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Root aerenchyma – formation and function

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ABSTRACT

The formation of root aerenchyma, the prominent air spaces in the root cortex which are normally induced by waterlogging, has an important role in providing an internal pathway for oxygen transport between roots and the aerial environment. Along this pathway, O_2 is supplied to the roots and rhizosphere, while CO_2, ethylene, etc. move from the soil to the shoot and the atmosphere. The aerenchyma are formed either as part of normal development, or in response to stress (e.g. hypoxia, nutrient deficiency), by two known mechanisms: schizogeny and lysigeny. Aerenchyma formation increases porosity above the levels that appear in the usual intercellular spaces.

Key words: roots, aerenchyma, O_2 depletion, hypoxia

IZVLEČEK

AERENHIMI V KORENINAH RASTLIN – NASTANEK IN FUNKCIJA

Aerenhimi, zračni prostori v primarni skorji korenine, se razvijejo zaradi zasičenosti tal z vodo oz. poplavljenosti tal, ko pride do pomanjkanja kisika v tleh. Omogočajo transport kisika znotraj rastline od nadzemnega dela do korenin in s tem zagotavljajo zadostno količino kisika za normalno opravljanje metabolnih funkcij. Hkrati se omogoča oksigenacija rizosfere in odstranitev številnih plinov (npr. CO_2, etilena) iz nje. Znana sta predvsem dva načina njihovega nastanka: shizogeni in lizogeni način. Njihov nastanek poveča poroznost korenin nad vrednost, ki je dosežena z običajnmi intercelularji.

Ključne besede: korenine, poroznost, aerenhimi, pomanjkanje kisika, hipoksija

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1 INTRODUCTION

Roots, rhizomes and other plant organs usually obtain sufficient oxygen for aerobic respiration directly from gaseous spaces in the soil. However, the diffusion of O_2 from the air into the soil can be effectively blocked when soil becomes flooded or waterlogged. In such cases, the respiratory consumption by plant roots, soil fauna and microorganisms can totally deplete the oxygen. Periods of oxygen deficiency can trigger functional and developmental responses that promote acclimation to hypoxic or anoxic conditions. Low oxygen concentrations lead to long-term morphological adaptations (Geigenberg, 2003) such as developing aerenchyma in roots (Colmer, 2003; Drew et al., 2000; Malik et al., 2003; Gunawardena et al., 2001a) or development of impermeable barriers to radial O_2 loss (ROL) in basal zones of roots (Colmer, 2003; Malik et al., 2003).

2 FUNCTIONAL AND MORPHOLOGICAL ADAPTATIONS OF PLANTS TO HYPOXIA

Plants differ widely in their capacity to adapt to oxygen deficiency (Colmer, 2003). Most plant species that are not adapted to waterlogging exhibit injury symptoms, wilting, leaf senescence and epinasty are likely to be the first. What follows is a rapid decline in or even its termination (Marshner, 1995). Adapted plants have developed various mechanisms. (1) When oxygen partial pressure limits the production of ATP by mitochondria, the anaerobic metabolism can contribute to cell survival in the short term by allowing ATP regeneration (Drew, 1997). (2) Seedlings and adult plants of many species have the ability to achieve rapid elongation growth of shoot organs when they are submerged (Sauter, 2000), so that plant contact with the atmosphere permits the exchange of gases via air channels (aerenchyma). (3) Plants can avoid hypoxic events by adjusting the life cycle, e.g. seed dispersal timing (Crawford and Braendle, 1996). (4) True tolerance to anoxia is found only in some specific cases in the plant kingdom, e.g. some seeds (rice) have the ability to germinate under anoxic conditions (Sauter, 2000).

Usually, the roots of plants are not suddenly exposed to anaerobic conditions. Under most circumstances, a gradual transition from normoxia to hypoxia to anoxia provides an opportunity for acclimation before conditions become lethal (Drew, 1997; Mustroph and Albrecht, 2003). There are three different mechanisms known for sensing hypoxia or anoxia: haemoglobin gene expression linked to increased activity of alcohol dehydrogenase (ADH) (Silva-Cardenas et al., 2003), changes in the concentration of cytosolic Ca^{2+} (Drew, 1997) and ethylene (Evans, 2003; Drew et al., 2000; Colmar, 2003; Armstrong and Drew, 2002; Voesenek et al., 2006). Sensing may occur if only a part of the root (e.g. the root apex) is exposed to hypoxic conditions. The local sensing causes a response along the whole root (Malik et al., 2003).

When roots cannot obtain enough oxygen for respiration, they react with fermentative processes where ethanol and lactic acid are formed (Sorrell, 1999). Accumulation of lactic acid causes cytoplasmic acidosis which inhibits lactic dehydrogenase (LDH). As a consequence, fermentation is switched to production of ethanol rather than to

lactate (Armstrong and Drew, 2002). The synthesis of ethylene by roots is strongly promoted by hypoxia, but blocked by anoxia (Drew, 1997). In hypoxic roots, the synthesis of ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) is stimulated. ACC is then transported to the shoot where its oxidative conversion to ethylene takes place. The formation of ethylene leads to epinastic leaves (Marschner, 1995), influences root growth and morphology, and triggers aerenchyma formation. After oxygen depletion, many old roots die; however, in many species, numerous new roots with well-developed aerenchyma can be formed closer to the soil surface or at the stem (Marchner, 1995). Roots of various types have different responses to low oxygen partial pressures. In wheat, an increase in the number of nodial roots was found in nonaerated hydroponic solution, but there was no change for seminal roots (Wiengweera et al. 1997). Trought and Drew (1980) reported on death in seminal wheat roots and stagnation in development of less susceptible nodial roots occurring under severe hypoxia.

3 AERENCHYMA

Aerenchyma in roots provide a low-resistance internal diffusion pathway for supplying oxygen to the root apex (Armstrong in Drew, 2002; Colmer 2003). Depending on the species and environmental conditions, aerenchyma are formed over virtually the entire body of a plant, with the exception of meristems, vascular bundles, sclerenchyma and covering tissues (Raven, 1996). Air channels of aerenchyma are usually formed in cortex of roots, rhizomes and stems. These are often termed lacunae (Armstrong, 1979). In roots, aerenchyma are formed behind the apical meristem (Marschner, 1995; Malik et al., 2003).

Aerenchyma occurs as one of two basic types named shizogenous and lysigenous. Lysigenous gas-spaces form via cell lysis, while schizogenous spaces form by means of cell separation during tissue development (see Evans, 2003). Lysigenous aerenchyma can develop in both mature as well as in newly-developing roots; older roots, however, lose the capability of their formation (Thomson et al., 1990). Besides improving aeration, lysigenous air spaces also reduce the number of oxygen consuming cells (Sauter, 2000).

In maize roots (*Zea mays*), aerenchyma are formed by the death of cells in the mid cortex in a zone behind the root apex. Lyzigeny subsequently spreads radially and longitudinally to form gas spaces separated by radial bridges of living cells linking the stele and epidermis (Gunawardena et al., 2001b). The cell walls and cell contents of the cells at the place of future channels are completely digested and leave no apparent trace (Gunawardena et al., 2001a). Also in maize roots, aerenchyma are specially initiated by ethylene, produced endogenously or applied (Gunawardena et al., 2001a; Bouranis et al., 2003). However, it can also be induced by nutrient starvation, which is not related to ethylene synthesis or accumulation in the root tips. In contrast, deficiencies of N or P (Drew et al., 2000) or S (Bouranis et al., 2003) depress ethylene production, but they strongly enhance the sensitivity of cortical cells to ethylene, resulting in a more rapid lysis of cells in the presence of very low oxygen concentration (Drew et al., 1989). It is interesting that omission of potassium under similar conditions did not induce aerenchyma (Drew et al., 2000).

In anoxia, the formation process of aerenchyma is interrupted (Evans, 2003), as ACC syntase activity is strongly depressed even if roots have previously been acclimated by hypoxia and have had high levels of initial enzyme activity (He et al., 1994). Consequently, the tissue remains intact until death by necrosis occurs (Evans, 2003).

Tolerance to hypoxia can be directly related to ventilation efficiency which is acquired through the development of air spaces within the tissue (Justin and Armstrong, 1987, Evans, 2003). Gas transport in the pathway may be simple diffusion or a consequence of pressure flow (see Raven, 1996). The oxygen can be supplied from photosynthesis or from the atmosphere (Evans, 2003). In common reed (*Phragmites australis*), the gas flow rate from the atmosphere can be enhanced by Venturi-convection driven by wind, which sucks the air into the belowground system via dead culms cut off above the ground level. Accordingly, high wind speed can considerably enhance aeration (Marshner, 1995).

Belowground oxygen transport not only enables the root mitochondrial respiration, but it also affects rhizospheric oxygen concentration. Oxygenation of the rhizosphere around the growing tip reduces the harmful effects of anoxic soils (such as phytotoxins and organic compounds, Fe^{2+}, Mn^{2+}) on roots while supplying the demand of soil organisms competing with the root tip for oxygen (Sauter, 2000; Armstrong and Drew, 2002; Evans, 2003). In many wetland species, aerenchymal diffusion pathways also remove gases (carbon dioxide, ethylene, methane) from hypoxic soil (Colmer, 2003).

While a certain amount of oxygen should be released from the roots to provide oxygenation of the rhizosphere at the very proximal parts of the root system; such radial oxygen loss should be minimized in other parts of the roots. The anatomical adaptations that help minimize O_2 loss to the surrounding soil include suberized hypodermis and a layer of lignified cells immediately interior to the hypodermis (Drew et al., 2000).

4 POROSITY OF ROOTS

Aerenchyma formation increases the porosity of roots, i.e. the volume of gas-filled spaces in relation to the total tissue volume, above the usual levels contributed by intercellular spaces (Colmer, 2003). Porosity can differ between root types (e.g. in wheat: 12% in seminal roots versus 15% in nodial roots; Thomson et al., 1990). Malik et al. (2003) also report increases porosity by the formation of aerenchyma along the entire root length. Purnobasuki and Suzuki (2004) report that root porosity rapidly increased between 0 and 10 mm from the root apex. Higher porosity is characteristic of plants adapted to growth in anaerobic conditions, since it enhances the internal movement of gases (Justin and Armstrong, 1987).

There are various approaches known to measure root porosity. One of the best known methods is that described by Raskin (1983). The basic idea is to measure the mass difference between intact and water infiltrated roots. With efficient vacuum infiltration, it can be presumed that water fills all gas spaces in the root tissue, and from the weight of this water, the volume of the root air spaces can be calculated.

Owing to difficulties inherent to the weighing of wet tissue in air – i. e. liquid adheres to the root surface – the weight can easily be overestimated. To solve this problem, Raskin (1983) introduced the method based on Archimedes' Principle, where the roots are weighed submerged. Vacuum infiltration is ensured by subjecting the submerged tissue to low pressures (cca 500 mm Hg, 670 hPa) for three periods of 40 s. The percentage of root porosity is calculated as a ratio between gas volume in the root and volume of the root (for modified calculations after Raskin (1983), see Thomson et al., 1990).

Another approach is measurement of aerenchyma in cross-sections taken at the appropriate distance (see below) behind the root tip. Conventional sample preparation techniques can be used to prepare samples for microscopy (see Gunawardena et al., 2001a; Gunawardena et al., 2001b). Analysis of aerenchyma channels could be made by the point counting method (Mustroph and Albrecht, 2003) and various image-analysis methods (Malik et al., 2003; Gunawardena et al., 2001b; Purnobasuki and Suzuki, 2004). In our study presented in Figures 1 and 2, we used image analysis to estimate the dimensions of aerenchyma in mofette grown maize (*Zea mays*). At the mofettes (natural CO_2 springs), a geogenic CO_2 can easily displace O_2 in the soil, causing transient or more stable hypoxic or even anoxic conditions around roots (see Pfanz et al., 2004).

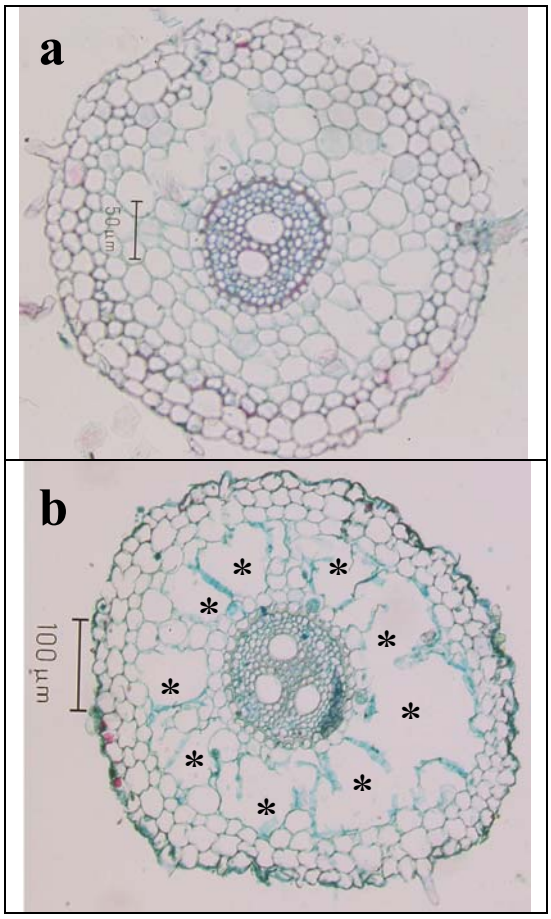

Figure 1: Aerenchyma in maize roots (*Zea mays* L.) growing in soil with varying geogenic CO_2 enrichment, natural CO_2 spring Stavešinci (NE Slovenia).
Maize roots were fixed in 70% ethanol. Specimens were dehydrated in a graded ethanol series. The dehydrated tissue was then taken through a graded mixtures of ethanol and xylol (3:1, 1:1, 1:3, 24

hours each step) and thereafter embedded in paraffin wax. Cross-sections (10 µm) were cut 10-40 mm behind the root tip. Slices were stained by safranin and astrablau. a = root without aerenchyma, b = root with aerenchyma; note intercellular channels in the root cortex indicated by asterisks.

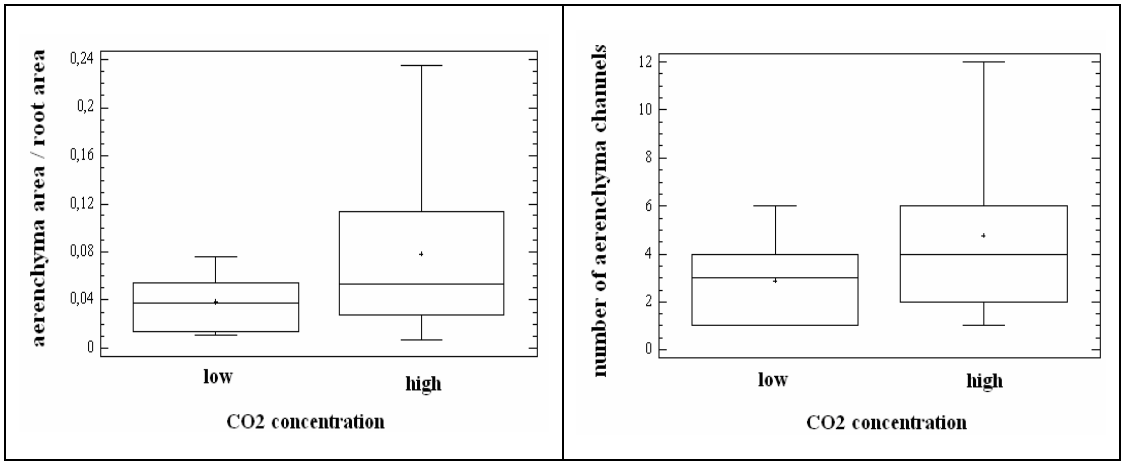

Figure 2: Aerenchyma in maize roots (*Zea mays* L.) growing in soil with varying geogenic CO_2 enrichment, at natural CO_2 spring Stavešinci (NE Slovenia). The aerenchyma in digitized images, similar to that presented in Figure 1, were measured using an image-analysis program Analysis (Soft Imaging System GmbH). Aerenchyma developed in all analysed roots independently of gas regime. In the late season, the tendency towards more extensive aerenchyma can be seen in high CO_2 grown plants compared to those grown under slightly enriched or normal CO_2 regime. Box-Whiskers plots present median, quartiles, range and extreme values. The central box represents the values from the lower to upper quartile (25 to 75 percentile). The middle line represents median. The horizontal line extends from the minimum to the maximum value, excluding the extreme values.

In estimating aerenchyma using the method described, we met certain difficulties. The major problem of precise estimation of aerenchyma cross-section surface was inherent to the quality of microscopic slices. In our case, their thickness was 10 µm. In some specimens, the problem was how to make precise resolution between the lumen of air channels and adjacent cortical cells. For this reason, only slices of sufficient quality were used for image analysis, and manual selection of measuring objects was used during analysis. It can therefore be recommended that less thick sections (ultra-thin, 1 µm) should be used. Another approach related to the problem described was used by Purnobasuki and Suzuki (2004). In their research, digital images of root cross-sections were adjusted to maximize contrast by converting the image to black and white. Aerenchymal spaces were then filled, resulting in a solid, black silhouette of the root selection, and the number of pixels was quantified. Aerenchyma area was determined by returning to the original black and white image, then inverting it to form a negative image, and pixels comprising the lacunae were quantified. The porosity was then estimated by dividing total area of intercellular spaces with total cross-section area. Since with such procedure the cell lumens are also included in the measured area of intercellulars one can presume that this area can be overestimated.

An additional and important issue in aerenchyma determination is at what distance from the root tip the samples for microscopy should be taken. When we focus only on maize, there are contradictory opinions about where to take slices. He et al. (1994)

reported that the first sign of aerenchyma formation is at 10 mm behind the tip, but Bouranis et al. (2003) found air channels 2 mm behind the root tip, where aerenchyma have developed in response to nutrient deprivation. Gunawardena et al. (2001a) sectioned 10 mm behind the tip. On the other hand, Malik et al. (2003) evaluated the extent of aerenchyma channels in the region 20 mm behind the apex. In our study, root cross-sections were taken 10-40 mm behind the root tip. The accuracy of estimations could have been improved if the morphology of root tissues had been studied at several distances from the apex.

With field sampled plant material, we sometimes encounter the problem of insufficient amounts of root material appropriate for slicing. This might not be a problem if roots are sampled from plants grown in hydroculture. Indeed, these techniques are most frequently used when basic mechanisms of plant response to hypoxia are studied (Thomson et al. 1990 or Malik et al. 2003). Nevertheless, studies on field-grown plants are still needed when ecologically relevant questions are addressed.

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