

Graft union formation in elepidote rhododendrons

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Abstract

Light microscopy was used to study the graft union formation of splice graft in the elepidote rhododendrons cultivar 'Cunningham's White'. The first visible reaction after grafting was the appearance of a necrotic layer between graft partners consisting of fragmented and compressed cells. Callus was visible six days after grafting and was formed from recent cambial derivatives and phloem rays cells. New cambium and tracheary elements were differentiated from callus cells. Newly differentiated cambium was slightly curved or had an S-shape, and it depended on rootstock and scion tissue matching. When initially the gap between the graft partners remained wide, callus produced more new cambiums: one of them emerged between pre-existing cambium of graft component and an uncommon one – in the pith region. The possible function of this uncommon cambium is discussed.

Key words: callus development, cambium, grafting, rhododendron, vegetative propagation.

Introduction

Rhododendron can be propagated either by seed or by vegetative methods. One of the most widely used vegetative propagation method is propagation by grafting. Grafting is essential for propagation of many woody plant species, cultivars, and hybrids because of existing difficulties in their propagation by cuttings and by micropropagation.

The development of graft union is a process of forming a functional unit through the interaction of organs, tissues or cells from the same or different plants (Shanfa 2000). A number of detailed studies have been made about graft union formation with woody and herbaceous plants (Hartman et al. 1997). The anatomical changes that occur during graft union formation are in the approximate order of occurrence, following grafting in many plants. These include the death of layers of cells at the graft interface, cohesion of scion and rootstock, generation of callus and establishment of vascular continuity and a new stem centre (Miller, Barnett 1993). According to Moore (1984), the development of a compatible graft includes three events: cohesion of rootstock and scion, proliferation of callus cells at the graft interface, and vascular differentiation across the graft interface. However, insufficient information is available about graft union formation of elepidote rhododendrons. Detailed information about this process is essential for the propagation of difficult-to-root cultivars. The elepidote rhododendrons cultivar 'Cunningham's White' is widely and successfully used as a rootstock for rhododendron grafting, because it has good grafting union formation potential (Cox, Cox 1988). The objective of this study was to investigate the structural development of graft union formation in this cultivar.

Materials and methods

Plant material

Investigation was carried out in December, 2001 and 2002, using the elepidote rhododendrons cultivar 'Cunningham's White' [*Rh. caucasicum* × *Rh. ponticum* var. *album*] both as rootstock and scion. The plant material was obtained from the Botanical Garden of the University of Latvia and the Experimental Nursery of Rhododendron Breeding "Babīte", University of Latvia. Rootstocks for grafting were 2-year old plants propagated by cuttings. Dormant scion material – new shoots, were collected from shrubs of rhododendron shortly before grafting. Scions were splice grafted to the rootstock. During the grafting procedure a diagonal cut was made at the top of rootstock and an appropriate cut at the base of the scion. The scion and rootstock were fitted together and wrapped with rubber grafting tape (Albrecht, Sommer 1991). The grafted plants were covered with a polyethylene tent and kept at 23 °C during the day and 20 °C at night, with a 16-h photoperiod in the growth chamber.

Histological analysis

Collections of five graft unions were made 3, 6, 9, 12, 15, 18, 25, 32, 39 days after grafting. Transverse sections in the middle of graft unions were cut by manual microtome and a razor. The sections were stained with safranin-astra blue, rinsed in water, dehydrated in an ethanol-xylol series and permanently embedded in Canadian balsam (Braune et al. 1999).

Sections were examined with an Olympus CH30RF200 light microscope and photographed using a Leica DMLS light microscope by digital camera Canon Power Shot S40.

Results and discussion

The anatomical changes that occur during graft union formation of elepidote rhododendrons are comparable to graft formation of other woody and herbaceous plants. Graft union development can be varied temporally between each of the grafts. It depends not only on the physiological condition of graft partners, but also on the success of the grafting technique.

The rootstock and scion anatomy was similar, consisting of epidermis, cortex, phloem, cambium, secondary xylem, primary xylem and pith (Fig. 1). A detailed stem anatomy of the rhododendron cultivar 'Cunningham's White' was described in our previous investigation (Kondratovičs, Megre 1999).

The first visible reaction after grafting was the appearance of a necrotic layer between graft components consisting of fragmented and compressed cells, observed three days after grafting (Fig. 1). Subsequently the necrotic layer became thinner and eventually disappeared, presumably being absorbed by the developing callus. A similar observation was described by Stoddard and McCully (1980).

There are different opinions regarding the origin of callus in different species. All living undamaged cells – parenchyma cells from pith to cortex and also cambium – are capable of callus formation. Usually, callus is formed from various tissues, but with different intensities of cell division (Dormling 1963). The cambial region was observed to play

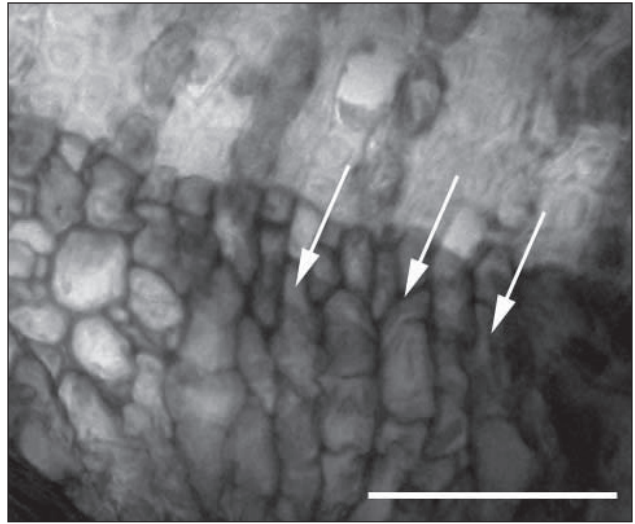
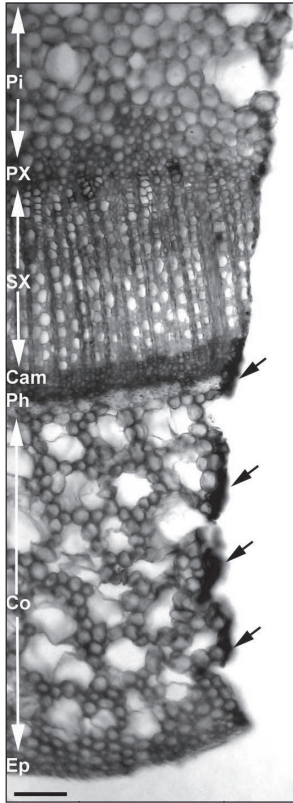


Fig. 2. Callus cell formation from recent cambial derivatives (arrows) in a 6-day-old graft union. Bar = 50 μm .

Fig. 1. Transverse section through a 3-day-old graft union shows scion anatomy and layer of necrotic tissues (black arrows). Ep, epidermis; Co, cortex; Ph, phloem; Cam, cambium; SX, secondary xylem; PX, primary xylem; Pi, pith. Bar = 100 μm .

an essential role as a callus producer in *elepidote rhododendrons*. Callus was produced from recent cambial derivatives (Fig. 2) and phloem ray cells (Fig. 3), and became visible six days after grafting. Callus formation from phloem ray cells is characteristic for many plants, such as *Pseudotsuga menziesii* (Copes 1969), *Picea sitchensis* (Miller, Barnett 1993), *Lycopersicon esculentum* (Fernandez-Garcia et al. 2004), but there are different views regarding the importance of the cambium as a callus producer. As in our work, Fuji and Niko (1972) suggested that the cambium has an essential role in callus formation, while other observations have suggested that cambium contributed little to early callus formation (Copes 1969) or not at all (Sharples, Gunnery 1933 cited in Larson 1994). Callus formation from xylem ray parenchyma cells has been observed in *Pinus sylvestris* (Dormling 1963) and in apple (Soumelidou et al. 1994). However, our study showed that xylem rays are not capable of producing callus in *elepidote rhododendrons*. In the subsequent days the callus growth was so abundant that it became impossible to determine the origin of any individual cell or group of cells. Callus continued to proliferate and to fill the space between scion and rootstock.

Tracheary elements were formed from callus and they could be recognized by the presence of reticulate secondary walls (Fig. 4). In *Picea sitchensis* graft union, such cells might assist in transport of water across the graft interface (Weatherhead, Barnett 1986), but it is possible that these cells formed merely as a result of the influence of auxin and nutrients on the callus, with no specific function (Soumelidou et al. 1994).

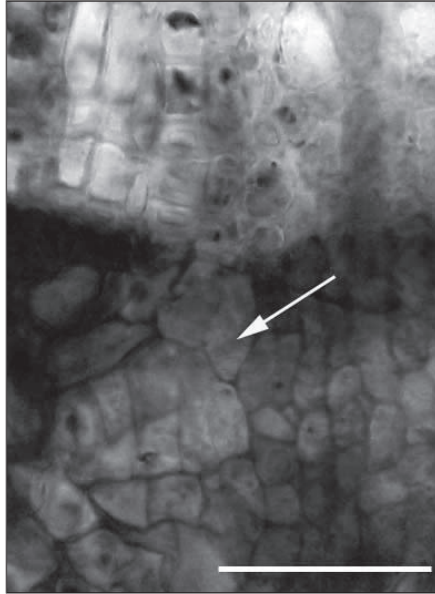


Fig. 3. Callus cells formation from phloem ray cells (arrow) in a 6-day-old graft union. Bar = 50 μm .

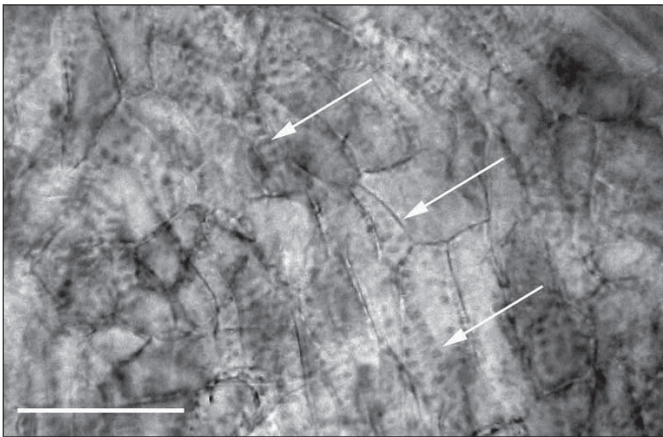


Fig. 4. Randomly arranged tracheary elements (arrows) by direct differentiation of callus cells in a 25-day-old graft union. Bar = 50 μm .

An important stage of graft union formation is the differentiation of callus cells by forming new cambium between pre-existing graft partner cambium. Differentiation of callus cells to form cambium between the cut ends of bud and rootstock cambium involves the formation of new cells aligned at right angles to the pre-existing cambium, as if in response to a stimulus flowing horizontally through the callus from one cambium to the other (Soumelidou et al. 1994). The new cambium was first recognizable 18 days after grafting (Fig. 5). New cambium shapes were variable, depending on rootstock and

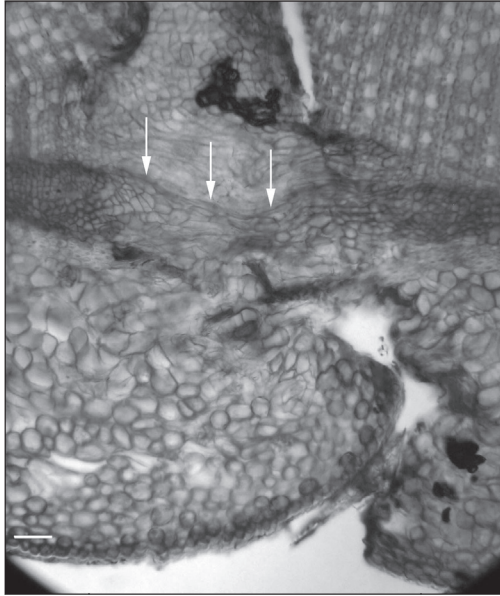


Fig. 5. Transverse section through a 18-day-old graft union shows a slightly curved newly formed cambium (arrows) between pre-existing cambium of the graft partners. Bar = 50 μm .

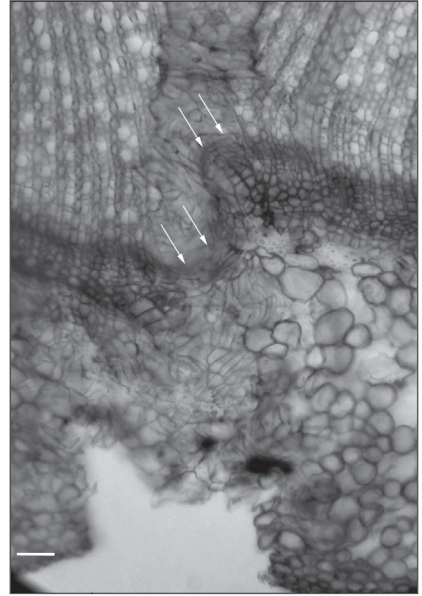


Fig. 6. Transverse section through a 25-day-old graft union displays a newly formed S-shape cambium (arrows) between pre-existing cambium of the graft partners. Bar = 50 μm .

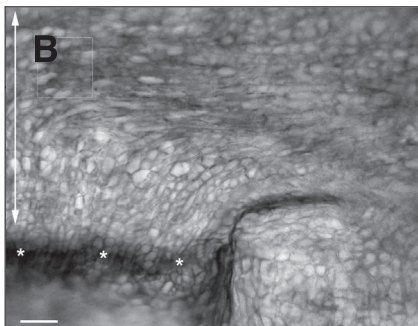
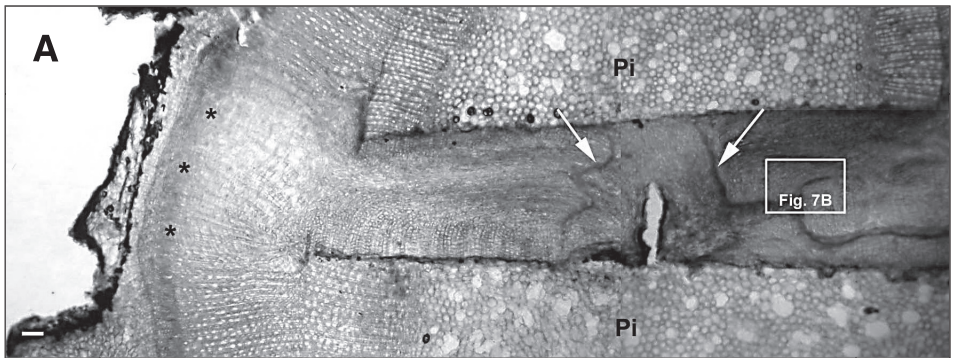


Fig. 7. Transverse section through a 39-day-old graft union. A, New cambium formation between pre-existing cambium (*) and between rootstock and scion piths (arrows). Pi, pith. Bar = 100 μm . B, newly formed cambium (*) and tracheary elements (arrows). Bar = 50 μm .

scion tissue matching. If graft partners were well matched, their pre-existing cambiums joined forming a slightly curved (Fig. 5) or S-shape (Fig. 6).

If physical pressure from binding tape, which is used to hold the graft partners together, is insufficient, a wide gap between the graft partners remains. Cambium development between pre-existing cambiums of graft partners was observed, as well as an uncommon cambium development between pith of rootstock and scion (Fig. 7A). Tracheary elements, which were formed partly by the differentiation of callus cells and partly by uncommon new cambium cells, were visible outward from the uncommon cambium (Fig. 7B). These elements may provide water transport, but it is not clear why they are formed in pith region. On the other hand, tracheary elements can also increase the mechanical endurance, which is essential for development of graft union. The functions of the uncommon cambium are yet unclear.

The graft union development of the elepidote rhododendrons cultivar 'Cunningham's White' is consistent with the graft union formation of other woody plants. Further study is required to determine the influence of uncommon cambium in graft union development.

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Mūžzaļo rododendru potējumu saaugšanas gaita

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Kopsavilkums

Izmantojot gaismas mikroskopiju analizēta mūžzaļo rododendru šķirnes 'Cunningham's White' potējumu (vienkāršā kopulēšana) saaugšanas gaita. Pirmā pamanāmā izmaiņa pēc potēšanas bija nekrotiska slāņa izveidošanās uz griezumam virsmas. Kalluss tika konstatēts sestajā dienā pēc potēšanas un tas veidojās no lūksnes staru un kambija šūnām. Koksnes elementi un jaunveidotais kambijs veidojās, diferencējoties kallusa šūnām. Jaunajam kambijam var būt dažāda forma – nedaudz izliekta vai S veida un tā ir atkarīga no potcelma un potzara kambiju savietošanas. Ja sākotnējā telpa starp potcelmu un potzaru ir plata, tad veidojas vairāki kambiji – viens starp potējuma partneru kambijiem un otrs, kura funkcija pagaidām nav skaidri zināma, – serdes rajonā.