THE CHANGE OF ROOT MORPHOLOGY OF PLANTAGO LANCEOLATA UNDER HYPOXIA CONDITIONS

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Abstract. Many plant species can adapt to flooding and hypoxia by forming a root system with an altered architecture: thicker, shorter and shallower adventitious roots, than under aerated conditions. The internal gas transport is often improved by increased root porosity and aerenchyma, which is tissue with large intercellular spaces. The raised root aeration allows better supply of oxygen to plant tissues and diffusion of oxygen into the rhizosphere (Radial Oxygen Loss, ROL). This phenomenon creates narrow, but well aerated zones in the hypoxic soil, where phytotoxins are oxidised and methanotrophic as well as nitrifying bacteria can live. The aim of the study was to determine the change of root architecture, porosity and ROL from roots of Plantago lanceolata plants originating from The Middle Vistula River Gorge. Selected plant species were subjected to transient flooding during 7 days of cultivation on aerated and stagnant oxygen-deficient hydroponic medium. We observed the formation of shorter hypoxic, adventitious roots (56-69 mm) than control roots (112-196 mm) with high porosity (stagnant 15-21 %, control 8.5-9.4%), and the diameter of aerated zone (halo) increased from control values of 0-1.5 mm to 2-2.5 mm under hypoxic conditions.

Keywords: Aerenchyma, hypoxia, Plantago lanceolata, porosity, Radial Oxygen Loss (ROL), soil flooding

INTRODUCTION

The global climate changes, which is the result of increased concentrations of greenhouse gases, leading to the increase of the quantity of precipitation. Similar to other countries, in Poland, flooding events occur every year during melting of snow in spring and heavy rainfalls in summer. Regular flooding events cause the necessity of water retention in river valleys. However, renaturalization of these
areas can initiate a number of processes that alter the environment. Physical processes limit gas exchange between the atmosphere and soil, leading to a decrease of oxygen availability for microorganisms and plant roots. Low oxygen hampers the performance of plants, potentially causing death but also initiating the range of plant adaptations. Such root adaptations comprise altered root architecture, formation of adventitious roots, increase of root porosity and Radial Oxygen Loss (ROL) caused by oxygen diffusion from roots to the rhizosphere.

In moist and wet soils roots are shorter, less branched, with relatively large diameter and low surface-to-volume ratio. They grow shallowly, because of oxygen shortage in deeper soil layers which are cut off from the air by excess of water. Aboveground parts of wetland plants grow usually very well under these conditions (Voesenek et al. 1988, Končalova 1990, Polakowski 1995). A very common form of root adaptation to soil flooding is the formation of adventitious roots which can appear after a few days of flooding. They are formed at the stem base, upper part of the main root or from the stem node, and penetrate upper, better aerated soil layers (Blom et al. 1994, Blom and Voesenek 1996, Vartapetian and Jackson 1997). These roots are characterised by a larger amount of aerenchyma than the control plants. Some plant species form adventitious roots without submergence, but even then are in an advantageous situation when their root system is flooded (Visser and Voesenek 2004). Plants forming adventitious roots tolerate flooding very well, after which the recovery is quick and complete, the growth of their stem is less impeded, epinasty is less strong, absorption of water is larger and stomata can be open more often than in plants of which adventitious roots had been removed. Earlier work shows that the emergence of these roots is regulated mainly by endogenous auxins and ethylene (Visser and Voesenek 2004, Dat et al. 2004).

Another adaptation is the formation of aerenchyma that facilitates diffusion of oxygen from the atmosphere or produced during photosynthesis to the root apex and, in the opposite direction, compounds such as CO₂, CH₄, C₂H₄, ethanol and acetaldehyde from plant tissues and flooded soil into the atmosphere (Thomas et al. 1995, Jespersen et al. 1998, Ram et al. 2002, Colmer 2003).

Many plant species form aerenchyma constitutively, but it is expanded in response to flooding. Plants adapted to areas with relatively dry soil do not contain constitutive aerenchyma, but it may be induced by environmental conditions, e.g. by flooding (Colmer 2003), hypoxia (Brailsford et al. 1993), high soil density or deficit of mineral components (N or P) (Voesenek et al. 2006, Drew et al. 2000).

Aerenchyma can be formed by schizogeny, through separation of cells (e.g. in roots of Rumex palustris, Visser et al. 1996) or lysigenous, by lysis of whole cells of some parts of the cortical parenchyma (e.g. in roots of Glyceria maxima)
(Smirnoff and Crawford 1983). This type of tissue is characterised by numerous intercellular spaces forming a system of channels which decrease the diffusion resistance for gases which are a reservoir of air for submerged roots (Visser et al. 1997, Drew et al. 2000). Additionally, aerenchyma limits the oxygen demand of organs containing this tissue by decrease of the number of respiring cells (Armstrong et al. 1972).

Increased root aeration enables oxygen supply to tissues but can have additional importance for plant development. It was noted that aerenchyma enables diffusion of oxygen from roots to the soil environment (Radial Oxygen Loss – ROL). Zones of aeration in the rhizosphere of rice (Oryza sativa) were described for the first time by Armstrong (1967, 1970 after Brune et al. 2000). The extent of aeration of the rhizosphere depends on many factors: plant species or variety, gradient of oxygen concentration along the plant roots, length of the diffusion pathway, length of the roots, resistance of oxygen diffusion in radial direction and its consumption by cells, biomass, leaf area, light, concentration of CO\textsubscript{2} in the water column, water level fluctuation, and various soil conditions such as moisture, porosity, degree of reduction, oxygen demand of microorganisms and roots, its temperature, presence of S\textsuperscript{2\textminus} (Colmer 2003, Mainiero and Kazada 2004, Armstrong and Armstrong 2005, Laskov et al. 2006, Sasikala et al. 2009).

The oxygen diffusion from plant roots growing on wet soil causes a narrow zone of well aerated soil along roots, in which aerobic biochemical processes lead to increased availability of nutrients and to oxidation of reduced phytotoxins, such as Fe\textsuperscript{2+}, Mn\textsuperscript{2+}, \text{H}_2\text{S}, \text{S}^{2\text{\textminus}}, \text{HS}^{-}, which occurs directly or by the activity of aerobic microorganisms (Engelaar et al. 1995, Jespersen et al. 1998, Braune et al. 2000, Sasikala et al. 2009).

However, diffusion of oxygen from roots to the soil causes a decrease of oxygen supply to the root apex, even by as much as 30-40% of oxygen provided by root aerenchyma (Armstrong 1979, Colmer 2003). Therefore, many plant species from wetlands have adventitious roots that contain barriers to radial gas diffusion, thereby decreasing ROL. This phenomenon increases internal oxygen concentrations, enables better root penetration in anaerobic soil and reduces penetration of toxins (Armstrong 1979, Končalova 1990, Colmer 2003). These barriers are mainly formed through suberization or lignification of walls of external cortical cell layers.

The aim of this study was to determine the root architecture, porosity and ROL from roots of \textit{P. lanceolata} plants habitating the river foreland in The Middle Vistula River Gorge grown on aerated and stagnant medium.
MATERIAL AND METHOD

Vegetation composition

To obtain information on plant species composition along a flooding gradient we have set two transects along elevation gradients in the polder in Kępa Sołecka (Opole Lubelskie District, Laziska Commune, N51° 8’ E21° 47’) and described the summer state of the vegetation in 20 plots (1m x 2m) using the Braun-Blanquet method (Braun-Blanquet 1928, Janecki 1999, Kucharczyk 2001, Rutkowski 2004, Matuszkiewicz 2008).

Seeds collection and plant cultivation

Seeds were collected from the plots in summer, placed on Petri dishes on moist filter paper and put into a growth chamber (photoperiod 12 h/12 h, photon flux density (PPFD) 20 μmol m⁻² s⁻¹, temperature 20°C/10°C). After germination, seedlings were transplanted on polyethylene grains soaked with 25% Hoagland solution modified by Visser et al. (1996) (2 mM Ca(NO₃)₂·4 H₂O, 1.25 mM K₂SO₄, 0.5 mM KH₂PO₄, 0.5 mM MgSO₄, 50 μM NaCl, 25 μM H₃BO₃, 2 μM MnSO₄·H₂O, 2 μM ZnSO₄·7H₂O, 0.5 μM CuSO₄·H₂O, 0.5 μM H₂MoO₄, 90 μM FeEDTA) at pH 5.6 and left for 3 weeks in a controlled climate room (photoperiod 16 h/8 h, PPFD 200 μmol m⁻² s⁻¹, air temperature 22°C).

Then plants were transferred to hydroponic cultivation in polyethylene containers (20 l) with 15 l modified 25% Hoagland solution and with aeration from pressurised air. On the surface of the solution a polyethylene float was placed, with 15 mm holes in it. The shoot base of the plants was wrapped with sponge material and put into a tight-fitting plastic cylinder (15 mm x 20 mm) to hold the sponge. These cylinders were then placed into the holes so that roots were immersed in the solution.

After 1 month we transferred 4 plants to containers with stagnant solution (0.1% agar in modified 25% Hoagland solution flushed with N₂ for 3 hours). Plants were grown under these condition for 1 week until they formed new adventitious roots (Visser et al. 1996).

Survival and condition

Plant survival was estimated at the end of the period of hydroponic cultivation on aerated and stagnant solution. Plants were considered alive when having green leaves with proper turgor (Blom et al. 1990, Nabben et al. 1999, van Eck et al. 2004). Plant condition was estimated based on the change of colour and the turgor of leaves and stems.

Root architecture, ROL and porosity

The root architecture was described on the basis of appearance, thickness and length of roots, and ability to form adventitious roots.
ROL was determined with a method using a glass cuvette (1000 mm x 350 mm x 18 mm) equipped with 2 outlets in the bottom part, set on a wooden stand (slightly tilted at an angle of 65°). We prepared the test solution (5 mM KCl, 0.5 mM CaSO₄ in 0.05% agar solution with 25 mg l⁻¹ methylene blue) by bubbling with N₂ during 12 hours. Directly before measurements the solution was reduced and decoloured with 0.5 M Na₂S₂O₄ · 2H₂O.

The cuvette was first filled with tap water, and 4 plants (2 from aerated and 2 from stagnant solution) were then fixed in the cuvette (so that roots were in the cuvette and shoots were in the atmosphere) together with an artificial root (glass capillary (inner diameter 0.2 mm) mounted with a silicon tube (length 10 mm, diameter 0.5 mm) that was sealed with a small glass stopper, to evaluate the rate of ROL (Sorell 1994, Roelofs et al. 1990).

After positioning the roots such that several single roots were well separated from the main root mass, the water was removed through the outlets, the cuvette was flushed with N₂ and then filled with the analytical solution. The diffusion of oxygen from roots into the solution caused the appearance of blue colouration around aerated parts of roots (halo) through the oxidation of methylene blue. We estimated the diameter and length of the halo and took pictures of aerated zones after 3 hours of the measurement.

The root porosity was determined in segments of roots (1 cm) taken from 10-20, 30-40, 70-80 mm from root apexes (according to the method of Visser and Bögemann 2003). They were stored separately in tubes with water. Directly before weighing, each segment was gently dried and transferred with tweezers into a pre-weighed gelatine capsule (type Helder-2, Bloklandpack bv, Ljsselstein, The Netherlands) to prevent water loss caused by evaporation. The segments were weighed (w₁) using a microbalance (Sartorius Micro M2P; Sartorius Instrumenten bv, Nieuwegen, The Netherlands). Then the segments were removed from the capsules, transferred into a holder with holes filled with water and infiltrated with water in a vacuum exsiccator (5 kPa, Emergo, Landsmeer, The Netherlands). After blotting adherent water, the root segments were weighed in the capsules (w₂). The root porosity was calculated using following formula:

\[
\text{Porosity (\% v/v) } = 100 \cdot \frac{(w_2 - w_1)}{(SW / w_2)},
\]

where: \(w_1\) – biomass of roots (mg), \(w_2\) – biomass of roots infiltrated with water (mg), \(SW\) – specific weight of infiltrated tissue being 1.03 g ml⁻¹ (Visser and Bögemann 2003).

Afterwards, the segments were preserved with 70% ethanol. We prepared cross-sections using a hand microtome (MIC 503, Euromex Microscopes BV, Arnhem, The Netherlands). These were observed and photographed (camera Olympus C3030, microscope BX-40F3, Olympus Optical Co., Hamburg, Germany). All porosity data were transformed using the formula \(\log (x+1)\) and ana-
lysed using SPSS 15.0 with one-way ANOVA (2006 Chicago, IL, USA).

RESULTS AND DISCUSSION

Our analysis indicates that the vegetation of the river foreland shows a zonation that is likely formed by flooding. On long-term flooded sandy deposits a zone of *Phalaridetum arundinaceae* Koch 1926 n.n. Libb. 1931 was found. Higher situated *Agropyro-Rumicion crispi* Nordh. 1940 em. RTx. 1950 and *Ranunculo-Alopecuretum geniculati* R.Tx.1937 were located. Close to the dike we found plants preferring soils rich in N and with medium moisture (*Arrhenatheretalia* Pawł. 1928 and *Molinietalia caeruleae* W. Koch 1926) with *Plantago lanceolata*.

During our hydroponic experiment we observed 100% of survival and a very good condition of all plants (green leaves with high turgor). All plants formed a reasonable number of adventitious roots. However, the plants from hypoxic medium were shorter (56-69 mm) than the control plants (112-196 mm) (p < 0.001, Tab. 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SEM (mm)</th>
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<tr>
<td>Control</td>
<td>142.75 ± 18.47</td>
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<tr>
<td>Hypoxia</td>
<td>63.75 ± 2.75</td>
</tr>
</tbody>
</table>

Two of the control roots and all roots from hypoxic condition showed ROL. The diameter of halo from control plants was significantly smaller than that from the plants from the stagnant medium (p < 0.05) and amounted to 0.75 mm and 2.13 mm, respectively. The aerated zones were located around the control roots showing ROL between 66 and 80 mm from the apex to the root base. In hypoxic roots they were formed from 1-2 mm to 11-14 mm from the apex (Photo. 1, Tab. 2).
In our experiment we found that hypoxia significantly increased root porosity (p < 0.001). In the control plants it grew with the distance from the apex from 4.8% (10-20 mm from the apex) to 21.8% (70-80 mm from the apex) (p < 0.05). However, in the roots from stagnant solution the porosity equalled from 12.2% (10-20 mm) to 21% (50-60 mm), with no significant difference (Fig. 1, Photo. 1).

During our experiments we used the stagnant agar solution to ensure the hypoxic conditions, because it has been shown that agar added to the nutrient solution prevents convective movement of the solution and thus limits gas movement (Wiengweera et al. 1997, Colmer et al., 1998). It thus helps to keep the medium hypoxic and promotes ethylene accumulation in the rhizosphere.
Similar to previous studies, we found that *P. lanceolata* adapted to flooding (Striker *et al.* 2007, Grimoldi *et al.* 2005). This relatively tolerant plant species (Banach *et al.* 2009) is common in Poland in roadsides, pastures, meadows, embankments, ruderal places, fallow lands, often as a weed. Under control condition it formed relatively deep, branched roots with constitutive aerenchyma. In response to soil hypoxia we observed induced additional development of lysigenous lacunae through its root cortex. *P. lanceolata* is able to aerate its rhizosphere but the aerated zone is relatively small (0-2.5 mm). Under aerated condition ROL is located in the middle and basal part of the root and it is limited probably by low root porosity (around 10%). During flooding, oxygen diffusion to the rhizosphere was visible in a narrow section behind the root apex. ROL oxidising the soil may protect the apex and laterals growing in waterlogged soil against soil toxins (Armstrong 1979, Končalova 1990).

The data suggest the development of a barrier to ROL in the basal zone of roots in response to stagnant conditions, which can enhance longitudinal O₂ diffusion in the aerenchyma to the apex by preventing the loss of excessive amounts of oxygen to the rhizosphere. The combination of soil aeration close to the apex and barriers in the basal zones enhance the root penetration into the aerobic sediments (Colmer 2003).
CONCLUSION

During our study we observed that *P. lanceolata* plants are well adapted to transient flooding and hypoxia. They can form porous adventitious roots with ROL in the middle and the basal zone of roots during normoxia and close to the root apex under hypoxic condition, suggesting the presence of an inducible barrier to ROL.

REFERENCES


ZMIENNOŚĆ MORFOLOGII KORZENI Plantago lanceolata w warunkach hipoksji

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Streszczenie. Wiele gatunków roślin adoptuje się do powodzi i hipoksji. Mogą formować system korzeniowy o zmienionej architekturze: grubse, krótsze i płytsze korzenie przybyszowe niż w na-tlenionych warunkach. Wewnętrzny transport gazów jest często poprawiany przez zwiększoną porowatość korzeni i aerenchymę, tkankę z dużymi przestworami międzykomórkowymi. Zwiększone natlenianie korzeni pozwala na lepszy transport tlenu i dyfuzyjne ją tętnienie (radialna utrata tlenu, ROL). Ten fenomen stwarza wąską, dobrze natlenioną, hipoksyczną strefę w glebie, gdzie fitotoksyny są utleniane a bakterie metanotroficzne i nitryfikacyjne mogą żyć. Celem badań było określenie zmiany architektury korzeni, porowatości i ROL z korzeni Plantago lanceolata zamieszkujących Małopolski Przelom Wisły. Wybrane gatunki roślin były poddane przejściowemu zalaniu przez 7 dni hodowli na natlenionym i hipoksycznym podłożu. Zaobserwowaliśmy formowanie krótkich korzeni przybyszowych (56-69 mm) niż u roślin kontrolnych (112-196 mm) o dużej porowatości (podłoże niedotlenione 15-21%, kontrolne 8,5-9,4%) a średnica strefy natlenionej (halo) zwiększyła się z wartości kontrolnej na poziomie 0-1,5 mm do 2-2,5 mm w warunkach hipoksycznych.

Słowa kluczowe: hipoksja, Plantago lanceolata, radialna utrata tlenu (ROL), zalanie gleby