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ANATOMICAL STRUCTURE AND ADVENTITIOUS ROOT FORMATION IN *Rhododendron ponticum* L. CUTTINGS

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Abstract. Cuttings of *R. ponticum* were treated or not with 'Ukorzeniacz AB' containing IBA and NAA, and placed in rooting medium (temp. 21°C). Transverse sections were made of the bases of the cuttings removed from the soil after 2; 3; 4; 5 and 6 weeks. Meaningful anatomical changes in anatomical structure of *R. ponticum* cuttings connected with wound-healing process could be seen during rooting. In first two weeks periderm in cortex and callus from cambium and phloem emerged as a response to wounding of cuttings. After 3 weeks initiation of wound xylem started in callus. It developed very intensively and did not depend on the way of cutting treatment. Application of auxins did not or not considerably influenced the anatomical changes in stem structure of *R. ponticum* during rooting – proliferation of new xylem was similar in control cuttings and in cuttings treated with rooting stimulator 'Ukorzeniacz AB', although it stimulated initiation of root primordia. Adventitious root formation occurred only in cuttings treated with 'Ukorzeniacz AB', in which numerous root primordia could be observed after 3 and more weeks of rooting. In control cuttings there was none of them even after 6 weeks of rooting.

Key words: rhododendrons, rooting, anatomy

INTRODUCTION

Rhododendron ponticum L. is an evergreen shrub coming from Asia Minor and south-west part of Iberian Peninsula. It achieves 4 m high and 10 m width. The inflorescence consists of 10–15 purple-pink flowers, which occur in June [Czekalski 1991]. The species characterizes by great anatomical and physiological conformability with most evergreen large flowered rhododendron cultivars. In Western Europe, USA and Canada they were therefore widely used as a rootstock for grafting. In Poland, especially in

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Lower Silesia a majority of *R.* Catawbiense-Hybridum Group cultivars are grafted on *R. ponticum* as well.

The most popular method of rhododendron production is propagation by stem cuttings, although most of the taxa are considered to have low ability to root regeneration. Detailed knowledge about the process of rooting in rhododendron cutting may be subservient to increase of intensiveness of their propagation, the study of it was therefore the aim of this research.

MATERIAL AND METHODS

The experiment with propagating *R. ponticum* by cuttings was carried out in the greenhouse of the Agricultural Experimental Station "Piastów", Department of Horticulture, Agricultural University in Wroclaw, Poland. One year old shoots were cut from about sixty years old shrubs growing in Wrocław University's Botanical Garden. The experiment was established on the 12th of November, 2001. After making the semi-hardwood cuttings (about 10–12 cm long), a 2–3 cm long stripe of cortex putting cambium aside were cut off. Half of cuttings were treated with rooting stimulator – 'Ukorzeniacz AB' containing: IBA – 0,05%, NAA – 0.3%, Benomyl – 0.1% and active carbon – about 5%, the others were treated with pure technical talk. The cuttings were inserted into a soil, consisted of white peat, pine bark and perlit 3 : 1 : 1; V : V : V. The medium was acid, its $pH_{(H2O)}$ was 4.2. It was heated to the temperature of 21°C. Low plastic tunnels were installed over the cuttings. The temperature inside the tunnels was 15–16°C.

For anatomical studies 3 cuttings per week were removed from the soil – after 2, 3, 4, 5 and 6 weeks of rooting. Transverse sections were made by hand of the 3–4 cm long base of cuttings. Sections were stabilized, stained with fuchsine and malachite green and dropped into Canadian Balm [Filutowicz and Kużdowicz 1951].

RESULTS

Anatomical structure of *R. ponticum* stems

One year old shoots of *R. ponticum* characterized by secondary structure typical for woody plants. In their transverse section it could be observed, from outside: epidermis with cuticle, cortex, endoderm, sclerenchyma, phloem and xylem creating continuous ring with cambium inside and pith. No pericycle could be distinguished in the stems.

Cortex consisted of two types of parenchymatous cells. Some of them had relatively thick walls. Among them there were big cells with very thin walls, which could be seen under great enlargement only. Schlerenchyma was built of cells with equally thick and strongly lignified walls. The ring of sclerenchyma was 1–5 cells thick and broken above pith rays. Xylem consisted of not numerous vessels, fibres and parenchymatous cells of rays and axillary parenchyma. Pith occupied the central part of stems. Similarly to cortex, it consisted of two types of cells: large with very thick walls and not numerous thin-wall-cells among them.

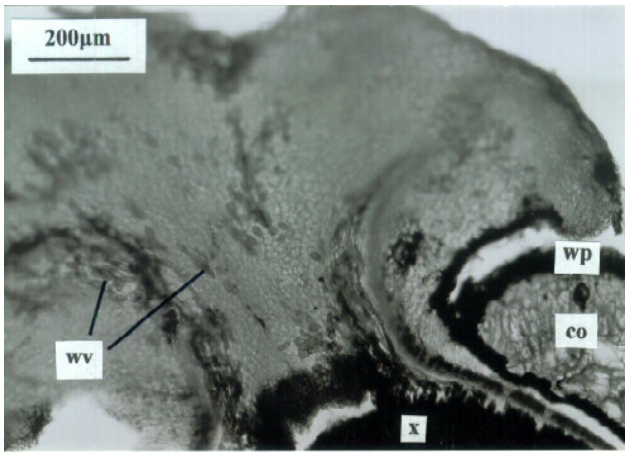

Fig. 1. Transverse section of callus proliferating at the base of *R. ponticum* control cutting after 5 weeks of rooting showing wound xylem differentiating from parenchymatous cells; wv – wound vessels, wp – wound periderm, co – cortex, x – xylem. Microscopic section stabilized, stained with fuchsin and malachite green

Ryc. 1. Przekrój poprzeczny przez kalus u podstawy sadzonki kontrolnej różanecznika pontyjskiego po 5 tygodniach ukorzeniania pokazujący ksylem różnicujący się z komórek parenchymatycznych; wv – naczynia przyranne, wp – peryderma, co – kora pierwotna, x – ksylem. Skrawek utrwalony, barwiony fuksyną kwaśną i zielenią malachitową

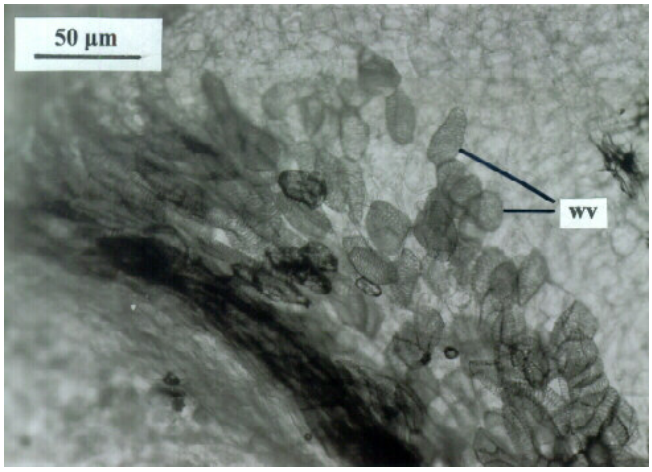

Fig. 2. Transverse section of callus proliferating at the base of *R. ponticum* control cutting after 5 weeks of rooting showing wound xylem differentiating from parenchymatous cells; wv – wound vessels. Microscopic section stabilized, stained with fuchsin and malachite green

Ryc. 2. Przekrój poprzeczny przez kalus u podstawy sadzonki kontrolnej różanecznika pontyjskiego po 5 tygodniach ukorzeniania pokazujący ksylem różnicujący się z komórek parenchymatycznych; wv – naczynia przyranne. Skrawek utrwalony, barwiony fuksyną kwaśną i zielenią malachitową

Changes in anatomical structure of stems during rooting of R. ponticum cuttings

Meaningful anatomical changes in anatomical structure of *R. ponticum* cuttings could be seen during rooting process. They develop in two ways: wound-healing process and root initiation with their further development. These changes differed depending on the way of cutting treatment.

The wound-healing process occurred on cut surface and it included tissues injured during forming of cuttings. In stems of *R. ponticum* the most significant response to wounding was callus proliferation. It developed only from cambium and phloem, whereas wound healing process in cortex ended up on formation of wound periderm, protecting dipper laid tissues from drying and penetration of microorganisms. In some places callus proliferation was very intensive and it achieved great size (fig. 1). After 3

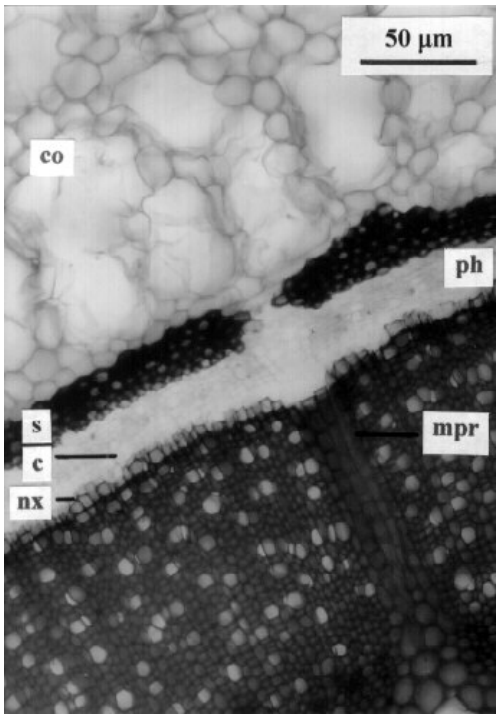

Fig. 3. Transverse section of the base of *R. ponticum* cutting treated with 'Ukorzeniacz AB' after 2 weeks of rooting; co – cortex; s – sclerenchyma, c – cambium, ph – phloem, nx – new xylem, mpr – multiseriate pith ray. Microscopic section stabilized, stained with fuchsin and malachite green

Ryc. 3. Przekrój poprzeczny przez podstawę sadzonki różanecznika pontyjskiego traktowanej 'Ukorzeniaczem AB' po 2 tygodniach ukorzeniania; co – kora pierwotna; s – sklerenchyma, c – miazga, ph – łyko, nx – nowe drewno, mpr – wielorzędowe promienie rdzeniowe. Skrawek utrwalony, barwiony fuksyną kwaśną i zielenią malachitową

weeks of rooting, initiation of wound xylem started in callus. The vessels differentiated from parenchymatous cells in result of wall lignification. The tracheary elements had oval shape with net perforation and they gradually elongated (fig. 2). Wound xylem development in callus, similarly to callus proliferation did not depend on the way of cutting treatment. The processes were equally intensive in both types of cuttings: control and treated with rooting stimulator.

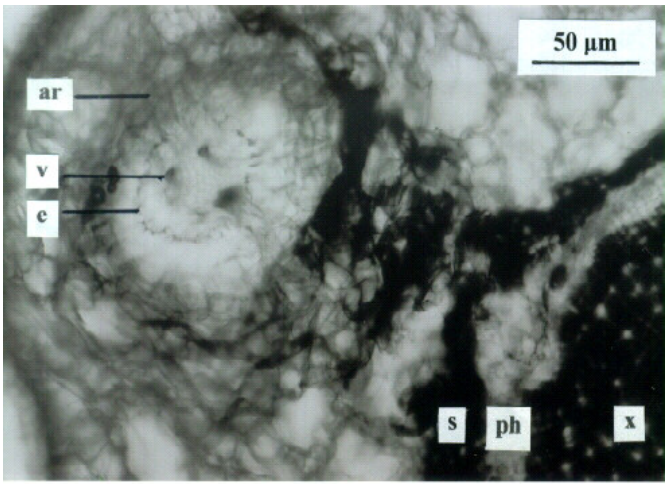

Fig. 4. Transverse section of the base of *R. ponticum* cutting treated with 'Ukorzeniacz AB' after 5 weeks of rooting showing adventitious root (ar), developing in cortex in connection with multiseriate xylem-phloem rays; s – sclerenchyma, ph – phloem, x – xylem, v – vessel, e – endoderm. Microscopic section stabilized, stained with fuchsin and malachite green

Ryc. 4. Przekrój poprzeczny przez podstawę sadzonki różanecznika pontyjskiego traktowanej 'Ukorzeniaczem AB' po 5 tygodniach ukorzeniania pokazujący korzeń przybyszowy (ar), rozwijający się w korze pierwotnej w pobliżu wielorzędowych promieni rdzeniowych; co – kora pierwotna; s – sklerenchyma, ph – łyko, x – drewno, v – naczynie, e – endoderma. Skrawek utrwalony, barwiony fuksyną kwaśną i zielenią malachitową

Another reaction taking place during rooting of *R. ponticum* cuttings was proliferation of new xylem. The process did not occur intensively and did not depend on treatment of cuttings as well: after two weeks of rooting the layer of new xylem was up to one vessel thick and it was not continues (fig. 3). After 5 weeks of rooting it was 5 cells thick in both combinations of cutting treatment.

Yet the most important process: adventitious root initiation was stimulated by auxins contained in 'Ukorzeniacz AB'. In cuttings treated with the rooting stimulator young adventitious roots could be observed after 3 weeks of rooting, whereas in control cuttings there was none of them even 6 weeks after removing them from the soil. Root primordia originated from cambium. They were fairly numerous – after 4 weeks there were up to 3 primordia or young roots in one section. They elongated in cortex, mostly parallel to stem axis. In their transverse section vessels of primery xylem and endoderm with Caspary's bands could be distinguished (fig. 4).

DISCUSSION

Adventitious roots could be divided into two types: preformed roots, which develop naturally on stems when they are still attached to parent plant and wound roots, which develop only after the cutting is cut off from the parent plant as a response to injuring the stem [Hartmann et al. 1990]. In rhododendrons, among them in *R. ponticum* stems, only the second type of roots is formed [Kondratovičs 1999, Krzymińska et al. 2001, Strzelecka and Czekalski 2001, Strzelecka 2003]. Adventitious root primordia originate de novo from cambium cells preserving meristematic character, whereas in other plants primordia can emerge from different tissues, like phloem, leaf traces, vascular rays, cortex and pith or other parenchymatous cells, e.g lenticels [Hartmann et al. 1990, Syros et al. 2004]. In case of *R. ponticum* root primordia initiate only in cuttings treated with rooting stimulator 'Ukorzeniacz AB'. This phenomenon allows to conclude that among others, *R. ponticum* belongs to easy-to-root taxa of *Rhododendron* genera and it corresponds with the data published by Czekalski [1991] and Bojarczuk [1995]. It could be included to the second of three classes created by Hartmann et al. [1990] in regard to adventitious root initiation: those, in which the naturally occurring cofactors are present in ample amounts, but auxins are limiting – with the application of them rooting is greatly increased.

In many difficult-to-root plant species initiation of root primordia is connected with callus proliferation. Very often root initials arise in the vicinity of wound xylem differentiating from callus cells [Cameron and Thomson 1969, Hejnowicz 1973]. Although proliferation of callus and formation of tracheary elements in *R. ponticum* cuttings was extremely intensive, it did not preceded initiation of root primordia.

According to many authors [Tureckaia 1961, Davies et al. 1982] callus proliferation, as well as wound xylem differentiation in plant cuttings is stimulated by auxin application. Similar response was observed in rhododendrons [Tureckaia 1961, Strzelecka 2003]. However in *R. ponticum* cuttings this phenomenon was not observed: in both combination of cutting treatment - control and treated with auxins these two processes were occurring with similar intensiveness. The same reaction was related to proliferation of new xylem, although in other taxa of rhododendrons this process leaded to formation of two-three layers of xylem [Krzymińska et al. 2001, Strzelecka and Czekalski 2001].

CONCLUSIONS

1. Meaningful anatomical changes in anatomical structure of *R. ponticum* cuttings connected with wound-healing process could be seen during rooting.

2. Application of auxins did not or not considerably influenced the anatomical changes in stem structure of *R. ponticum* during rooting – proliferation of new xylem was similar in control cuttings and in cuttings treated with rooting stimulator 'Ukorzeniacz AB', although it stimulated initiation of root primordia.

3. Adventitious root formation occurred only in cuttings treated with 'Ukorzeniacz AB', in which numerous root primordia could be observed after 3 and more weeks of rooting. In control cuttings there was none of them even after 6 weeks of rooting.

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BUDOWA ANATOMICZNA I POWSTAWANIE KORZENI PRZYBYSZOWYCH W SADZONKACH RÓŻANECZNIKA PONTYJSKIEGO (*Rhododendron ponticum* L.)

Streszczenie. Sadzonki różanecznika pontyjskiego, kontrolne i traktowane preparatem Ukorzeniacz AB zawierającym IBA i NAA, umieszczano w podłożu o temperaturze 21°C. Do analizy anatomicznej pobierano 3 sadzonki, po 2, 3, 4, 5 i 6 tygodniach od czasu umieszczenia ich w podłożu.

Podczas ukorzeniania sadzonek zachodziły w nich kompleksowe zmiany dotyczące budowy anatomicznej, związane z procesem gojenia się rany. W pierwszym tygodniu, w odpowiedzi na zranienie podstawy sadzonek w obrębie kory pierwotnej powstała

ochronna peryderma, a z kambium i komórek miękiszowych łyka następowała silna proliferacja kalusa, w którym po 3 tygodniach komórki miękiszowe różnicowały się w naczynia o siatkowatej perforacji. Zastosowane auksyny bardzo nieznacznie lub wcale nie wpływały na zmiany w budowie anatomicznej pędu sadzonek w trakcie ukorzeniania – tworzenie nowego drewna zachodziło z jednakową intensywnością w sadzonkach kontrolnych i traktowanych Ukorzeniaczem AB, wpływał on natomiast na powstawanie primordiów. Inicjacja korzeni przybyszowych zachodziła tylko w sadzonkach traktowanych Ukorzeniaczem AB, w których obserwowano liczne zawiązki korzeni przybyszowych po 3 i więcej tygodniach ukorzeniania. W sadzonkach kontrolnych nie obserwowano tworzenia się primordiów nawet po 6 tygodniach ukorzeniania.

Słowa kluczowe: rododendron (różanecznik) pontyjski, korzenie, budowa

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