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Growth and differentiation of root endodermis in *Primula acaulis* Jacq.

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Abstract

Adventitious roots of *Primula acaulis* Jacq. are characterized by broad cortex and narrow stele during the primary development. Secondary thickening of roots occurs through limited cambial growth together with secondary dilatation growth of the persisting cortex. Close to the root tip, at a distance of *ca.* 4 mm from the apex, Casparian bands (state I of endodermal development) within endodermal cells develop synchronously. During late, asynchronous deposition of suberin lamellae (state II of endodermal development), a positional effect is clearly expressed - suberization starts in the cells opposite to the phloem sectors of the vascular cylinder at a distance of 30 - 40 mm from the root tip. The formation of secondary walls in endodermis (state III of endodermal development) correlates with the beginning of secondary growth of the root at a distance of *ca.* 60 mm. Endodermis is the only cortical layer of primrose, where not only cell enlargement but also renewed cell division participate in the secondary dilatation growth. The original endodermal cells additionally divide anticlinally only once. Newly-formed radial walls acquire a typical endodermal character by forming Casparian bands and deposition of suberin lamellae. A network of endodermal Casparian bands of equal density develops during the root thickening by the tangential expansion of cells and by the formation of new radial walls with characteristic wall modifications. These data are important since little attention has been paid up till now to the density of endodermal network as a generally significant structural and functional trait of the root.

Additional key words: Casparian bands, cell division, density of endodermal network, primary growth and differentiation, secondary dilatation growth.

Introduction

Endodermis, the innermost uniseriate layer of the root cortex, is characterized by the development of specific wall modifications which regulate physiological processes of the root. The endodermis may develop in 3 stages, which are species specific. The special wall modification of the state I (primary endodermis) are Casparian bands, corresponding in the neighbouring radial and transversal cell walls. In this way a continuous network of Casparian bands is formed around the vascular cylinder; usually this process occurs synchronously. Functionally, the endodermal network represents the apoplastic barrier, controlling by the selection of ions radial centripetal movement of solutes in the root (Peterson *et al.* 1993, Clarkson 1996). During the state II (secondary endodermis) the suberin lamellae are deposited on the inner surface of endodermal walls, usually including the area occupied by Casparian bands. So, the function of endodermis as an apoplastic barrier is increased. The program of the state III of development (tertiary endodermis) is the formation of cellulosic secondary cell wall. The asynchrony in development during state II and III results in a possibility of all three states occurring

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at the same distance from the root tip (von Guttenberg 1943, 1968, Van Fleet 1961, Barnabas and Peterson 1992).

In the secondary thickening roots of dicotyledons with persisting cortex, the behaviour of endodermis is determined by the timing of secondary thickening and by the intensity of this process. Two distinct types of growth are involved in the early secondary growth of these roots: cambial growth and secondary dilatation growth of the cortex. From the viewpoint of root morphogenesis, they are complementary one to another. Thickening of vascular cylinder occurs through the activity of vascular cambium. Secondary dilatation of the cortex occurs through the reactivation of cortical cells of primary origin. It occurs through cell enlargement, additional division, or both. Prominent renewed radial cell division in endodermis, described in earlier anatomical works was summarised and completed by von Guttenberg (1943, 1968). More details with documentation for herbaceous species can be found, *e.g.*, in the Root Atlas (Kutschera and Sobotik 1992) and in our previous work (Lux and Luxová 2001).

Root anatomy of *Primulaceae* was investigated by Luhan (1951). She concluded, that the roots of alpine members of genus *Primula* do not exhibit secondary thickening and additional division of their endodermal cells does not occur, whereas a weak thickening takes place in other analysed species. One of these species is *Primula acaulis*, with the individual endodermal cells once divided additionally by a radial wall. Sensitive procedures which distinguish between non-lamellar suberin of Casparian bands and lamellar suberin (Brundrett *et al.* 1988, 1991) allowed the study of complicated differentiation processes of endodermal sheath. They were used in the present study, the objective of which was to characterize growth and differentiation processes of root endodermis of *Primula acaulis* and resulting changes of endodermal network density. The reduction of the endodermal network density during secondary dilatation growth does not take place in this species contrary to the older data.

Materials and methods

Plants: Root samples of *Primula acaulis* Jacq. were taken from mature plants growing in natural conditions. Genus *Primula* is characterized by homorhizia determined by an early cessation of primary root, substituted by adventitious stem roots. Minimum 10 - 15 adventitious roots of five different plants were taken for investigation of all developmental stages of endodermis.

Paraffin sections: Segments from distal parts of the roots, approximately 5 mm long, were fixed in *Craf II* (Sass 1951), from proximal parts of the roots in FAA (formalin - acetic acid - alcohol, Johansen 1940). After dehydration in tertiary butyl alcohol the samples were embedded in paraffin. The material was cut with a rotary microtome; transversal and both radial and tangential sections from 7 to 10 μm in thickness were stained with hematoxyline or tannic acid - ferric chloride (Sass 1951).

Semithin sections: Root samples approximately 2 mm long were fixed by 5 % (m/v) glutaraldehyde in phosphate buffer (0.1 M, pH 7.0), postfixed in 2 % (m/v) osmium tetroxide, dehydrated in ethanol and propylene oxide, and embedded in Spurr embedding medium. Sections approximately 1 μm thick were cut using ultramicrotome (*LKB Nova,* Bromma, Sweden) and glass knives and stained by toluidine blue and basic fuchsin (Lux 1981). Sections were observed using a *Zeiss* Photomicroscope (Jena, Germany). Photographs were taken with 100 ASA black and white negative film.

Histochemistry for observation of cell wall modifications: Roots for fluorescence microscopy were sectioned, stained and observed within 3 h of the excavation from the soil. Intact, undamaged roots were rinsed briefly in tap water, and then hand-sectioned. Suitable sections were stained by berberine-aniline blue according to Brundrett *et al.* (1988) for visualisation of endodermal Casparian bands. Visualisation of endodermal suberin lamellae was realized according to Brundrett *et al.* (1991). Freehand sections of roots were stained by Fluorol yellow 088 in polyethylene glycol-glycerine. Sections were observed using *Olympus* and *Nikon Optiphot II* (Kawasaki, Japan) microscopes, both with UV illumination and set of filters allowing wavelengths > 420 nm to pass. For estimation of cellulose and lignin, routine microtechnique reactions were performed (Luxová 1962). Photographs were taken with 400 ASA colour negative film.

Results

Primula acaulis is a homorhizic species with limited thickening of roots and a persisting primary cortex. Dimorphic endodermis maturing by state III of development (Fig. 1*A*,*B*) has a low frequency of short cells.

State I of development: The characteristic of the state I is a synchronous formation of Casparian bands throughout the entire circumference of the endodermal cylinder at distance of ca 4 mm from the root tip (Fig. 2*A*,*B*). The distance from the tip at which they first appear correlates with the maturation of protoxylem cells. Thin-walled endodermal cells are at first approximately isodiametric in the cross section, later they expand tangentially (primary dilatation growth). The Casparian bands are formed at the centre of radial walls.

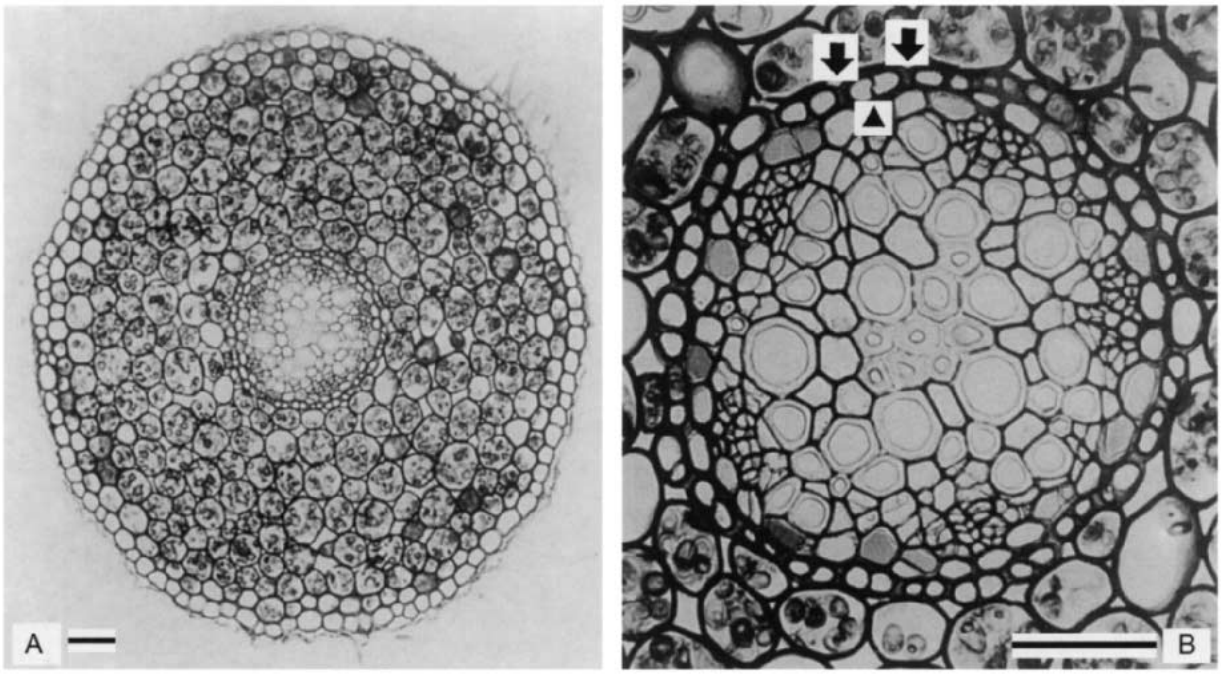

Fig. 1. Transverse section of mature basal part of *Primula acaulis* adventitious root (bar = 50 μm); *A* - Thick primary cortex represents storage tissue containing numerous starch grains. Stele is narrow, only limited thickening occurs by cambial activity. Note endodermis in state III of development. *B* - Higher magnification of A exhibiting stele surrounded by endodermis. Each endodermal cell is additionally anticlinally divided, a regular pattern of alternating thick original (arrows) and thin newly-formed radial walls (arrowheads) is formed in this way. Paraffin embedding, tannic acid - ferric chloride staining.

State II of development: Suberin lamellae depositions in endodermal cells occur at a distance of 30 - 40 mm from the root tip. Contrary to the synchronous formation of Casparian bands the suberization occurs asynchronously. Along the endodermal cylinder an intermediate zone is formed, where cells gradually pass from the state I of development to the state II. During this process the relationship between the development of secondary endodermis and the development of vascular cylinder, is evident. The first endodermal cells with suberin lamellae appear next to the ontogenetically more advanced phloem poles (Fig. 2*C*, *D*). Thin suberin lamellae are deposited on the whole inner surface of endodermal cells including Casparian bands.

State III of development together with the secondary growth: The termination of suberization of some ontogenetically advanced endodermal cells and their transition to the state III of development is correlated with the onset of secondary growth in primrose root, occurring at a distance of approximately 6 cm from the root apex. In the endodermis of this species cells enlargement is combined with additional cell division during the secondary dilatation growth. The renewed division of original endodermal cells starts after their asynchronous suberization, thus being also asynchronous.

Endodermal cells of primrose are usually divided anticlinally only once, thus a single new radial wall appears in individual cells (Fig. 1*B*). A regular pattern of alternating original and newly-formed radial walls is formed in this way. Exceptionally the original endodermal cell does not divide or the anticlinal division can be asymmetrical and a larger daughter cell can divide anticlinally once more. In tangential sections it is also possible to see additional transversal walls (Fig. 2*E*).

Cell walls of all original endodermal cells are gradually thickened during state III of development, by the deposition of secondary wall material. Their radial walls are usually the thickest. Thickened walls do not have the affinity for lignin stains. Newly formed radial walls exhibit a typical endodermal character (Fig. 2*F*, *G*). Originally thin Casparian bands are situated closer to the

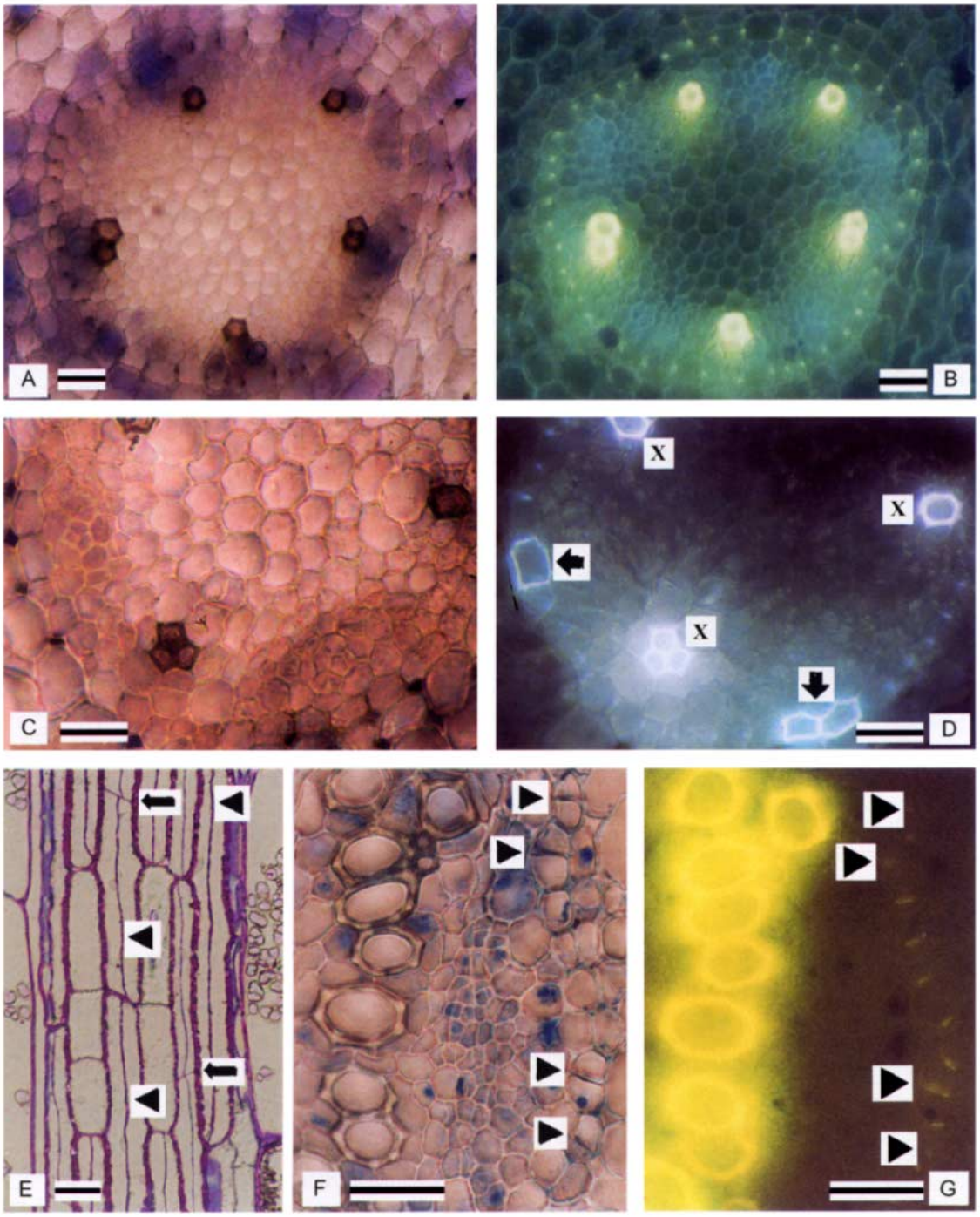

Fig. 2. Transverse (*A* - *D* and *F* - *G*) and tangential (*E*) sections of *Primula acaulis* roots in different ontogenetic stages (*bar* = 20 μm): *A*,*B* - fresh, hand-cut section at a distance of 4 mm from the apex viewed with white light optics (*A*) and epifluorescence optics (*B*); synchronous formation of Casparian bands in endodermal cell (state I of endodermal development) correlates with the maturation of protoxylem cells (berberine-aniline blue staining); *C*,*D* - fresh, hand-cut section at a distance of 30 mm from the apex; asynchronous formation of suberin lamellae (state II of endodermal development) starts in cells opposite to phloem poles; visualisation of suberin lamellae by Fluorol yellow 088; *C* - viewed with white light optics, *D* - viewed with epifluorescence optics, endodermal cells with suberin lamellae (*arrows*), and xylem poles (x); *E* - secondary thickening root exhibiting endodermal cells additionally radially divided (*arrowheads*); occasionally also additional transversal division of endodermal cells occurs (*arrows*); note thick original cell walls and thin newly-formed radial and transversal walls; semithin section, toluidine blue - basic fuchsin staining; *F*,*G* - fresh, hand-cut section at a distance of 6 cm from the apex viewed with white light optics (*F*) and epifluorescence optics (*G*); four endodermal cells are additionally anticlinally divided (*arrowheads*); Casparian bands are developed in newly-formed radial walls; berberine-aniline blue staining.

inner tangential walls. Casparian bands and suberin lamellae are formed in this case fast one after another. The differing thickness of newly-formed walls demonstrates their asynchronous origin.

Density of endodermal network: The diameter of endodermis is small and its cells are tiny in the level of synchronous formation of Casparian bands. More proximally, 30 - 40 mm from the root tip, at the level of the onset of asynchronous deposition of suberin lamellae, the circumference of endodermis is increased approximately by a factor of 1.2. The original density of the endodermal network is slightly reduced by the limited increase of cell size. During the secondary thickening of the primrose adventitious root, the circumference of endodermal cylinder is further increased. However, the activity of vascular cambium is limited in these roots. The maximum increase in width of endodermis circumference is by a factor of 2. The persistence of endodermis is not compromised by the secondary thickening of the vascular cylinder. Suberized endodermal cells, that have the capacity to reactivate, divide additionally in anticlinal direction. The newly-formed radial walls rapidly acquire endodermal character by formation of Casparian bands and by deposition of suberin lamellae. By doubled circumference of endodermal cylinder and by single additional anticlinal division of original endodermal cells, doubling by the number of circumferential cells, the endodermis density remains approximately the same.

Changes in growth correlations: The coordinated mechanism of cambial growth and secondary dilatation growth of the cortex can be impaired in some roots. An example is a root with the extensive development of cortex, which occurs through increased number of cortical cell layers with a very weak cambial activity, but with abundant additional division of endodermis (Fig. 3). Alternatively, the additional anticlinal division of some endodermal cells in the state III of development was observed in secondary non-thickening lateral roots (not shown). This occurred in the region of their penetration through the cortex of the main root. Changes like this demonstrate a defect in the coordination of both cambial and dilatation growth processes.

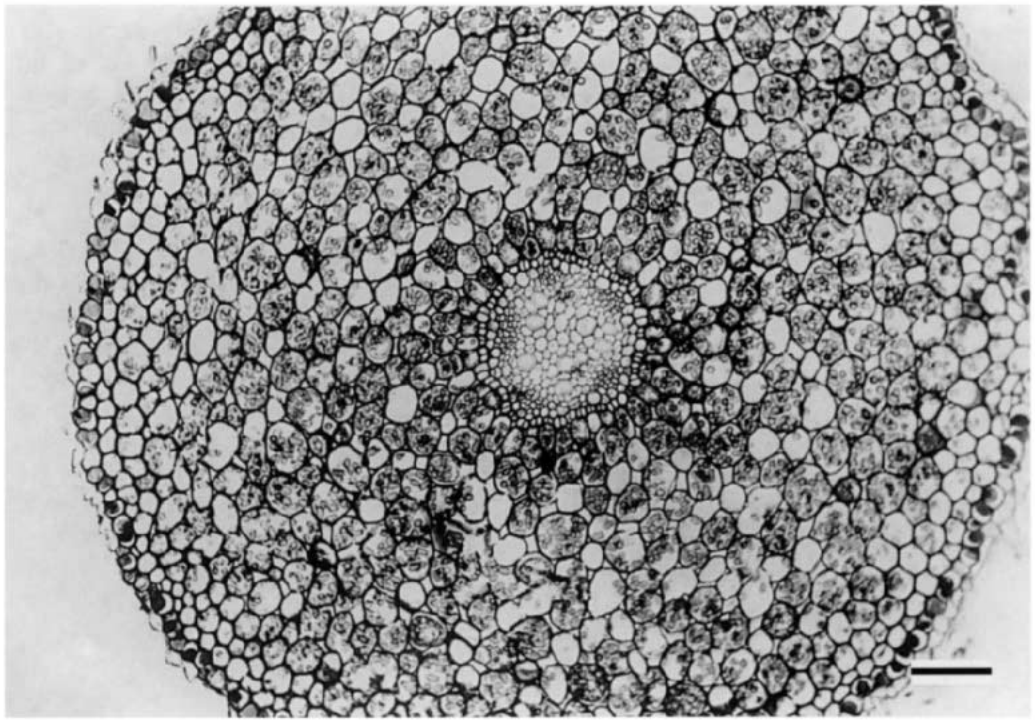

Fig. 3. Transverse section of exceptionally developed primrose root with extensively developed cortex (compare with Fig. 1*A*) and very weak cambial activity in the stele. Nevertheless almost all endodermal cells are additionally anticlinally divided (*bar* = 100 μm). Paraffin embedding, tannic acid - ferric chloride staining.

Discussion

Differentiation of specific wall modifications in primrose endodermis has a similar course as in the dimorphic endodermis of onion (Barrowclough and Peterson 1994). The early synchronous formation of Casparian bands classifies the primrose as a species with functional apoplastic barrier formed by continuous Casparian

network from the youngest distal region of the root. Asynchronous deposition of suberin lamellae starts late in endodermal cells located opposite to the phloem poles. This positional effect may be explained by increased levels of nutrients - sucrose or substances which distribution is regulated by sucrose - in cells close to the phloem (Heimsch 1960, Barlow and Adam 1989). Suberization gradually spreads over the whole endodermal perimeter. However, in some developmentally delayed cells this process occurs only during the secondary growth of the root.

Asynchrony of endodermal development, starting with the state II appears as an important formative character later, during the secondary growth of the root. In this species both cell enlargement and additional cell division take part in the secondary dilatation growth of endodermal cells. This process correlates with the state III of endodermal development. As a result of asynchronously renewed anticlinal division of individual suberized cells along the root axis the cell number is gradually increasing in circumference of endodermis. This course of endodermal dilatation corresponds with the production of cambium derivatives and with the increasing diameter of vascular cylinder. Hypothesis of maintaining the role of apoplastic barrier by endodermis even during renewed cell division, expressed by Bond (1930), was proved by Weerdenburg and Peterson (1984). The authors observed apoplastic dye exclusion by endodermal cells of garden balsam, sunflower and broad bean, which were additionally divided. Contrary to some other species where original endodermal cells continue to divide anticlinally, resulting in formation of cell packets (an example is *Gentiana campestris* L. with 24 newly-formed radial cell walls in original cell, Luhan 1954), primrose endodermal cell divides anticlinally only once.

According to the older data (Bond 1930, von Guttenberg 1968) renewed divisions of endodermal cells occur in the state I or III of development. This difference is species specific and can be expressed also by the different development of newly-formed radial walls. During the additional divisions of endodermal cells in developmental state I Casparian bands are formed quickly; new cell wall differentiation can proceed further according with the state of endodermis maturation. During additional division of endodermal cells in state III, as in the case of primrose, the new cellulosic walls remain, according to the above mentioned authors, without Casparian bands and without suberin lamellae. These walls were characterized as cellulosic prop walls also in *Primula acaulis* by Luhan (1951). However, using fluorescent techniques, the typical endodermal character of these newly-formed radial walls has been proved. Casparian bands development is here quickly followed by deposition of suberin lamellae in new walls.

The feature, which called only limited attention up till now, is endodermis density. The common tissue density represents the dry matter investment per tissue volume (Ryser 1998). The density of endodermal network formed by Casparian bands can be considered as a criterion. The density of endodermal network in primrose changes during primary growth by increase of cell size and by the increase of Casparian band width. During the secondary dilatation growth, the change of cell size is accompanied by other factors determining the density of the endodermal network in many species: the frequency of renewed cell divisions and the way of differentiation of newly-formed walls which can be modified or unmodified. The extending endodermal network of primrose maintains approximately the same density even during the secondary dilatation growth. It occurs by an increase of cell size in combination with their single anticlinal division and by the modification of newly-formed walls typical for endodermis. A different process takes place in the species with intensive tangential increase of original endodermal cells combined with their multiple additional division without the capability to form Casparian bands and suberin lamellae in the newly-formed radial walls. Intraspecific and interspecific differences and changes of endodermal network density require further attention from the viewpoint of structure as well as function.

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