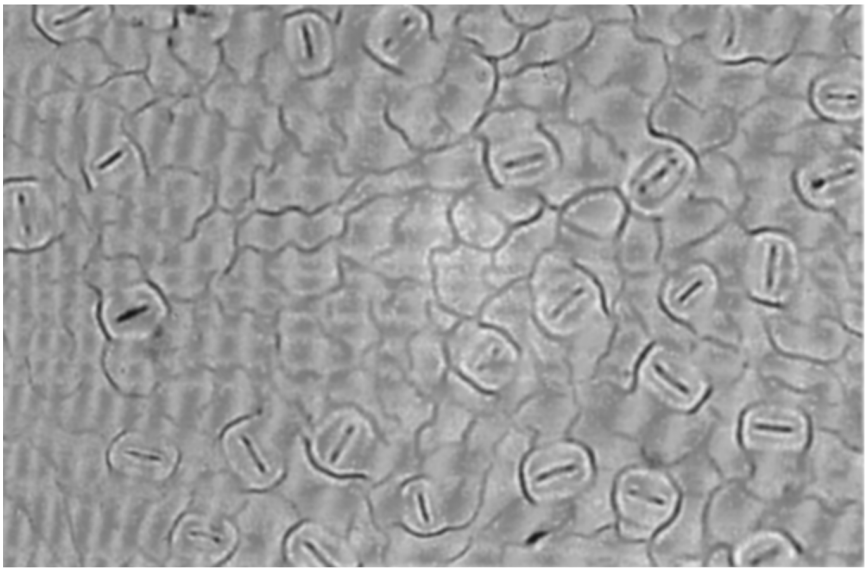

Fig. 2. – Photomicrographs of the abaxial leaf surfaces of *in vitro* grown (from root initiation) *Q. robur* NL 100 plants at a 400X magnification.

development and density can be affected by water availability, light intensity, temperature, humidity and CO_2 intensity (SCIUTTI and MORINI, 1993) and osmotic concentration of culture medium (ZAID and HUGHES, 1995). Increase in the light intensity from 20 µmol m^{-2} sec^{-1} to 40 µmol m^{-2} sec^{-1} during the acclimatization period caused a decrease in stomatal density. This was supported by investigations in sweetgum (LEE *et al.,* 1988).

Stomatal differentiation was also studied by analyzing variations in the stomatal index (stomata per 100 epidermal cells). Leaves from greenhouse grown plants of *Q. robur* NL 100 had the lowest stomatal index values as compared to acclimatized and *in vitro* grown leaves *(Fig. 3)*. Stomatal index and density values were closely matched for the leaves of greenhouse grown plants. In contrast, stomatal index and density values were varied for the leaves of acclimatized and *in vitro* grown leaves. The variation between the stomatal index and stomatal density could be due to the total number of epidermal cells. Similar results were obtained with *Prunus cerasifera* and *Malus pumila* (ZACCHANI *et al.,*1997).

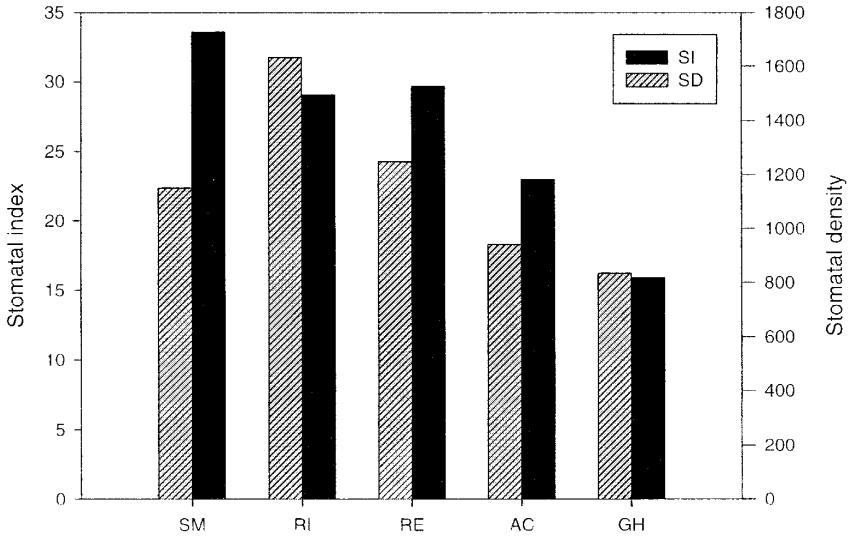

Fig. 3. – Stomatal density and index of leaves of *in vitro* cultured, acclimatized and greenhouse grown *Q. robur* NL 100. Each treatment is an average of 12 leaves (on three different leaves) (SM: shoot multiplication, RI: root initiation, RE: root elongation, AC: acclimatization, GH: greenhouse).

Water loss experiment

The % of moisture loss of *in vitro* grown leaves was considerably greater than that of acclimatized and greenhouse grown plants. This is true at each of 30 min, 60 min, 90 min and 120 minute intervals of air-drying *(Table 1)*. Leaves from shoot multiplication phase lost approximately 90% of their water content within 30 min. Leaves from rooted shoots lost 80% of their water content within 90 min. Acclimatized plants had an average water loss of approximately 45% and 65% after 30 min and 120 min, respectively. In contrast, greenhouse grown plants lost 14% and 25% of their water content after 30 min and 120 min respectively *(Table 1)*. *In vitro* grown leaves shrivelled whereas those from acclimatized plants showed slight wilting. Greenhouse grown plants remained nearly intact. This clearly demonstrates the inability of *in vitro* grown non acclimatized plants to resist to desiccation. Similar results were reported for apple (SHAKLE *et al.,* 1990) and grape (FILA *et al.,* 1998).

The present study showed that the rapid leaf wilting and the consequent desiccation of *in vitro* grown *Q. robur* NL 100 were a result of excessive water loss. This phenomenon might be associated with high density and heterogeneous functionality of stomata. Normal looking elliptical stomata would respond to stimuli, while other circular stomata would be unable to close completely, keeping the leaf percent of moisture loss at high level. In conclusion, the acclimatization process under high irradiance and gradual decrease in relative humidity apparently reduced the excessive water loss of leaves by restoring stomatal function and thereby avoided wilting of the leaves and the enhancing their survival after transfer to the greenhouse.

Table 1. – The percent of water loss from detached leaves of *in vitro* grown, acclimatized and greenhouse grown *Q. robur* NL 100 (SM: shoot multiplication, RI: root initiation, RE: root elongation, AC: acclimatization, GH: greenhouse).

Phase	30	60	90	120
SM	88.1 ± 4.1	88.1 ± 4.1	88.1 ± 4.1	88.1 ± 4.1
RI	40.5 ± 3.7	80.6 ± 2.4	80.6 ± 4.3	80.6 ± 4.3
RE	50.9 ± 5.2	72.6 ± 5.9	79.2 ± 5.1	82.5 ± 5.0
AC	44.6 ± 6.1	58.8 ± 7.5	64.7 ± 8.7	64.1 ± 8.1
GH	14.0 ± 2.2	14.6 ± 2.2	22.2 ± 2.0	24.9 ± 3.2

Each treatment combination is an average of 12 replicates

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Investigations on the Correlation Pattern in Even-aged Stands of Larch

VI. Relationships Between Single Tree Height and Diameter Measurements and Individual THIESSEN Polygon Areas

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Summary

The objective of this study was to investigate the relationships between single tree measurements for height and diameter and available individual areas per tree (determined by the areas of THIESSEN polygons) in even-aged stands of larch. Extensive data sets from a field trial with 27 entries of *Larix europaea, Larix leptolepis* and their hybrids were available. Single tree measurements for height and diameter of this trial have been analyzed for 6 stages of stand development (ages: 11, 13, 19, 50, 53, 56 years). The correlation between height and area was negative and small (–0.17, –0.18, –0.22, –0.20, –0.28, –0.31), while the correlations between diameter and area are separated into two clearly differentiated parts: negative with small numerical values (–0.15, –0.14, –0.17) for the early period of stand development (up to 19 years) and positive with small numerical values (+0.19, +0.22, +0.24) for the late period of stand development (up to 56 years).

In this study, the areas of THIESSEN polygons have proven to be poor predictors of tree height and tree diameter. But, they are a reasonable measure of two-dimensional area available to comparable individuals and may provide useful independent variables for prediction approaches in even-aged populations.

Key words: larch, height, diameter, Thiessen polygon tesselation, competition index, trait-area relationships.

Zusammenfassung

Für die Untersuchung der Beziehungen zwischen Einzelbaummessungen für Höhe und Durchmesser und den verfügbaren individuellen Standräumen (hier bestimmt durch die Flächen von THIESSEN Polygonen) in gleichaltrigen Lärchenbeständen wurde eine Versuchsfläche mit 27 Sorten von *Larix europaea, Larix leptolepis* und ihren Hybriden ausgewählt, die zu 6 verschiedenen Zeitpunkten der Bestandesentwicklung (Alter: 11, 13, 19, 50, 53, 56 Jahre) einzelbaumweise vermessen wurde. Die Korrelation zwischen Höhe und Standraum ist

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