

Simultaneous graft union and adventitious root formation during vegetative propagation in elepidote rhododendrons

Dace Megre*, Uldis Kondratovičs, Kristīne Dokāne

Department of Plant Physiology, Faculty of Biology, University of Latvia, Kronvalda Bulv. 4, Rīga LV-1586, Latvia

*Corresponding author, E-mail: dace.megre@lu.lv

Abstract

Light microscopy was used to carry out a detailed study of cutting graft (grafting on unrooted cuttings) in simultaneous graft union and adventitious root formation using elepidote rhododendron cultivars 'Cunningham's White' as rootstock and 'Catawbiense Grandiflorum' as scion. The first visible reaction was the appearance of a necrotic layer in scion, rootstock and the base of rootstock, observed on day 2 after experimental initiation. The next observable reaction was callus formation from cells in the cambial region. The wound vascular cambium was first recognizable on day 21, and the cambium bridge (a continued strand of wound vascular cambium in callus tissue) between rootstock and scion on day 30. Simultaneously, root initial formation from phloem ray was observed on day 30. Root initials differentiated into root primordias and they continued to develop till emergence at the surface of the rootstock base. Cutting graft is a quick propagation technique that reduces substantially time from unrooted cutting graft to field plant. It may be useful as a method of rooting of varieties showing poor rooting potential.

Key words: grafting, rhododendron, rooting, vegetative propagation.

Introduction

Rhododendrons can be propagated both by generative and vegetative methods. Vegetative propagation is an important commercial method of regenerating large quantities of genetically uniform plant material. One method of rhododendron vegetative propagation is grafting on unrooted cuttings (cutting graft), which involves simultaneous graft union and adventitious root formation. Cutting graft is a quick propagation technique, which reduces substantially the time from unrooted cutting graft to field plant (Ackerman et al. 1997).

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). Several authors have defined the sequence of structural events during the healing of the grafts both in woody and herbaceous plants. Hartman et al. (2002) provides a review of the sequence of events: the necrotic layer formation, proliferation of new parenchymatous cells from both rootstock and scion that soon intermingle and interlock, filling up the space between the scion and rootstock forming the callus bridge. The wound-repair xylem

and phloem, as well as new cambial cells differentiate from the newly formed callus. New cambium forms a continuous cambial connection between scion and rootstock – cambium bridge. In the last step of the graft establishment process, the newly formed cambial layer in the callus bridge begins typical cambial activity forming new vascular tissue.

In general adventitious roots develop either directly from the stem or indirectly via wound tissue (Grönroos, Arnold 1987). A direct and indirect pattern of root formation may be present in both herbaceous and woody systems (Altamura 1996). Adventitious roots in woody plant stem cuttings usually originate from living parenchyma cells such as phloem, vascular ray and cambium (Hartmann et al. 2002). In most species that are difficult-to-root, and initiation of roots occurs from callus tissue (Hamman 1998).

Simultaneous rooting and grafting has been successfully used in propagation of *Rhododendron* (Eichelser 1967), *Rosa* (Macdonald 1986), *Leucospermium* and *Leucadendron* (Ackerman et al. 1997). However, detailed information about anatomical changes during the simultaneous adventitious root and graft union formation is not available. The objective of the present research was to determine the anatomical changes in elepidote rhododendron cutting grafts during simultaneous graft union and adventitious root formation.

Materials and methods

The investigation was carried out from October 2003 to February 2004 using the rhododendron cultivar ‘Cunningham’s White’ as a rootstock and ‘Catawbiense Grandiflorum’ as a scion. The plant material was obtained from the Experimental Nursery of Rhododendron Breeding “Babite”, University of Latvia. Rootstock and scion materials for grafting were approximately 9 to 12-cm-long current year shoots collected from shrubs of rhododendron shortly before grafting. Scions were side veneer graft to the rootstocks. During the grafting procedure a long shallow cut was made into one side of the rootstock and reverse side of the scion. The scion and rootstock were fitted together and wrapped with woolen yarn. The rooting medium consisting of peat moss and pine needles (1:1) was placed in plastic beds, and the bases of cutting grafts were inserted to a depth of 20 mm. Plastic beds were covered with a polyethylene tent and kept at 23 °C during the day and 20 °C the night, with 16-h photoperiod in the growth chamber.

Two to three graft unions were collected within 58 days after experiment initiation. Cutting-grafts were fixed in solution containing 37 % formaldehyde, glacial acetic acid, 95 % ethanol and distilled water (10:5:50:35, v/v/v/v). Transverse sections were cut by a manual microtome and a razor, or by a rotary microtome (Leica RM2145). The hand sections were stained with Astra Blue - Safranin, rinsed in water, dehydrated in an ethanol-xylol series and permanently embedded in Canada balsam (Braune et al. 1999). At the same time, serial cross sections (25 µm in thickness) were obtained by rotary microtome, dehydrated in an ethanol/tert-butyl alcohol series, embedded in Histowax (Ruzin 1999), stained with Astra Blue - Safranin (Braune et al. 1999) and mounted on glass slides in Canada balsam.

Sections were examined with an Olympus CH30RF200 light microscope and photographed using a Leica DM2000 light microscope equipped with a digital camera Canon Power Shot S40.

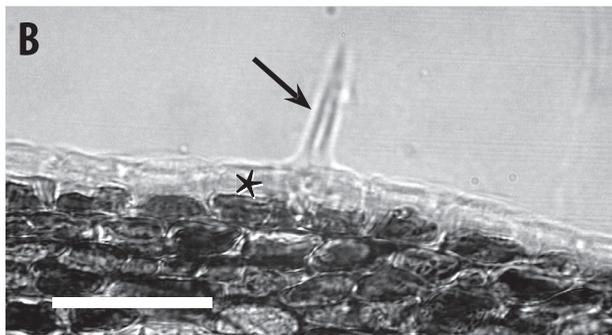
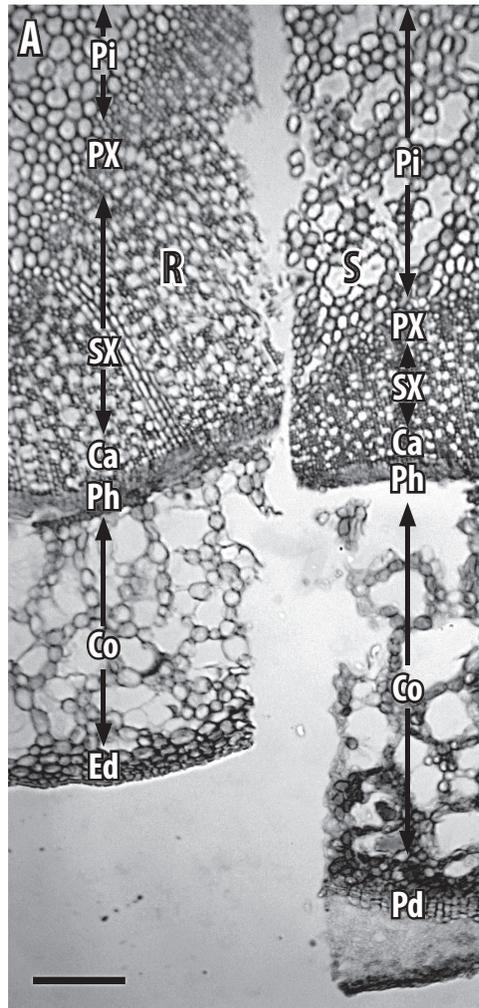


Fig. 1. Transverse sections through a 2-day-old cutting graft. A, anatomy of graft union. Ed, epidermis; Pd, periderm; Co, cortex; Ph, phloem; Ca, cambium; SX, secondary xylem; PX, primary xylem; Pi, pith; R, rootstock; S, scion; B, trichomes (arrows) of scion epidermis (*). Bars 200 μm (A) and 50 μm (B).

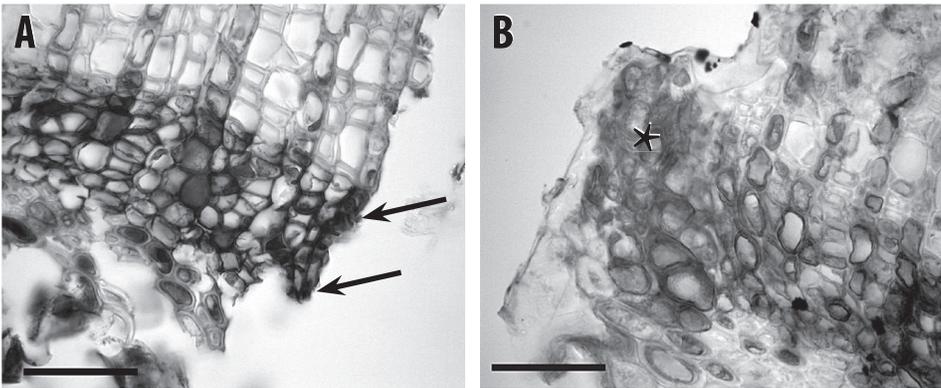


Fig. 2. A, transverse section through a 2-day-old cutting graft shows layer of necrotic tissues (arrows). B, callus formation (*) from the cambial region in a 4-day-old cutting graft. Bars 50 µm.

Results

For successful grafts, it is necessary to match like tissue of stock and scion precisely as it is possible. However, due to small variation in the diameter of the scion and rootstock, improper matching of corresponding tissue often occurs in the graft union. We investigated graft unions with well matched vascular tissues in this study.

The stem anatomy of rootstock and scion were quite similar and typical for woody stem consisting of epidermis, cortex, phloem, cambium, secondary xylem, primary xylem and pith (Fig. 1A). Scion was distinguishable from rootstock only by the presence of trichomes on the epidermis (Fig. 1B). The appearance of a necrotic layer consisting of fragmented and compressed cells was observed on day 2 after experimental initiation in scion, rootstock and the base of rootstock (Fig. 2A).

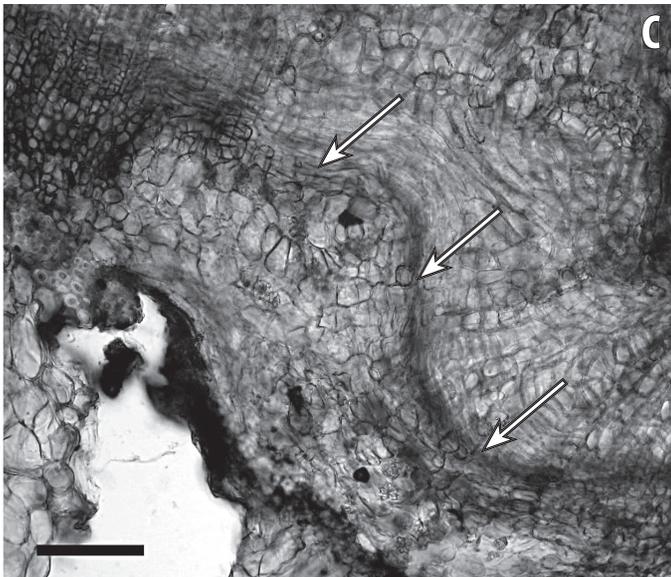
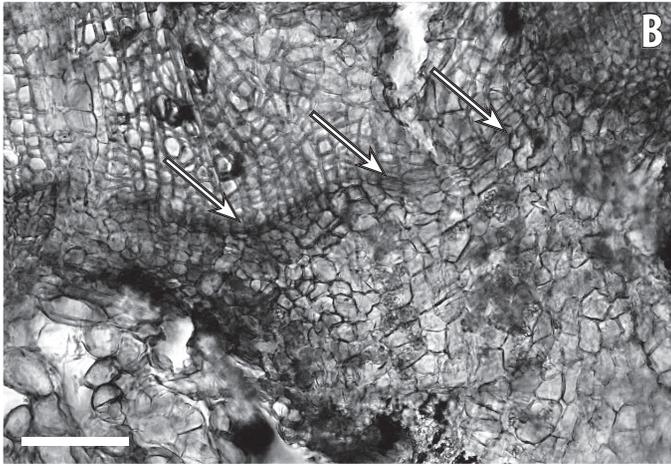
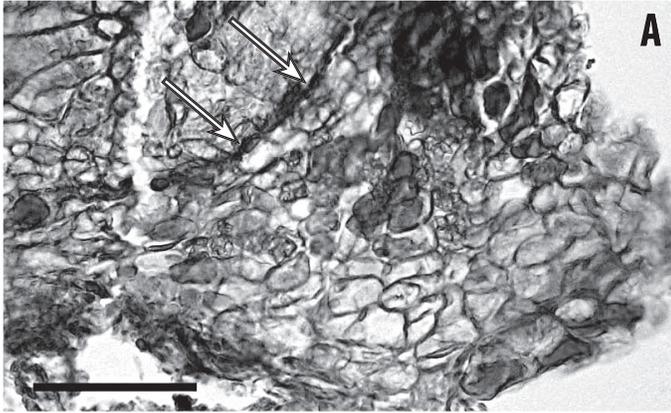
The next observable reaction was callus formation from cells of cambium region (Fig. 2B) on day 4 in graft union and later in the base of rootstock. Callus continued to proliferate and filled the space between rootstock and scion forming a callus bridge. Simultaneously, callus developed at the base of rootstock.

The wound vascular cambium was first recognizable on day 21 (Fig. 3A), and a cambium bridge between rootstock and scion was observed on day 30 (Fig. 3B). Cambium bridge shapes of well-matched graft partners were variable: slightly curved (Fig. 3B) or S-shape (Fig. 3C). Simultaneously, root initial formation from phloem ray was observed on day 30 (Fig. 4A). Subsequently, root initials differentiated into root primordias (Fig. 4B). The root primordia continued to develop until the emergence at the surface of the rootstock base.

Discussion

The graft unit development and adventitious root formation of cutting graft is comparable to graft unit formation and rooting of other woody cuttings. It is known that success

Fig. 3. Different forms of cambium bridge. A, wound vascular cambium (arrows) within callus in a 21-day-old cutting graft; B, a slightly curved cambium bridge (arrows) in a 30-day-old cutting graft; C, S-shape cambium bridge (arrows) in a 36-day-old cutting graft. Bars 100 µm.



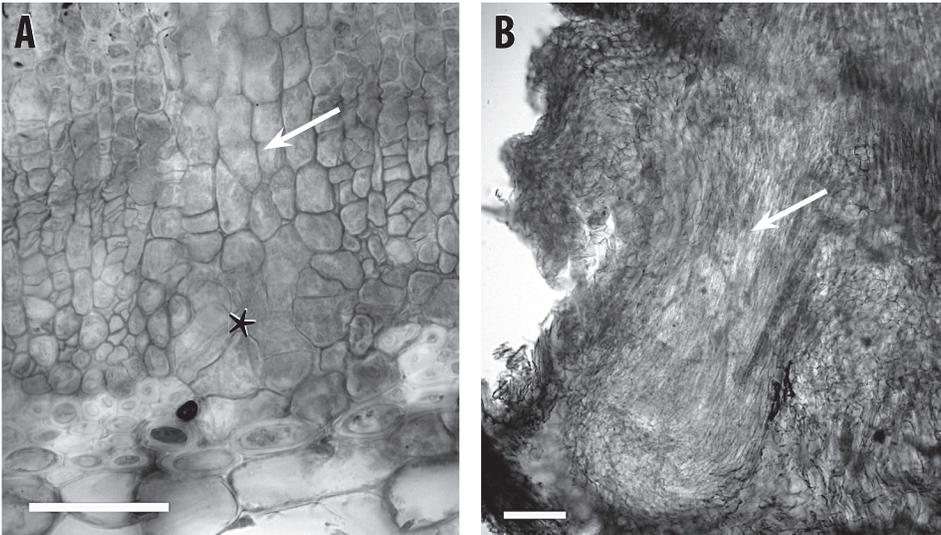


Fig. 4. Adventitious root formation. A, root initial formation (*) from phloem ray cells (arrow) in a 30-day-old cutting graft; B, root primordia (arrow), development in a 44-day-old cutting graft. Bars 50 μm (A) and 100 μm (B).

in grafting of higher plants mainly depends on two essential factors: the physiological compatibility between rootstock and scion, and proper matching of the different tissue of graft partners. (Kollmann et al.1985). At the same time, success in rooting of cuttings depends on two factors: the cutting being in a favourable physiological state at the time of excision and environmental conditions that support the expression of the inherent rooting potential (Cameron et al. 2003).

Grafting includes the formation of a necrotic layer and its subsequent reduction or elimination (Stoddard, McCully 1980). Our results confirm this observation. The formation of callus tissue at the graft interface is the first response to grafting (Pina, Erra 2005). New callus formation is a primary response of wounding that occurs in compatible and incompatible grafts (Moore, Walker 1981) and callus formation is independent of other events in graft development (Pina, Erra 2005). Different views of the origin of callus in various species are present in the literature. Callus can be formed from the cambial region, cortex, pith and xylem ray parenchyma (Hartmann et al. 2002). Our study showed that the cambial region is the main callus producer in cutting grafts of elepidote rhododendrons.

An important step of graft formation is wound vascular cambium formation within callus and the following vascular tissue development. Cambium bridge shapes were variable, depending on rootstock and scion tissue matching (Megre et al. 2004). If the graft partners were well matched, the wound cambium bridge shape can be slightly curved or S-shape. This shape suggests the existence of a high degree of coordinated activity between the cut edges of the pre-existing cambia (Soumelidou et al 1994).

Formation of adventitious roots and wound vascular cambium in the elepidote rhododendron occurred simultaneously. We observed adventitious root formation from phloem ray cells (direct rooting) but callus formation on the base of rootstock and indirect rooting was not seen. Similarly, leaf bud cuttings of *Ficus pumila* form adventitious roots

from phloem ray cells (Davies et al. 1982). However, root primordia can be differentiated from basal callus of some individual mature leaf bud cuttings, and neither these nor the few primordia elongate into well-developed roots (Davies et al. 1982). Direct root formation has been described for *Ficus pumila* (Davies et al. 1982), apple rootstock M26 (Zhou et al. 1992), *Pinus taeda* (Diaz-Sala et al 1996), and *Rhododendron* (Kondratovics, Megre 1999).

The used method is useful for propagating of those woody varieties, which have poor rooting potential, but show histological and physiological compatibility with the rootstock. The studies will be continued to find optimal graft partner combinations.

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Mūžzaļo rododendru potējumu saaugšana un adventīvo sakņu veidošanās to veģetatīvās pavairošanas gaitā

Dace Megre*, Uldis Kondratovičs, Kristīne Dokāne

Augu fizioloģijas katedra, Latvijas Universitātes Bioloģijas fakultāte, Kronvalda bulv. 4, Rīga LV-1586, Latvija

*Korespondējošais autors, E-pasts: dace.megre@lu.lv

Kopsavilkums

Izmantojot gaismas mikroskopiju, pētīja vienlaicīgu potcelma apsākņošanu un potējuma saaugšanu mūžzaļo rododendru šķirnēm ‘Cunningham’s White’ (neapsākņots potcelms) un ‘Catawbiense Grandiflorum’ (potzars). Pirmā pamanāmā izmaiņa bija nekrotisku šūnu slāņa izveidošanās potcelma un potzara potējuma griezumā vietā un potcelma pamatnē otrajā dienā pēc eksperimenta sākšanas. Nākamā atbildes reakcija bija kallusa veidošanās kambija tuvumā – vaskulārais rētu kambijs pirmoreiz tika novērots 21. dienā, bet kambija tilts (potcelma un potzara veidotā vaskulārā rētu kambija apvienošanās rezultāts) starp potcelmu un potzaru – 30. dienā. Vienlaicīgi 30. dienā novēroja adventīvo sakņu iniciāļu veidošanos potcelma pamatnē no lūksnes stariem. Turpmākajā gaitā sakņu iniciāļi diferencējās par sakņu aizmētņiem, kuri attīstījās, līdz kļuva redzami uz potcelma pamatnes virsmas. Potēšana uz neapsākņotiem spraudņiem ir kombinēts veģetatīvās pavairošanas paņēmieni, ko var izmantot, lai ātri iegūtu kokaugu stādāmo materiālu. To var izmantot kā metodi, lai pavairotu tās šķirnes, kurām ir zems apsākņošanās potenciāls, bet kuras labi saaug ar potcelmu.