

# MORPHOLOGICAL AND ANATOMICAL JUVENILITY MARKERS IN SILVER BIRCH (*BETULA PENDULA* ROTH) IN VITRO SHOOTS

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*During plant development, woody plants progress from a juvenile phase to the reproductive mature phase. This process is marked by morphological, physiological, and biochemical changes, including loss of competence to vegetative reproduction. During in vitro stabilisation, mature tissues undergo rejuvenation, however, the exact degree of juvenility attained is not known and differing regeneration capabilities are observed between genotypes. Markers indicating juvenility state would assist the in vitro rejuvenation process and increase the efficiency of successful micropropagation. Several leaf and stem morphological and anatomical traits were analysed as possible juvenility markers in mature and rejuvenated silver birch in vitro shoots. The results showed that juvenility state of birch in vitro shoots varies between different genotypes and between plants from the same genotype. Mature birch in vitro shoots had approximately two times larger and thicker leaves compared to rejuvenated and juvenile shoots. Stem radius, including cortex, phloem, xylem and pith width as well as phloem width : stem radius and phloem width : xylem width ratio was significantly larger in mature shoots. Periderm development and loss of rooting ability were observed in mature shoots. These anatomical and morphological traits could be used as possible juvenility/maturation markers.*

**Keywords:** vegetative reproduction, woody plants, in vitro culture, rejuvenation, shoot anatomy, morphology.

## INTRODUCTION

Birch (*Betula pendula* Roth) is a widespread tree species in the Baltic Sea region (Brus *et al.*, 2012), and its timber is used for plywood, timber, and wood-based panel production, as well as for pulpwood and bioenergy production (Dubois *et al.*, 2020). The pressure for forest productivity is increasing in order to meet the demand for wood processing industries (Silva *et al.*, 2018). Micropropagation is an effective vegetative propagation technique to obtain clonal material of desired superior genotypes that can be used in breeding as well as for direct establishment of highly productive forest stands (Jones *et al.*, 1996).

In the mature phase, the vegetative propagation capacity of woody plants declines abruptly. Explants obtained from mature trees are often characterised by slow growth and re-

duced responsiveness to plant growth regulators, resulting in low morphogenic ability (McCown, 2000). During micropropagation, mature plant material undergoes rejuvenation via axillary or adventitious shoot proliferation, resulting in juvenile characteristics including high multiplication and rooting rate (Welandar, 1993; Sánchez *et al.*, 1997). However, partial rejuvenation is reported, where shoots exhibited mature traits soon after acclimatisation compared to seedlings of the same age (Brand and Lineberger, 1992). To assess the level of rejuvenation, different phase-change markers can be used, for example, differences in foliar and stem morphology, reduced regeneration potential, cellular and subcellular anatomy, plant hormone types and concentrations and the concentrations of other chemicals. However, variation of juvenile-to-adult traits is often species-dependent (Feng *et al.*, 2016).

Markers indicating juvenility of birch *in vitro* shoots would increase the efficiency of successful *in vitro* culture initiation and micropropagation. To date, only a few morphological, physiological and molecular differences have been observed in mature, juvenile and rejuvenated birch *in vitro* shoots (e.g., juvenile and rejuvenated birch *in vitro* shoots were characterised by thin stems, small leaves, short internodes, and dense pubescence) (Brand and Lineberger, 1992).

The aim of this study was to assess the utility of morphological and anatomical characteristics as markers of juvenility and rejuvenation in silver birch *in vitro* shoots.

## MATERIALS AND METHODS

**Plant material.** Buds from three different clones of mature (approximately 25 years old) silver birch (*Betula pendula* Roth) elite trees growing in a progeny trial in the Ķegums region in Latvia were selected and initiated *in vitro* to obtain shoot cultures. Woody Plant Medium (WPM) was used (Lloyd and McCown, 1980) with an additional 20 g·l<sup>-1</sup> sucrose, 6 g·l<sup>-1</sup> agar and 0.5 mg·l<sup>-1</sup> zeatin. Cultures were incubated at 25 ± 3 °C, with a 16/8 h light/dark period provided by cool-white fluorescent lamps (photosynthetically active radiation with a photon flux density 90 μmol m<sup>-2</sup>·s<sup>-1</sup>). After six months, both rejuvenated shoots (characterised by a high proliferation rate and high rooting capacity) and mature shoots (characterised by a low proliferation rate and zero rooting capacity) were observed from each clone and selected for closer morphological and anatomical evaluation. Seeds from the same clones were obtained, surface sterilised, initiated *in vitro* and cultivated at the same conditions as mentioned above. Obtained seedlings were used for evaluation as juvenile controls.

**Morphological evaluation.** Mature, rejuvenated and juvenile shoots from each clone were used to determine morphological traits: length and width of the third leaf from the apex, the number of stomata in leaf lower epidermis per 1 mm<sup>2</sup>, the number of trichomes on the lower and upper epidermis of a leaf per 10 mm<sup>2</sup>, and the number of trichomes on the epidermis of stem and petiole per 1 mm<sup>2</sup>, taken from the third node from the apex. Rooting ability were evaluated by determining the number of rooted plants.

**Anatomical evaluation.** Shoots from each clone and seedlings were fixed in FAA solution (37% formaldehyde : 95% ethanol : acetic acid : distilled water (10 : 50 : 5 : 35) (Ruzin, 1999). After fixation, tissues were dehydrated in an ethanol-tert-butyl alcohol series, and embedded in Histowax (HistoLab) (Ruzin, 1999). Serial 20 μm cross sections from the third node of the stem and third leaf from the apex were prepared using a rotary microtome (Leica RM2145). Samples were further treated using a modified protocol (Braune *et al.*, 1999). Samples were deparaffinised in UltraClear™-ethanol series, stained with astra blue-safranin and placed on glass slides in UltraKitt™. An average of 10 stem and leaf cross sections from each clone and each shoot type

were examined and photographed using a Leica DM5500B light microscope equipped with a digital camera Leica DFC490. Stems were evaluated for stem radius, phloem width, xylem width, pith width, cortex width, presence of periderm as well as phloem : stem radius ratio, xylem : stem radius ratio and phloem : xylem ratio. Leaves were evaluated for total leaf thickness, palisade and spongy parenchyma width. Only one clone was used to evaluate leaves. All measurements were performed in Image Pro 6.2.

**Statistical analysis.** The quantitative data obtained in the study were analysed with R.3.5.1. Analysis of variance (ANOVA) and Post-Hock Tukey HSD test at significance level  $\alpha = 0.05$  were used to evaluate data.

## RESULTS

Significant morphological and anatomical differences were identified between mature, rejuvenated and juvenile birch *in vitro* shoots. Several traits were similar between juvenile and rejuvenated shoots and differed significantly from mature shoots. One of these traits was rooting ability (Table 1). Seedlings and rejuvenated shoots had 100% rooting frequency, while mature shoots did not root or rooted poorly, for example clone 2, which had a rooting frequency of 16.7 %. A similar pattern was observed for leaf traits. Rejuvenated shoots and seedlings of all three clones had more similar leaf size, which was on average 10.7 and 8.6 mm in length and 7.8 and 6.9 mm in width, respectively. Leaf size in mature shoots, on the other hand, was about two times longer and wider — 16.3 mm and 12.3 mm, respectively. Similar patterns were observed for leaf anatomical traits. Mature shoots had significantly thicker leaves compared to seedlings and rejuvenated plants, which resulted from larger palisade and spongy parenchyma layers (Table 2, Fig. 1). Several stem anatomical features also showed a clear correlation with juvenility state and could be used as juvenility markers (Table 2). Stem radius of juvenile and rejuvenated shoots were more similar to each other (average 276.4 μm and 373.7 μm, respectively); however, stem radius of mature shoots was significantly larger (average 740.8 μm). Analysis of stem structures indicated that the increase in radius was due to an increase in size of all structures, including xylem, phloem, pith and cortex, which were significantly larger in almost all mature shoots compared to seedlings and rejuvenated shoots. Mature shoots also had significantly higher phloem width : stem radius and phloem : xylem width ratios compared to seedlings and rejuvenated shoots. However, there were no significant differences in xylem width : stem radius ratio between mature and rejuvenated shoots or seedlings. In addition, secondary dermal tissue — periderm was observed in all mature shoots, but most seedlings and rejuvenated shoots had dermal tissue of only one cell layer — epidermis, except for clone 1, where periderm development was observed in 25% of rejuvenated shoots (Table 1). Some leaf and stem traits showed differences between shoot variants, but did not correspond to juvenility state. For example, the highest number of stomata in the leaf lower epidermis per 1 mm<sup>2</sup> was observed in rejuvenated shoots, having an average of 238.1 stomata, fol-

Table 1. Morphological traits of mature, rejuvenated and juvenile birch (*Betula pendula*) *in vitro* shoots of three different clones

| Traits                                                             | Clone          | Mature          | Rejuvenated     | Seedling       |
|--------------------------------------------------------------------|----------------|-----------------|-----------------|----------------|
| Leaf length (mm)                                                   | Clone 1 (mean) | 20.0 ± 5.0 a    | 9.3 ± 0.8 b     | 7.8 ± 0.8 b    |
|                                                                    | Clone 2 (mean) | 16.7 ± 1.7 a    | 10.8 ± 0.7 b    | 8.8 ± 0.3 c    |
|                                                                    | Clone 3 (mean) | 15.5 ± 0.9 a    | 12.4 ± 0.4 a    | 6.7 ± 0.3 b    |
|                                                                    | Overall mean   | 16.3 ± 3.5 a    | 10.7 ± 2.1 b    | 8.6 ± 1.4 c    |
| Leaf width (mm)                                                    | Clone 1 (mean) | 17.5 ± 2.5 a    | 8.0 ± 0.8 b     | 6.4 ± 0.4 b    |
|                                                                    | Clone 2 (mean) | 13.3 ± 1.7 a    | 8.0 ± 0.3 b     | 7.1 ± 0.3 b    |
|                                                                    | Clone 3 (mean) | 11.0 ± 0.8 a    | 7.2 ± 0.7 b     | 6.7 ± 0.3 b    |
|                                                                    | Overall mean   | 12.3 ± 3.4 a    | 7.8 ± 1.5 b     | 6.9 ± 1.2 b    |
| Number of stomata per 1 mm <sup>2</sup>                            | Clone 1 (mean) | 141.3 ± 9.8 b   | 250.0 ± 22.7 a  | 112.9 ± 8.5 b  |
|                                                                    | Clone 2 (mean) | 198.4 ± 58.5 ab | 229.0 ± 15.6 a  | 108.3 ± 7.5 b  |
|                                                                    | Clone 3 (mean) | 195.5 ± 15.6 a  | 230.2 ± 24.5 a  | 103.9 ± 7.5 b  |
|                                                                    | Overall mean   | 181.7 ± 57.9 b  | 238.1 ± 100.9 a | 108.3 ± 26.0 c |
| Number of trichomes on leaf upper epidermis per 10 mm <sup>2</sup> | Clone 1 (mean) | 29.4 ± 4.0 b    | 50.8 ± 5.8 a    | 2.7 ± 1.0 b    |
|                                                                    | Clone 2 (mean) | 33.1 ± 8.5 a    | 58.0 ± 10.3 a   | 6.2 ± 1.3 b    |
|                                                                    | Clone 3 (mean) | 17.6 ± 3.3 a    | 26.6 ± 9.4 a    | 10.1 ± 1.8 a   |
|                                                                    | Overall mean   | 22.0 ± 12.6 b   | 47.6 ± 25.3 a   | 6.2 ± 5.1 b    |
| Number of trichomes on leaf lower epidermis per 10 mm <sup>2</sup> | Clone 1 (mean) | 11.5 ± 3.6 b    | 75.7 ± 6.0 a    | 5.4 ± 0.8 b    |
|                                                                    | Clone 2 (mean) | 6.8 ± 5.2 b     | 53.9 ± 8.8 a    | 9.1 ± 1.4 b    |
|                                                                    | Clone 3 (mean) | 23.1 ± 3.5 a    | 27.4 ± 2.6 a    | 4.6 ± 1.7 b    |
|                                                                    | Overall mean   | 19.4 ± 12.1 b   | 54.9 ± 26.0 a   | 7.7 ± 5.5 b    |
| Number of trichomes on petiole per 1 mm <sup>2</sup>               | Clone 1 (mean) | 104.0 ± 33.6 a  | 86.4 ± 10.1 a   | 101.8 ± 32.4 a |
|                                                                    | Clone 2 (mean) | 61.9 ± 10.2 ab  | 56.0 ± 10.7 b   | 96.6 ± 5.9 a   |
|                                                                    | Clone 3 (mean) | 112.3 ± 10.7 a  | 76.8 ± 6.4      | 155.7 ± 36.4 a |
|                                                                    | Overall mean   | 101.1 ± 37.0 ab | 72.3 ± 27.5 b   | 105.8 ± 39.0 a |
| Number of trichomes on stem per 1 mm <sup>2</sup>                  | Clone 1 (mean) | 64.8 ± 2.7 b    | 82.7 ± 18.8 a   | 137.6 ± 18.2 a |
|                                                                    | Clone 2 (mean) | 67.2 ± 6.4 a    | 80.0 ± 27.9 a   | 112.7 ± 5.7 a  |
|                                                                    | Clone 3 (mean) | 58.8 ± 4.8 b    | 73.6 ± 9.2 b    | 124.6 ± 32.9 a |
|                                                                    | Overall mean   | 61.7 ± 11.3 b   | 79.3 ± 37.1 b   | 120.4 ± 35.3 a |
| Rooted plants (%)                                                  | Clone 1 (mean) | 0               | 100             | 100            |
|                                                                    | Clone 2 (mean) | 16.7            | 100             | 100            |
|                                                                    | Clone 3 (mean) | 0               | 100             | 100            |
|                                                                    | Overall mean   | 5.6             | 100             | 100            |

Means with the same letter are not significantly different ( $p \geq 0.05$ ).

lowed by mature shoots with an average of 181.7 stomata, while seedlings had the lowest number (average of 108.3 stomata per 1 mm<sup>2</sup>). A similar pattern was observed for the number of trichomes on the leaf surface per 10 mm<sup>2</sup> (Table 1). The highest number of trichomes on both adaxial and abaxial leaf surfaces were observed for rejuvenated shoots — 47.6 and 54.9, while the lowest number was observed for seedlings (6.2 and 7.7 trichomes). With regard to the number of trichomes per 1 mm<sup>2</sup> on petioles and stem epidermis, a different tendency was observed — seedlings had the highest number of both trichome number on petioles and stems (105.8 and 120.4 trichomes, respectively), followed by mature and rejuvenated shoots, which had similar trichome numbers on stems.

DISCUSSION

During *in vitro* stabilisation, shoots from mature woody plant individuals show gradual rejuvenation, resulting in the

return of species-specific morphological, anatomical and physiological juvenility traits (Mullins *et al.*, 1979; Struve and Lineberger 1988; McCown 2013). However, *in vitro* rejuvenation in non-*de novo* shoots can be incomplete and persists only when specific culture conditions are maintained (Bastías *et al.*, 2016). Different juvenility states occur in birch *in vitro* shoots of the same clone as there were significant differences in morphological and anatomical features of the *in vitro* shoots derived from the same clone in this experiment. For many woody plant species, morphological, anatomical and physiological traits can be used as specific markers to determine the degree of shoot juvenility (Frydman and Wareing 1973; Zimmerman *et al.*, 1985). In this experiment the most significant differences were observed for morphological and anatomical features such as rooting ability, length and width of leaves, leaf thickness, palisade parenchyma thickness, stem anatomy, including stem radius, phloem, xylem, pith and cortex width, and presence of secondary dermal tissue — the periderm. High rooting ability is a characteristic trait of juvenile plants,

Table 2. Anatomical traits of mature, rejuvenated and juvenile birch (*Betula pendula*) *in vitro* shoots of three different clones

| Traits                             | Clone          | Mature          | Rejuvenated    | Seedling       |
|------------------------------------|----------------|-----------------|----------------|----------------|
| Stem radius (μm)                   | Clone 1 (mean) | 654.6 ± 9.7 a   | 408.2 ± 13.9 b | 236.5 ± 5.8 c  |
|                                    | Clone 2 (mean) | 942.0 ± 45.2 a  | 442.4 ± 11.5 b | 330.8 ± 7.6 c  |
|                                    | Clone 3 (mean) | 884.3 ± 24.1 a  | 279.7 ± 25.3 b | 238.9 ± 2.5 c  |
|                                    | Overall mean   | 740.8 ± 132.5 a | 373.7 ± 89.3 b | 276.4 ± 60.3 c |
| Xylem width (μm)                   | Clone 1 (mean) | 193.5 ± 20.0 a  | 122.6 ± 13.9 b | 70.8 ± 2.9 c   |
|                                    | Clone 2 (mean) | 317.6 ± 13.3 a  | 72.4 ± 3.9 c   | 140.8 ± 6.2 b  |
|                                    | Clone 3 (mean) | 177.8 ± 25.0 a  | 61.0 ± 7.8 b   | 46.3 ± 2.4 b   |
|                                    | Overall mean   | 205.3 ± 73.3 a  | 94.3 ± 53.5 b  | 84.1 ± 46.0 b  |
| Phloem width (μm)                  | Clone 1 (mean) | 66.9 ± 4.2 a    | 16.9 ± 1.6 b   | 14.0 ± 0.5 b   |
|                                    | Clone 2 (mean) | 170.4 ± 5.2 a   | 15.5 ± 2.6 b   | 13.3 ± 0.6 b   |
|                                    | Clone 3 (mean) | 86.0 ± 6.5 a    | 9.3 ± 0.8 b    | 16.9 ± 0.5 b   |
|                                    | Overall mean   | 84.5 ± 37.6 a   | 14.7 ± 5.9 b   | 14.7 ± 3.5 b   |
| Cortex width (μm)                  | Clone 1 (mean) | 148.0 ± 7.1 a   | 100.8 ± 4.7 b  | 99.0 ± 4.2 b   |
|                                    | Clone 2 (mean) | 216.9 ± 24.2 a  | 143.0 ± 3.2 b  | 45.6 ± 3.2 c   |
|                                    | Clone 3 (mean) | 326.4 ± 14.0 a  | 135.3 ± 5.5 b  | 79.9 ± 6.5 c   |
|                                    | Overall mean   | 191.63 ± 74.0 a | 125.1 ± 22.1 b | 79.6 ± 26.7 c  |
| Pith width (μm)                    | Clone 1 (mean) | 125.6 ± 9.9 a   | 96.3 ± 6.5 b   | 51.8 ± 3.0 c   |
|                                    | Clone 2 (mean) | 197.5 ± 6.4 a   | 126.6 ± 7.1 b  | 39.5 ± 2.9 c   |
|                                    | Clone 3 (mean) | 238.8 ± 22.2 a  | 79.8 ± 5.9 b   | 44.4 ± 2.7 c   |
|                                    | Overall mean   | 158.3 ± 55.8 a  | 93.3 ± 23.2 b  | 46.4 ± 10.9 c  |
| Xylem width:stem radius ratio      | Clone 1 (mean) | 0.29 ± 0.05 a   | 0.30 ± 0.05 a  | 0.19 ± 0.02 a  |
|                                    | Clone 2 (mean) | 0.34 ± 0.05 ab  | 0.17 ± 0.01 b  | 0.42 ± 0.03 a  |
|                                    | Clone 3 (mean) | 0.20 ± 0.02 a   | 0.20 ± 0.03 a  | 0.28 ± 0.05 a  |
|                                    | Overall mean   | 0.28 ± 0.1 ab   | 0.25 ± 0.1 b   | 0.36 ± 0.13 a  |
| Phloem width:stem radius ratio     | Clone 1 (mean) | 0.10 ± 0.008 a  | 0.04 ± 0.004 b | 0.06 ± 0.003 b |
|                                    | Clone 2 (mean) | 0.18 ± 0.001 a  | 0.02 ± 0.009 b | 0.04 ± 0.002 b |
|                                    | Clone 3 (mean) | 0.10 ± 0.002 a  | 0.03 ± 0.002 c | 0.07 ± 0.003 b |
|                                    | Overall mean   | 0.11 ± 0.04 a   | 0.04 ± 0.01 b  | 0.05 ± 0.01 b  |
| Phloem width:xylem width ratio     | Clone 1 (mean) | 0.37 ± 0.04 a   | 0.14 ± 0.02 b  | 0.31 ± 0.03 a  |
|                                    | Clone 2 (mean) | 0.54 ± 0.06 a   | 0.22 ± 0.05 b  | 0.10 ± 0.01 c  |
|                                    | Clone 3 (mean) | 0.48 ± 0.03 a   | 0.12 ± 0.01 b  | 0.27 ± 0.05 ab |
|                                    | Overall mean   | 0.41 ± 0.1 a    | 0.15 ± 0.05 b  | 0.16 ± 0.1 b   |
| Plants having periderm (%)         | Clone 1 (mean) | 100             | 25             | 0              |
|                                    | Clone 2 (mean) | 100             | 0              | 0              |
|                                    | Clone 3 (mean) | 100             | 0              | 0              |
|                                    | Overall mean   | 100             | 8.30           | 0              |
| Leaf thickness (μm)                | Clone 1 (mean) | 123.2 ± 6.2 a   | 97.8 ± 3.4 b   | 87.5 ± 2.5 b   |
| Palisade parenchyma thickness (μm) | Clone 1 (mean) | 25.3 ± 1.4 a    | 19.9 ± 3.7 b   | 19.1 ± 0.6 b   |
| Spongy parenchyma thickness (μm)   | Clone 1 (mean) | 65.6 ± 3.5 a    | 50.6 ± 1.6 b   | 42.1 ± 1.6 c   |

Values with the same letter are not significantly different. ( $p \geq 0.05$ ).

which decreases during maturation (Sánchez *et al.*, 1997). In this study, mature shoots rooted poorly (clone 2 — 16.7%) or not at all (clones 1 and 3), while juvenile and rejuvenated shoots had a rooting frequency of 100%, which indicates that changes occur in the juvenility state of *in vitro* shoots. This was also observed in a study with *Malus*, in which *in vitro* shoots obtained from mature trees reached 75% rooting in the fourth subculture and 100% in the ninth subculture (Noiton *et al.*, 1992). Leaf morphology is one of the most commonly used markers of phase change (Poething 1990). Silver birch and other woody plants have smaller and simpler leaves in the juvenile phase compared to the mature phase (Poething 1990). Leaf morphology has

been observed to change during *in vitro* cultivation and resemble seedling leaf morphology, indicating rejuvenation. This has also been reported for *in vitro* cultured plants of *Morus* (Patel *et al.*, 1983), *Sambucus* (Hrib *et al.*, 1980) as well as silver birch (Brand and Lineberger 1992). In this study, the leaves of rejuvenated shoots resembled seedling leaves in their length, width and anatomical traits. They differed significantly from mature shoots of the same clone, which had significantly longer, wider and thicker leaves. Significant differences have also been observed between the anatomical structures of the stem and leaves of mature and juvenile shoots (Hartmann *et al.*, 2011). Under optimal *in vitro* conditions, periderm formation is not usually observed

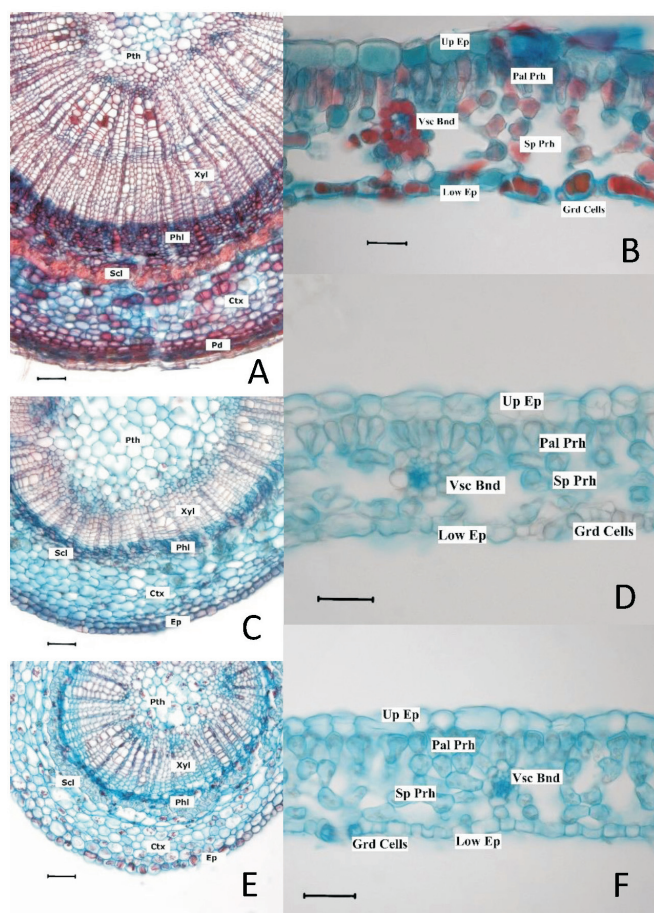


Fig. 1. Cross sections of stems and leaves of mature, rejuvenated shoots and seedlings. A – mature shoot stem, B – mature shoot leaf, C – rejuvenated shoot stem, D – rejuvenated shoot leaf, E – seedling stem, F – seedling leaf. Pth – pith, Xyl – xylem, Phl – phloem, Scl – sclerenchyma, Ctx – cortex, Ep – epidermis, Pd – periderm. Up Ep – upper epidermis, Pal Prh – palisade parenchyma, Vsc Bnd – vascular bundle, Sp Prh – spongy parenchyma, Low Ep – lower epidermis, Grd Cells – guard cells of the stomata. Scale bar represents 30 µm.

in rejuvenated shoots (Ballester *et al.*, 1999; Iliev *et al.*, 2001). In addition, sclerenchyma is usually less developed and has a lesser degree of lignification (Hartmann *et al.*, 2011). In this study, mature shoots had developed a periderm and had significantly larger stem radius, phloem, xylem, pith and cortex width compared to rejuvenated shoots and seedlings. Mature shoots had higher phloem width : stem radius and phloem width : xylem width ratios compared to juvenile shoots. Differences in stem anatomy could be due to different hormonal levels between mature, juvenile and rejuvenated shoots, which affect secondary growth of the stem. Auxin and gibberellins could be the most important hormones involved. A high endogenous auxin: gibberellin ratio stimulates xylem growth, while a low ratio stimulates phloem development. Other hormones, like cytokinins, jasmonic acid and ethylene, as well as the competence of cambium cells towards hormones, could also be involved (Ursache *et al.*, 2012). The number of trichomes can be used as a precise marker of juvenility in some species, for example *Arabidopsis thaliana* (Telfer *et al.*, 1997). Higher trichome density is usually observed in juvenile organs compared to mature organs. The stem of sil-

ver birch seedlings is densely covered with trichomes, but the shoots of mature birch are bare or warty (Brand and Lineberger 1992). In this experiment, seedlings had more trichomes on stems compared to rejuvenated and mature shoots, indicating that both mature and rejuvenated shoots may have a higher degree of maturation than seedlings. This is also supported by the fact that some rejuvenated shoots had developed periderm tissues (Table 2). However, *in vitro* shoots had a higher number of trichomes on leaf upper and lower epidermis compared to seedlings and mature shoots, which showed that trichome number may not be the most suitable juvenility marker for birch *in vitro* shoots.

## CONCLUSIONS

The results of this study show that juvenility state can vary between birch *in vitro* shoots of the same clone, and juvenility can be affected by currently unknown factors during cultivation. The best morphological and anatomical markers to assess juvenility state in silver birch (*Betula pendula* Roth) *in vitro* shoots were the length, width and thickness of leaves, stem radius, including cortex, phloem, xylem and pith width as well as phloem width : stem radius and phloem width : xylem width ratio. These traits could be used as juvenility/maturation markers, as values for all these traits were significantly larger in mature individuals. Further work is necessary to evaluate more subtle differences in juvenility state of birch *in vitro* shoots as well as the influence of culture conditions. Potentially, recently discovered phase-change regulators miR156/157 and their target genes could provide more insight and maybe be used as molecular juvenility markers (Wang *et al.*, 2011).

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## MORFOLOĢISKIE UN ANATOMISKIE JUVENILITĀTES MARĶIERI PARASTĀ BĒRZA (*BETULA PENDULA* ROTH) *IN VITRO* DZINUMOS

Augu attīstības laikā kokaugi progresē no juvenilās fāzes līdz reproduktīvai nobriedušai fāzei. Šo procesu iezīmē morfoloģiskas, fizioloģiskas un bioķīmiskas izmaiņas, kā arī kompetences trūkums veģetatīvās pavairošanas metodēm. *In vitro* stabilizēšanas laikā nobriedušie audi tiek pakļauti rejuvenilizācijai, tomēr bieži vien juvenilitātes pakāpe nav nosakāma, un starp genotipiem ir novērojamas atšķirīgas reģenerācijas spējas. Marķieri, ar kuru palīdzību varētu noteikt juvenilitātes pakāpi, palīdzētu *in vitro* pavairošanas procesā un palielinātu veiksmīgas mikropavairošanas efektivitāti. Vairākas morfoloģiskās un anatomiskās īpašības tika analizētas kā iespējamie juvenilitātes marķieri nobriedušos un rejuvenilizētos parastā bērza *in vitro* dzinumos. Rezultāti liecināja, ka bērzu *in vitro* dzinumu juvenilitātes pakāpe atšķiras starp dažādiem genotipiem un starp augiem no viena genotipa. Nobriedušiem bērzu *in vitro* dzinumiem novēroja apmēram divas reizes lielākas un biežākas lapas salīdzinājumā ar rejuvenilizētiem un juveniliem dzinumiem. Stumbra rādiuss, ieskaitot korteksu, floemu, ksilēmu, un serdes platumu, kā arī floemas platuma: stumbra rādiusa un floemas platuma: ksilemas platuma attiecība nobriedušiem dzinumiem bija ievērojami lielāka. Nobriedušos dzinumos novērota peridermas attīstība un apsākšanas spēju zudums. Šīs anatomiskās un morfoloģiskās īpašības varētu izmantot kā iespējamās juvenilitātes/nobriešanas marķierus.