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Differences in anatomical structure and lignin content of roots of pedunculate oak and wild cherry-tree plantlets during acclimation

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Abstract

The lignin contents and anatomical structure of roots of wild cherry (*Prunus avium* L.) and pedunculate oak (*Quercus robur* L.) plantlets were compared to explain differences in response during transfer from *in vitro* to *ex vitro* conditions. Lignification of cell walls increased significantly in both oak and cherry roots during the period of acclimation and finally lignin content of root tissues of *in vitro* propagated plantlets reached the levels not significantly different from seedlings grown in soil. Later on when secondary tissues appeared, lignified secondary xylem constituted most of the tissues of both species. The most conspicuous interspecific difference in root structure was the presence of phi-thickenings in cortical layers just outer to endodermis in cherry roots cultivated *ex vitro*. Formation of phi-thickenings was avoided *in vitro* and their presence thus seems to be under environmental control. Suberised well established exodermis was present in roots of oak but not detected in those of cherry. Very early development of exodermis in oak roots, preceding suberisation of endodermis, was recorded *in vitro* but not in well aerated soil. While multilayered and well-developed cork occurred in oak, only thin walled and less suberised secondary dermal tissues were found in cherry.

Additional key words: exodermis, *in vitro*, lignin, phi-thickenings, *Prunus avium, Quercus robur*, root development.

Introduction

Interactions of plant with its environment have made plants to evolve a variety of strategies and mechanisms, which allow them to deal successfully with changes in their surroundings and survive stress situations. Such, artificially induced, situation is also the transfer of plants cultivated under *in vitro* conditions to *ex vitro* environment. This step is often the main limiting and loss-making part of micropropagation technologies. Losses are often connected to insufficient acclimation (Bigot and Engelman 1987), which should result in structural and biochemical changes allowing plant to survive in field conditions. Rooting in agar media was proved of being effective part of the technology (Vietez *et al*. 1981, Pinker *et al*. 1995) but *in vitro* formed root system needs some acclimation period, which might be facilitated by semi sterile conditions, to gain its functionality and resistance in outer environment. Evaluation of morphological and functional quality of root system was by some authors connected to lignin content of the root tissues (Gaspar and Coumans 1987) and subsequently, as the quality of root system seems to be a key factor of plantlet survival in the field (Pinker *et al*. 1995), to fitness of whole plantlet. The genus *Quercus* has a wide distribution in the European countries and is of enormous ecological and economical value. *Prunus avium* is a fast-growing hardwood timber tree with potential for wide use in forestry.

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Abbreviations: BAP - benzylaminopurine; IBA - indole-3-butyric acid; MS medium - Murashige and Skoog medium; NAA - 1-naphthaleneacetic acid; WPM - woody plant medium.
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Differentiation of root cortex is a key factor determining the transport of compounds into and out of the root and, subsequently, to other parts of plant body. The innermost layer is in roots generally differentiated into endodermis – an apoplastic barrier crucial for selective transport into the root stele *via* symplast (Clarkson a Robards 1975). Exodermis, similarly to endodermis, poses properties of apoplastic barrier but differentiates in sub-epidermal layers of cortex periphery. This localisation enables exodermis to shield the tissues of middle part of cortex. Exodemis is present in most, about 90 %, of studied plant species from various environments – hydro-, hygro-, meso- as well as xerophytes (Von Guttenberg 1968, Peterson and Perumalla 1984, 1990). Its formation might be even induced by environmental conditions (Zimmerman and Steudle 1998). Studies dealing with structure, function, development and its plasticity under various environmental conditions are still very rare (Peterson *et al*. 1982, Peterson and Perumalla 1984, Hose *et al*. 2001, Soukup *et al*. 2002) and limited to only a few model species. Therefore the aim of this paper was to compare anatomical structure of roots and lignin contents of wild cherry and pedunculate oak plantlets during transfer from *in vitro* to *ex vitro* conditions.

Materials and methods

Plants: Shoots of wild cherry (*Prunus avium* L.) were multiplicated in Murashige and Skoog (MS, 1962) medium supplemented by 0.3 mg dm^{-3} of benzyl-aminopurine (BAP), 0.1 mg dm^{-3} indole-3-butyric acid (IBA), and 200 mg dm^{-3} glutamine, pH 5.8. Woody plant medium (WPM, Lloyd and McCown 1981) supplemented by 0.2 mg dm^{-3} of BAP, 0.1 mg dm^{-3} IBA, and 400 mg dm^{-3} glutamine, pH 5.8, was used for multiplication of shoots of pedunculate oak (*Quercus robur* L.). The shoots were cultivated under white fluorescent tube (irradiance of 30 μmol m^{-2} s^{-1}, 16-h photoperiod, temperature of 24° C) for 4 - 6 weeks.

Rooting in agar: 2 - 3 cm long multiple shoots were placed onto agar rooting medium [mg dm^{-3}]: 237.50 KNO_3, 206.25 NH_4NO_3, 55 $CaCl_2.H_2O$, 46.25 $MgSO_4$, 21.25 KH_2PO_4, 8.14 $Na_2EDTA/FeSO_4$, 2.07 H_3BO_3, 5.63 $MnSO_4$, 2.87 $ZnSO_4$, 0.27 KI, 0.083 Na_2MoO_4, 0.083 $CuSO_4$, 0.008 $CoCl_2$, 40 myo-inositol, 0.4 thiamine, 0.2 nicotinic acid, 0.2 pyridoxine, 0.4 glutamine, 1.7 glycine) and 10 g dm^{-3} saccharose. 1-naphthaleneacetic acid (NAA) was used for rooting of oak (6.3mg dm^{-3}) and cherry (2.8 mg dm^{-3}). Transferred shoots were cultured in black boxes in air-conditioned room at 25 °C. After 7 d the shoots were re-transferred into fresh medium of the same composition but without NAA, and cultivated under white fluorescent light with a continuous irradiance for oak and 16-h fotoperiod for cherry (30 μmol m^{-2} s^{-1}). This procedure proved optimal for used species in previous experiments (Malá, unpublished results).

Acclimation in *Perlite*: Plantlets grown in agar medium were transferred into side slit cells (*BCC* growing trays, Landskrona, Sweden; height 100 mm, upper diameter 50 mm) with *Perlite* (beads of expanded basic silicates). They were cultivated for 30 d under continuous irradiation (30 μmol m^{-2} s^{-1}), temperature of 25 °C, and relative humidity the first 15 d of culture of 90 % and then of 70 %. The plantlets were irrigated by WPM (oaks) and by MS (cherries) mineral nutrients 3 times a week.

Acclimation in peat + soil mixture: Plantlets were transferred into peat:soil:*Perlite* mixture (2:1:1) and cultured in *BCC* growing trays (height 190 mm, upper diameter 67 mm) for 15 d under the same irradiance and temperature and relative humidity of 70 %. The plantlets were irrigated by water 3 times a week.

Seed derived plantlets were cultivated in the same way and used for comparison to *in vitro* originating ones.

Anatomical analyses: The anatomical structure of roots was studied using fresh free-hand sections or fresh serial sections cut by means of hand microtome. Sections were taken from 5, 20, and 40 mm from the root tip and at the root base if the roots were longer than 40 mm.

HCl-phloroglucinol and Mäule reactions (Johansen 1940) were used to detect lignin. Staining the sections with Sudan Red 7B according to Brundrett *et al*. (1988) was used to localise suberin. The excess dye was subsequently washed with 1 % (m/v) sodium dodecyl-sulphate to minimise the precipitation of the dye on the sections.

Extraction of cell walls and determination of lignin: The lignin content of purified cell walls was estimated after derivatization with thioglycolic acid by the method of Bruce and West (1989). The produced lignothio-glycolic acids were extracted from cell walls with alkali, precipitated and spectrophotometrically (*Helios* α, *Unicam*, UK) quantified at 280 nm. The whole root system of plantlets was sampled.

Results and discussion

Growth characteristics: Oak and cherry plantlets significantly differed in their growth parameters as might be expected from slower and faster growing species. First roots appeared on the sixth day on the cherry shoots and tenth day on the oak shoots. Differences in the root growth rate are obvious from the Fig. 1. Similarly, faster growth was recorded for shoots of cherry (*ANOVA*, $P < 0.05$, $n = 150$). Both species significantly differed in percentage of plantlets losses during the initial steps of acclimation. Losses of oak plantlets were 5 % in *Perlite* and 0 % in peat + soil mixture while for cherry it was 10 % in *Perlite* and 5 % in peat + soil mixture. From this perspective cherry plantlets seem to be faster growing but more vulnerable to stresses related to the acclimation.

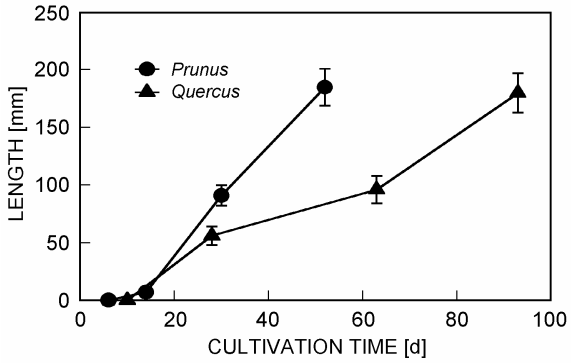

Fig. 1. Root length of cherry and oak plantlets ($n = 150$) as it changed during the cultivation. The length was always measured after each cultivation step of the sequence from agar to soil (corresponds to individual points). The slope of the curve may be related to average elongation (growth rate).

Lignification and suberisation of root tissues: Lignification and suberisation of cell walls during differentiation of the tissues substantially affect mechanical properties and apoplastic permeability of cortical tissues and, thus, also the way of communication between root and surrounding environment (Schreiber *et al*. 1999). Root abilities to exclude potentially harmful substances, prevent infections, and maintain water and ion relations are crucial to keep plant internal homeostasis and consecutively plant survival. Some authors considered lignification to be one of the most convincing characteristics of a degree of cell differentiation in plant tissues (Fukuda and Komamine 1980). Compared to oak, cherry roots contained in general slightly higher amount of lignin in cell walls of roots (Fig. 2; $P < 0.001$) but the difference was significantly pronounced only in agar and *Perlite* (Tukey-Kramer Multiple-Comparison Test, $\alpha = 0.05$). Such a significant difference was found neither in other cultivation steps later on nor in the roots of plants derived from the seeds. The biggest increase in lignin content of cell walls of root tissues occurred during period of cultivation in *Perlite* – the first *ex vitro* acclimation step. This increase was connected with beginning of production of lignified secondary xylem, but also with earlier onset of differentiation of root tissues. While the roots of *in vitro* cultivated plants of both species did not contain secondary tissues even 4 cm behind the root tip they were present within 2 cm from the tip of oak (Fig. 3) and usually between 2 and 4 cm in cherry roots (Fig. 4) from plantlets transferred to *Perlite* and during further cultivation. Major part of root volume at these later stages of cultivation was composed of secondary tissues in which secondary xylem presented the major contribution to lignin content. From comparison of Figs. 3 and 4, which present detection of lignification with HCl-phloroglucinol, it is obvious that oak root possessed secondary lignified tissues earlier (closer to the root tip) and it might be expected that lignin content would be higher there. On the other hand, not-lignified secondary thickening of cell walls (*e.g.* sclerenchymatous fibers; Fig. 3*a*) might be related to "dilution" of lignin in total mass of cell walls and to non-significant differences or even lower relative content of lignin in cell walls of oak roots. Lamberts and Porter (1992) presented general tendency in slow growing species to have more of cell wall material per dry mass (Dijkstra and Lambers 1989). Unfortunately those data are hardly comparable to ours, as they do not include lignin content per dry mass of cell walls presented here.

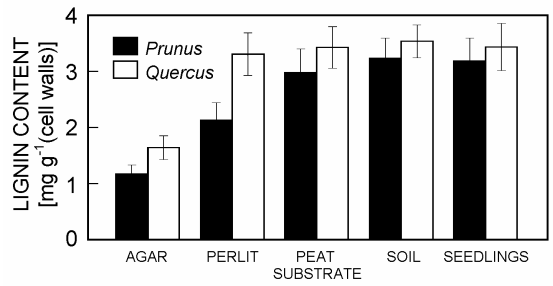

Fig. 2. Contents of lignin in roots of oak and cherry plantlets propagated *in vitro* ($n = 6$). Treatments represent particular steps of subsequent cultivation sequence. Data from seedlings cultivated *ex vitro* from the very beginning were used for comparison and presented in last two columns (seedlings).

From the above mentioned results it is obvious that lignin content itself is not a reliable interspecific measure of cell differentiation and/or survival during acclimation. Suberised cell layers (discussed later), which seem to be better developed in oak, might be more related to higher resistance of oak to stress conditions.

Lignin in root tissues of both species grown in peat-soil mixture, later in acclimation, and in outdoor soil beds was not significantly different from lignification of roots of seed-derived plantlets (Fig. 2). The *in vitro* derived

plantlets thus reached during the acclimation level of lignification comparable to seedlings. The structure of roots initiated *in vitro*, which are usually not appropriately developed to perform well in *ex vitro* conditions, is markedly developed during the acclimation in semi-sterile *Perlite*. Procedure including acclimation in *Perlite* seems to be more convenient than rooting the micro-cuttings directly in a peat substrate, in which they also form well-developed and resistant root system but higher mortality makes this attitude less effective. Moreover, direct rooting in peat substrate is applicable only for some species.

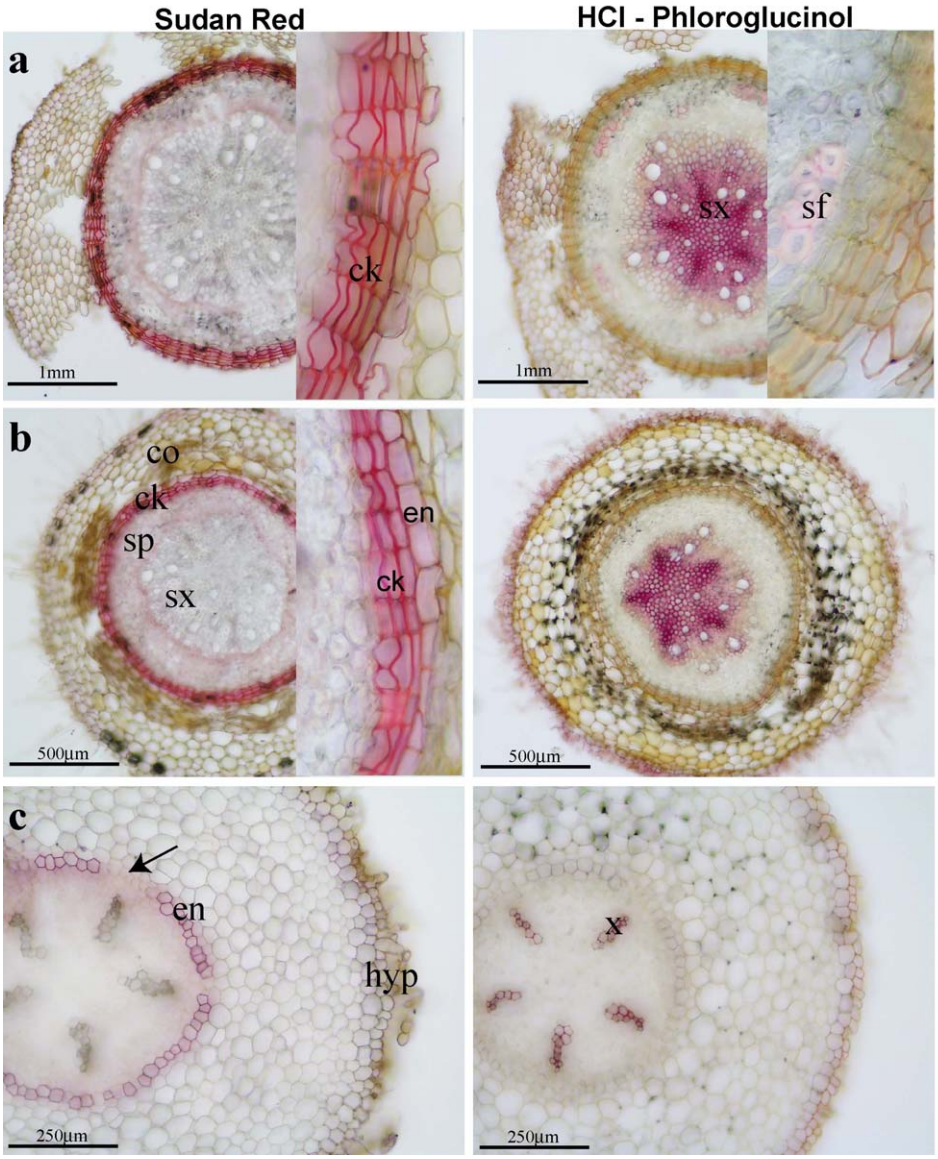

Fig. 3. Structure of oak roots grown in *Perlite*. Micrographs taken from the same position behind the root tip are arranged in rows. Results of particular reaction are arranged in columns. 4 cm (*a*): The cortex was almost shed and surface was covered by well-differentiated cork (ck). Detected lignification was found in secondary xylem and phloem sclerenchyma fibres (sf). 2 cm (*b*): Formation of secondary xylem (sx) and phloem (sp) increased the volume of tissues in central part of the root. Cortex (co) surrounding them was compressed (*arrow*) and disrupted later on. Living tissues were surrounded by multilayered cork (ck) with heavily suberised cell walls. Secondary xylem (sx) strongly responded to lignin tests. 0.5 cm (*c*): Complete suberin lamellae were apparent in endodermis (en) and gaps of cell wall suberisation (*arrow*) were still present in endodermal cylinder. Xylem yielded positive tests for lignins. Suberisation and lignification were observed also in hypodermal layers (hyp).

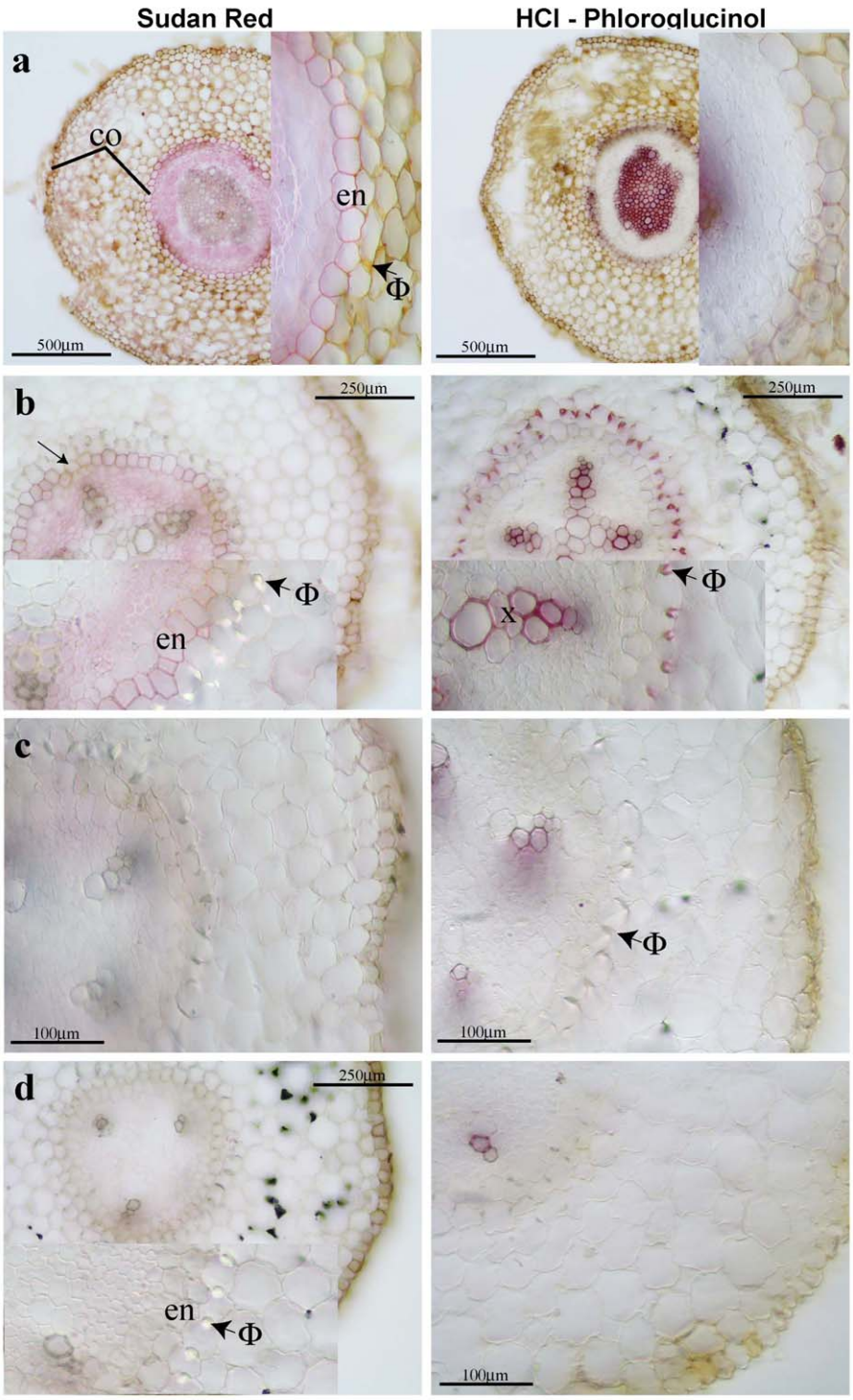

Fig. 4. Structure of cherry roots grown in *Perlite*. The arrangement of micrographs is the same as in Fig. 3. 6 cm (*a*): Tissues in the base of root produced by secondary growth. Secondary xylem and phloem increased their volume and cortex (co) peripheral to endodermis (en) was disrupted. Notice remaining phi-thickenings (Φ) in the cortex. 4 cm (*b*): Phi-thickenings exhibited strong response to lignin detection. Xylem (x) was another site of detected lignification in this part of root. Endodermis (en) possessed complete suberin lamellae but their continuity around the cylinder still was not complete (*arrow*). 2 cm (*c*): Phi thickenings became more strongly pronounced but still without any detectable lignification. 0.5 cm (*d*): Phi-thickenings were already present, still without any detectable lignification. Casparian bands of endodermis, in this part of the root, were seen as dark dots in radial cell walls, set slightly towards the centre. Lignification was detected only in early xylem elements. Suberisation or lignification were not detected in outer cortex at any position along the root.

Suberised cell layers: Endodermis is a generally present apoplastic barrier in roots (Peterson 1992). It restricts the movement of water and solutes between cortex and stele via apoplast (Clarkson and Robards 1975) and thus makes this exchange of material, to some extent, under active control of the plant. Casparian bands of endodermis in both species were formed close to the root tip (up to 3 mm). They are noticeable at first as dark dots occupying only part of radial cell walls without any detectable staining for suberin. In the later stage of root development, the endodermal cells with complete suberin lamella form cylinder. Deposition of suberin lamellae was delayed in endodermal cell walls facing xylem poles (Figs. 3, 4).

Intact suberised hypodermal layers (exodermis) were present in oak roots without substantial secondary growth (Fig. 3). Contrary, exodermis was not detected in roots of cherry from *ex vitro* conditions (Fig. 4). Similarly to endodermis, exodermis is considered to provide an apoplastic barrier shielding deeper cortical layers of the roots. From this point of view, tissues of cortex of cherry root without exodermis might be easier affected by unfavourable conditions, toxic compounds (Enstone and Peterson 1992, Soukup *et al*. 2002) and pathogens (Kamula *et al*. 1994) from their surroundings than oak.

The very early suberisation of exodermal cell walls of oak roots from *in vitro* conditions is also very interesting (Fig. 5). Suberisation in exodermis of oak was detected even closer to the root tip than in endodermis. This sequence of development was previously reported only for *Phragmites* (Soukup *et al*. 2002) and hypothesised being typical for wetland plants. There it maintains the oxygen economy of roots and minimises negative impact of unfavourable conditions of flooded sediment. The lower oxygen availability in agar of *in vitro* cultivation system (in range of 1,5 - 5 mg dm^{-3}) might be connected also with early development of oak exodermis. This developmental pattern was reversed after transfer

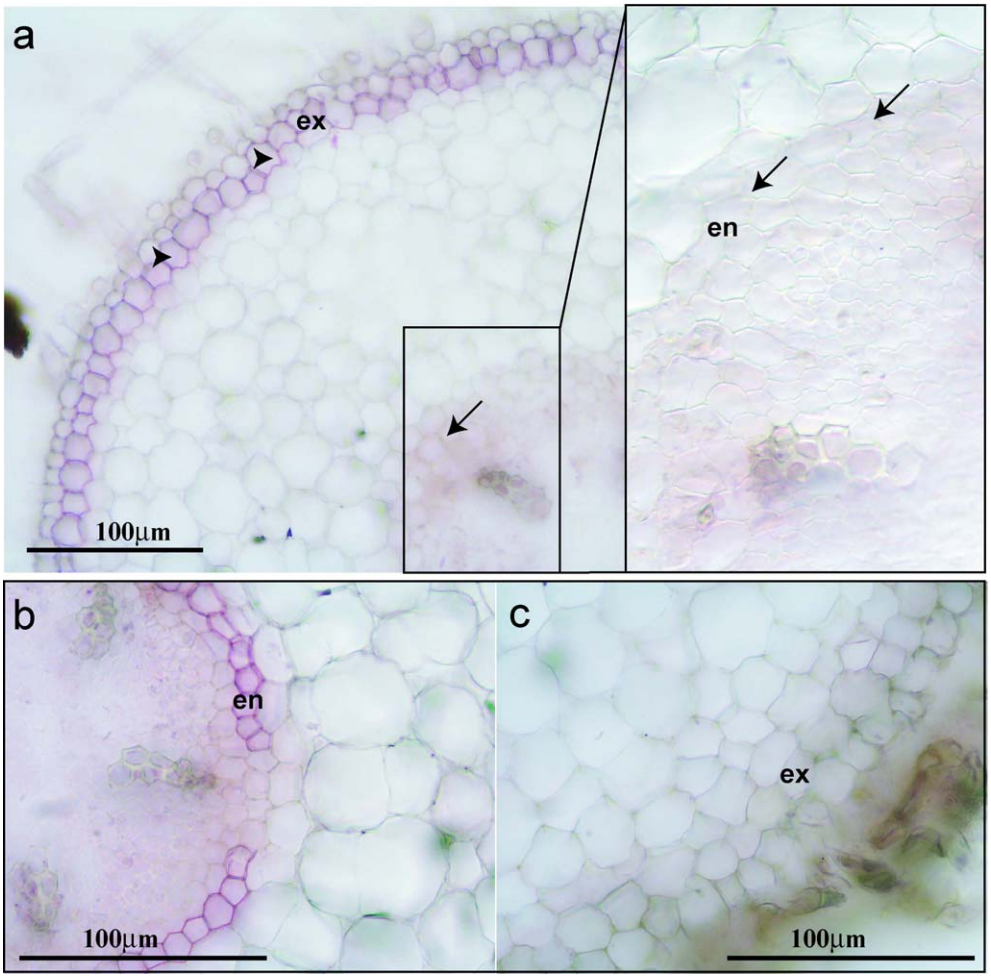

Fig. 5. Very early suberisation of exodermis (*ex*) was observed in the oak roots which were grown *in vitro* (*a*). Detectable suberisation appeared even closer to the root tip than that of endodermis (*en, arrow*). Inverse developmental sequence of endodermis and exodermis suberisation was recorded in roots from well aerated *Perlite* (*b*, *c* - both images were taken from the same section, 3 - 5 mm from the tip, Sudan Red 7B).

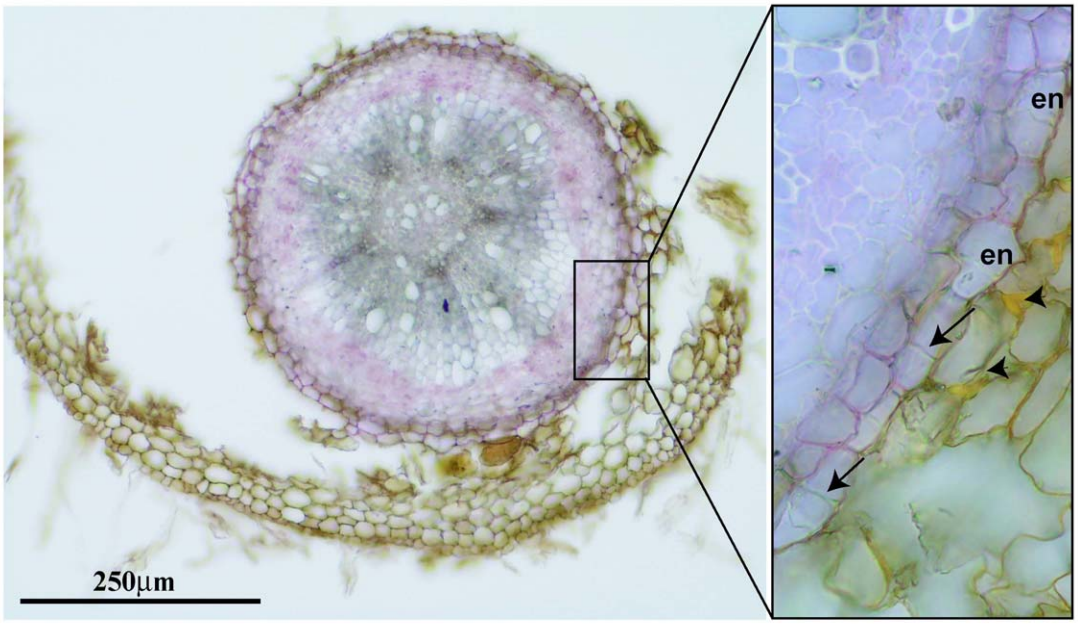

Fig. 6. Two suberised layers covered increasing volume of secondary tissues of cherry root. One of them originates in endodermis (*en*) increasing its surface by radial division (*arrows*). Remaining phi-thickenings (*arrowheads*) are visible in sloughed cortex (root base, root length 120 mm, Sudan Red 7B).

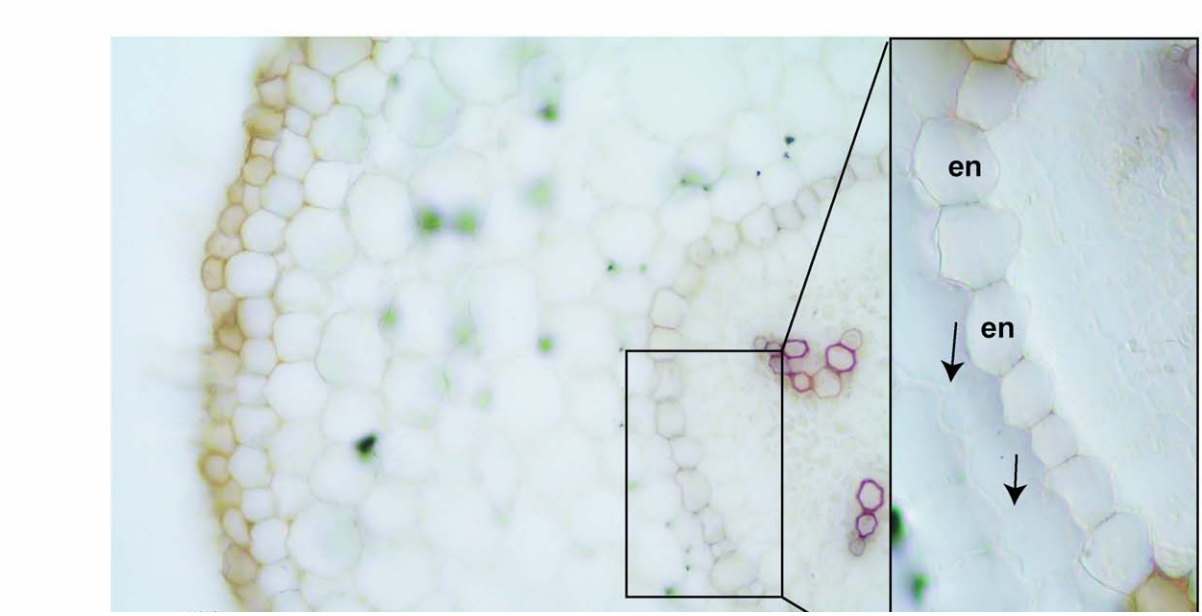

Fig. 7. Phi-thickenings were absent in cherry roots grown *in vitro*. *Arrows* indicate the cell layer outer to endodermis (*en*) where phi-thickenings normally form (40 mm from the tip of the 50 mm long root, HCl-phloroglucinol). Compare to the micrographs in Fig. 2.

to well-aerated *Perlite* where suberisation of exodermis took place further behind the apex than that in endodermis (Fig. 5). This change demonstrates plasticity of differentiation of oak cortex tissues under various environmental conditions.

In general, as the secondary growth proceeds, the volume of vascular tissues increases, primary cortex is sloughed and secondary protective tissues are formed. Well-differentiated multiserial phellem (cork) was present in oak roots. Intensely suberised cork cells, originating from pericycle, were organised in radial rows (Fig. 3). Unlike in oak, only one to two-layers of cells

with suberised cell walls, without distinct radial organisation, were observed in roots of cherry (Fig. 6). One of them originates in endodermis increasing its surface by radial division (Fig. 6). The difference in secondary protective tissue formation between the species could be connected with different susceptibility to environmental factors of both species and explain higher endurance of oak plantlets during acclimation in comparison to wild cherry. The arrangement of root tissue might also reflect the "growth strategy" of the selected species. The slow growing oak develops more complete "protective layers" that might be connected to higher resistance to environmental stresses after the transfer while faster growing cherry produces more roots to cope with changing or unfavourable environment other way round.

Phi-thickenings: Conspicuous phi-thickenings (named because of their similarity to Greek letter Φ (Russow 1875) were formed in one or two cortical cell layers external to endodermis of *Prunus* (Fig. 4). Thickenings of radial and transverse walls of phi-layer were discernible within 5 mm from the root tip. Modified cell walls initially did not stain for lignin or suberin (Fig. 4). Lignification of phi-thickenings started slightly behind the point where early xylem got lignified. This phi-layer formed at the same distance from the root tip where narrow Casparian bands without detectable suberin were seen in endodermis. When secondary tissues were formed, all cell layers external to endodermis, including phi-layers, were sloughed (Fig. 4; root base).

Phi-thickenings were never observed in roots cultivated *in vitro*. Even after prolonged period of *in vitro* cultivation allowing formation of secondary tissues and sloughing of cortex layers (Fig. 7) the thickenings were not present. Therefore it seems that their formation is prevented under *in vitro* conditions.

As the presence of lignified phi-thickenings does not block the movement of apoplastic tracers (Peterson *et al.* 1981) and plasmalemma does not adhere to phi-thickening portion of cell wall (Haas 1976), they are not considered being an apoplastic barrier. This opinion was not accepted by Mackenzie (1979), who noted high frequency of plasmodesmata in outer tangential cell walls of these phi-layers. The mechanical function of phi-thickenings is also rejected by some and accepted by other authors (Russow 1875, Weerdenburg and Peterson 1983). Despite the unclear function (Von Guttenberg 1968, Mackenzie 1979, Fahn 1990, Peterson 1992), the presence of phi-thickenings was supposed to be consistent within a family and used as a systematic characteristic (Gerrath 2002). Our work confirms, together with work of Praktikakis *et al*. (1998) and Degenhardt and Gimmler (2000), that phi-thickenings formation is not constitutive but, at least partially, under control of environmental factors. This observation supports their yet unknown roles in interaction of root with surrounding environment.

More information and further research on root development and plasticity of the developmental sequence of tissues under various environmental conditions is required to make more general conclusions.

References

Bigot, C., Engelmann, F.: Vegetative propagation *in vitro* of *Cunninghamia lanceolata* (Lamb.) Hook. - In: Bonga, J.M., Durzan, D.J. (ed.): Cell and Tissue Culture in Forestry. Vol. III. Pp.114 - 127. Martinus Nijhoff Publishers, The Hague 1987.

Bruce, R.J., West, C.A.: Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of castor bean. - Plant Physiol. **91**: 889-897, 1989.

Brundrett, M.C., Kendrick, B., Peterson, C.A.: Efficient lipid staining in plant material with Sudan Red 7B or Fluorol Yellow 088 in polyethylene glycol. - Biotech. Histochem. **66**: 133-142, 1988.

Clarkson, D.T., Robards, A.W.: The endodermis, its structural development and physiological role. - In: Torrey, J.G., Clarkson, D.T. (ed.): The Structure and Function of Roots. Pp. 415-436. Academic Press, London 1975.

Degenhardt, B., Gimmler, H.: Cell wall adaptations to multiple environmental stresses in maize roots. - J. exp. Bot. **51**: 595-603, 2000.

Dijkstra, P., Lambers, H.: Analysis of specific leaf area and photosynthesis of two inbred lines of *Plantago major* differing in relative growth rate. - New Phytol. **113**: 283-290, 1989.

Enstone, D.E., Peterson, C.A.: The apoplastic permeability of root apices. - Can. J. Bot. **70**: 1502-1512, 1992.

Fahn, A.: Plant Anatomy (4th Edition). - Pergamon Press, Oxford 1990.

Fukuda, A.H., Komamine, A.: Establishment of an experimental system for the study of tracheary element differentiation from single cells isolated from the mesophyll of *Zinnia elegans.* - Plant Physiol. **65**: 57-60, 1980.

Gaspar, T., Coumans, M.: Root formation. - In: Bonga, J.M., Durzan, D.J. (ed.): Cell and Tissue Culture in Forestry. Vol. II. Pp. 202 - 217. Martinus Nijhoff Publishers, The Hague 1987.

Gerrath, J.M., Covington, L., Doubt, J., Larson, D.: Occurrence of phi-thickenings is correlated with gymnosperm systematics. - Can. J. Bot. **80**: 852-860, 2002.

Haas, D.L., Carothers, Z.B., Robbins, R.R.: Observation on the phi-thickenings and Casparian strips in *Pelargonium* roots. - Amer. J. Bot. **63**: 863-867, 1976.

Hose, E., Clarkson, D.T., Steudle, E., Schreiber, L., Hartung, W.: The exodermis: a variable apoplastic barrier. - J. exp. Bot. **52**: 2245-2264, 2001.

Johansen, D.A.: Plant Microtechnique. - McGraw-Hill Book Co., New York 1940.

Kamula, S.A., Peterson, C.A. Mayfield, C.I.: Impact of exodermis on infection of roots by *Fusarium culmorum*. -

Plant Soil **167**: 121-126, 1994.

Lambers, H., Porter, H.: Inherent variation in growth rate between higher plants: a search for physiological causes and ecophysiological consequences. - Adv. ecol. Res. **23**: 188-261, 1992.

Lloyd, G., McCown, B.H.: Commercially-feasible micropropagation of mountain laurel *Kalmia latifolia* by use of shoot tip culture. - Comb. Proc. Int. Plant Propag. Soc. **30**: 421-427, 1981.

Mackenzie, K.A.D.: The development of the endodermis and phi layer of apple roots. - Protoplasma **100**: 21-32, 1979.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassay with tobacco tissue cultures. - Physiol. Plant. **15**: 473-797, 1962.

Peterson, C.A., Emanuel, M.E., Weerdenburg, C.A.: The permeability of phi thickenings in apple (*Pyrus malus*) and geranium (*Pelargonium hortorum*) roots to an apoplastic fluorescent dye tracer. - Can. J. Bot. **59**: 1107-1110, 1981.

Peterson, C.A., Emanuel, M.E., Wilson, C.: Identification of a Casparian band in the hypodermis of onion and corn roots. - Can. J. Bot. 60: 1529-1535, 1982.

Peterson, C.A., Perumalla, C.J.: Development of the hypodermal Casparian band in corn and onion roots. - J. exp. Bot. **35**: 51-57, 1984.

Peterson, C.A., Perumalla, C.J.: A survey of angiosperm species to detect hypodermal Casparian bands. II. Roots with a multiseriate hypodermis or epidermis. - Bot. J. Linn. Soc. **103**: 113-125, 1990.

Peterson, R.L.: Adaptations of root structure in relation to biotic and abiotic factors. - Can. J. Bot. **70**: 661-675, 1992.

Pinker, I., Jesch, H.H., Klausch, A.: Rooting and acclimatization of *in vitro* propagated shoots of *Tilia cordata* "Wega". - Gartenbauwissenschaft **60**: 253-258, 1995.

Praktikakis, E., Rhizopoulou, S., Psaras, GK.: A phi layer in roots of *Ceratonia siliqua* L. - Bot. Acta **111**: 93-98, 1998.

Russow, E.: Betrachtungen über das Leitbündel aus phylogenetischem Gesichpunkt. - Schnakenburg's Anstalt, Dorpat 1875.

Schreiber, L., Hartmann, K., Skrabs, M., Zeier, J.: Apoplastic barriers in roots: chemical composition of endodermal and hypodermal cell walls. - J. exp. Bot. **50**: 1267-1280, 1999.

Soukup, A., Votrubová, O., Čížková, H.: Development of anatomical structure of *Phragmites australis*. - New Phytol. **153**: 277-287, 2002.

Vietez, A.M., Vietez, M.L., Ballester, A.: *In vitro* chesnut regeneration: anatomical and chemical changes during the rooting process. - In: Proc. IUFRO Sect. S2015.I Workshop *In Vitro* Cultivation for Tree Species. Pp. 149-152. IUFRO, Fontainebleau 1981.

Von Guttenberg, H.: Der primäre Bau der Angiospermenwurzel. - Gebrüder Borntraeger, Berlin 1968.

Weerdenburg, C.A., Peterson, C.A.: Structural changes in phi thickening during primary and secondary growth in root. 1. Apple (*Pyrus malus*) Rosaceae. - Can J Bot. **61**: 2570-2576, 1983.

Zimmermann, H.M., Steudle, E.: Apoplastic transport across young maize roots: effect of the exodermis. - Planta **206**: 7-19, 1998.