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Aerenchyma formation in maize roots

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Abstract

Maize (*Zea mays* L.) is generally considered to be a plant with aerenchyma formation inducible by environmental conditions. In our study, young maize plants, cultivated in various ways in order to minimise the stressing effect of hypoxia, flooding, mechanical impedance or nutrient starvation, were examined for the presence of aerenchyma in their primary roots. The area of aerenchyma in the root cortex was correlated with the root length. Although 12 different maize accessions were used, no plants without aerenchyma were acquired until an ethylene synthesis inhibitor was employed. Using an ACC-synthase inhibitor, it was confirmed that the aerenchyma formation is ethylene-regulated and dependent on irradiance. The presence of TUNEL-positive nuclei and ultrastructural changes in cortical cells suggest a connection between ethylene-dependent aerenchyma formation and programmed cell death. Position of cells with TUNEL-positive nuclei in relation to aerenchyma-channels was described.

Additional key words: *Zea mays*, hypoxia, ethylene, PCD, TUNEL reaction, TEM.

Introduction

Zea mays is one of the model organisms used to study the plant's response to flooding, especially lysigenous aerenchyma formation in the root cortex. Generally, aerenchyma facilitates gas exchange between flooded and non-flooded plant organs and due to its relatively small fraction of living cells decreases the metabolic requirements of the flooded organ. When compared with typical wetland plants, in which aerenchyma forms constitutively, *Zea mays* is classified as a plant with hypoxia-inducible aerenchyma formation, similar to *Spartina alterniflora*, wheat or rye (Beers 1997, Watkin *et al*. 1998, Drew *et al*. 2000, Maricle and Lee 2002). In maize, aerenchyma formation is inducible also by mechanical impedance (He *et al*. 1996) or nutrient starvation (Jackson and Armstrong 1999, Drew *et al*. 2000, Bouranis *et al*. 2003, Evans 2004, Bouranis *et al*. 2006).

Lysigenous aerenchyma formation is considered to be a case of programmed cell death (He *et al*. 1996, Buckner *et al*. 1998, Drew *et al*. 2000, Gunawardena *et al*. 2001a,b, Bouranis *et al*. 2003, 2006). The best-known type of programmed cell death (PCD) is probably apoptosis. Its regulation and molecular basis are well mapped, with its typical steps being precisely described (Cohen 1993, Danon *et al*. 2000, Becker *et al*. 2002). The methods often used for apoptosis detection are electron microscopy, annexin binding (revealing changes in plasmalemma), or DNA-electrophoresis and TUNEL reaction, both capable of detecting a specific DNA fragmentation. The TUNEL reaction is based on the binding of short chains of labelled nucleotides on free 3´-OH groups in the DNA breaks (Danon *et al*. 2000). In plants, each case of PCD more or less differs from apoptosis, usually bearing some of its markers. The most frequently mentioned apoptotic markers in plant PCD are chromatin condensation, specific DNA fragmentation and the presence of "apoptotic bodies" (membrane-bound vesicles containing organelles and cytoplasm). A tonoplast rupture and a role of "metacaspases" (plant homologues of apoptosis-involved proteases – caspases) and phytohormones in the PCD signalling pathway can be classified as a plant-cell specific event (Jones 2000, De Jong *et al*. 2000, Danon *et al*. 2000, Beers and McDowell 2001, Woltering 2004).

Ethylene synthesis is connected with almost all biotic and abiotic stress conditions. In flooded plants, it

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Abbreviations: ACC - 1-aminocyclopropane-1-carboxylic acid; AOA - aminooxy-acetic acid hydrochloride; PCD - programmed cell death.

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promotes adventitious root formation, rapid elongation of submerged semiaquatic plants, permitting them to keep a part of their foliage above water, or increased alcohol dehydrogenase synthesis necessary for efficient metabolism in hypoxia (Peng *et al*. 2001). It was ascertained that ethylene is required for aerenchyma formation in hypoxic roots of maize (He *et al*. 1996, Drew *et al*. 2000), whereas in anoxia aerenchyma does not form, probably because oxygen is necessary for the conversion of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) into ethylene by ACC oxidase (Jackson *et al*. 1985, Brailsford *et al*. 1993, Ecker 1995).

The aim of our study was to examine in detail the conditions under which aerenchyma forms in maize roots and whether it is genotype-dependent. We focused on the relationship of aerenchyma to root length, connection with PCD, and its dependence on endogenous ethylene synthesis.

Materials and methods

Plants and cultivation: In most experiments, two maize (*Zea mays* L.) accessions, CE 240 or Cefran, were used. To test the dependence of aerenchyma formation on genotype, ten more accessions were examined: Celio, CE 704, CE 1302, Celux 225, 2023, 2001, 2002, 2023, 2232, and 2023 × CE 704. All the caryopses were provided by *Oseva*, Bzenec, Czech Republic.

Different cultivation designs were employed: *1*) Caryopses of the accession CE 240 were imbibed and germinated for 3 d. Seedlings were placed in containers with aerated or non-aerated ¼-strength Hoagland solution in a cultivation room (16-h photoperiod, irradiance 500 μmol m^{-2} s^{-1}, day/night temperature 25/18 °C, relative humidity 60 %) for 5 d. The oxygen content of the solution was measured daily with the Clark-type oxygen electrode. *2*) Seedlings were grown in a greenhouse in plastic pots (5 plants per a 0.75-dm^3 pot) in drained or water-flooded *Vermiculite* or sand for 7 d. In this case, the temperatures oscillated between 21 and 28 °C (during the day) and was approx. 20 °C (at night), irradiance was natural with *ca*. 15-h photoperiod. *3*) Caryopses of the accession Cefran (the accession with the most regular inner organisation of the cortex) were cultivated in water or in ¼-strength Hoagland solution in a "wrapped in paper" for 7 d. The maize caryopses were fixed with an adhesive tape between two sheets of filter paper that lined the vessel, were attached to the inner side of the vessel and formed kind of tunnels around growing roots. Once the filter paper stretched to the bottom of the container and was covered to a depth of 3 cm with the cultivation solution, the solution rose through the capillaries to the caryopses and seedlings. For inhibition of ethylene synthesis, various concentrations of an ethylene synthesis inhibitor, aminooxyacetic acid hemihydrochloride (AOA, 200 or 900 μM, provided by *MP Biomedicals*, *MGP* Zlín, Czech Republic), were used in water or ¼-strength Hoagland solution during the whole cultivation (Peng *et al*. 2001). To observe the effect of the dark, the cultivation vessel was covered with a large ventilated lightproof container or left uncovered.

Anatomical observations: Roots were harvested and then stored in 70 % ethanol. 150 - 200 μm thick free-hand transverse sections were cut from the root base, from the "¾ zone" (¼ of the root length from the base, ¾ from the tip), from the "1/2 zone" or from areas of known age, which was estimated by Indian ink marking, according to (Gunawardena *et al*. 2001a). The sections were observed with an *Olympus BX 51* (Tokyo, Japan) microscope and photographed with an *Apogee alta U4000* (*Apogee Instruments*, Roseville, CA, USA) camera. The area of aerenchyma was quantified with the image analyser *Lucia G* (*LIM*, Prague, Czech Republic) and expressed as "aerenchyma fraction", which is the area of lysigenous aerenchymatous channels *versus* the area of cortex, not including endodermis and hypodermis.

TUNEL reaction: The detection of DNA fragmentation *via* TUNEL reaction was modified from Jones *et al*. (2001). 2-d-old root segments, about 1 cm long, were taken from the same roots that had been used for anatomical studies. They were fixed for 24 h in 4 % paraformaldehyde in a vacuum at room temperature and cut with a hand microtome. The sections were subsequently permeated with 1 % Triton in 0.1 % sodium citrate for 3 h and treated with *Proteinase K* for 15 min. (20 cm^3 dm^{-3}, *Sigma-Aldrich*, St. Louis, MO, USA). The positive control was obtained after 15 min. of DNase I (10 mg cm^{-3}, *Sigma-Aldrich*) action. Then the reaction mixture of TUNEL enzymes and nucleotides 1:9 was applied for 1.5 h. The negative control was run concurrently and conducted without enzymes. The reaction was visualised with alkaline phosphatase (AP) conjugated anti-fluorescein antibodies (applied for 30 min., *TUNEL AP* kit, *Roche Diagnostic*, Manheim, Germany), followed by a 20-min. treatment with 5-bromo-4-chloro-3-indolyl phosphate and *p*-nitrobluetetrazolium chloride (*MP Biomedicals*). The activity of non-specific phosphatases was inhibited by adding 50 mM levamisole hydrochloride (*MP Bio-medicals*) to this mixture. The number of TUNEL-positive nuclei was counted on photographs of cross-sections while the cells with TUNEL-positive nuclei were digitally marked (with colour points). Their distribution in the middle cortex (the area between endodermis and hypodermis) was described by their number in each concentric cell tier. The first tier was the one neighbouring the endodermis.

TEM: The samples of root tissue with young aerenchyma were taken from plants cultivated in hydropony. The tissue was assessed according to length and age. The roots were fixed in 4 % glutaraldehyde, post-fixed in 1 % OsO_4 and, after gradual dehydration in ethanol series and gradual resin infiltration, embedded in Spurr resin (*Sigma-Aldrich*), and polymerised overnight at 60 °C. This five-day procedure was adjusted according to (Lux and Hudák 1987) and Lux, personal communication. Ultrathin sections were stained with lead citrate and uranyl acetate and examined with a *Philips 268 Morgagni* (Eidhoven, The Netherlands) transmission electron microscope equipped with a *Megaview 2* camera (*Olympus*) and *analySIS*® software.

Statistics: At least 5 roots were included in each experiment and at least 5 sections from the area of interest were taken from each root. The experiments were repeated at least twice. Some data have been transformed by square root to obtain normal distribution with equal variances. The statistical package *NCSS 2000* (© Jerry Hintze, Kaysville, USA) was used for the statistical evaluation.

Results

Aerenchyma formation: In the hydroponic culture, the oxygen content in the aerated (+O) and non-aerated (-O) solutions differed significantly. In +O treatment, it oscillated between 82.9 and 91.6 % of O_2 saturation, whereas in the -O treatment it decreased to 13.6 % on the fourth day of cultivation and started to increase again on the fifth day (to 20.25 %), data not shown. The aerenchyma appeared in 2-d-old root tissues in the hypoxic treatment (-O) but not in the +O treatment of the same age. It was, however, found in 3- and more-d-old root parts in +O, despite the fact that its proportion was significantly lower in comparison with -O root segments of the same age. Even though it was revealed that both the kind of treatment and its duration had influence on aerenchyma formation ($P < 0.0001$), the areas of aerenchyma in the -O treatment were actually almost the same as with a one day delay in the aerated one (Fig. 1). The effect of flooding on the aerenchyma formation in the roots of the same cultivar grown in *Vermiculite* or in sand was similar. Aerenchyma was again found in both treatments, flooded (hypoxic) and drained (control), although the differences between control and hypoxia-treated plants were higher (data not shown). The role of genotype was tested under the same conditions using 11 other accessions. The presence of aerenchyma was then semiquantitatively evaluated based on photographs of cross sections. Aerenchyma formed in all the accessions even though its amounts differed slightly among them, higher being in Celux225, 2232, CE1302, Cefran, Celio, 2002 and 2023 × CE704 (25 % and more formed by aerenchyma). There was a correlation between the area of aerenchyma and root length in all of these accessions (data not shown) and a significant regression was revealed in the "wrapped-in-paper" cultivation, where aerenchyma also formed (Fig. 2, Spearman's coefficient = 0.67, $\alpha = 0.05$, $P < 0.005$).

The role of ethylene: The effect of AOA, the ethylene synthesis inhibitor, was tested in the "wrapped-in-paper" cultivation. As control treatments, cultivations in water and in Hoagland solution were used. As there was no difference in the aerenchyma fraction between these two treatments, only the results from the water cultivations are presented. The addition of AOA reduced the aerenchyma formation, the effect being dependent on irradiance. When cultivated in a 16-h photoperiod, the 200 μM AOA reduced the aerenchyma fraction to 6.4 % of the control (Table 1). In darkness, the decrease was smaller, only 41.7 % of the dark control and there was no significant difference as against the control light treatment. Aerenchyma formation in darkness was totally suppressed by 900 μM AOA. The growth of roots and shoots was influenced by the application of AOA and by irradiance too ($P < 0.00$).

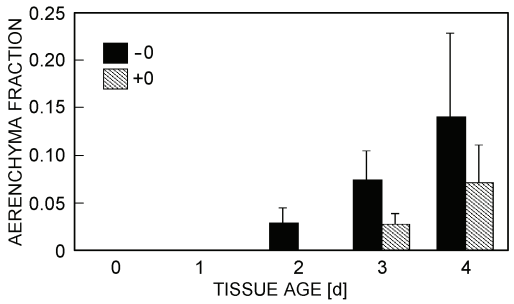

Fig. 1. Aerenchyma formation in primary roots of maize plants in non-aerated (-O) and aerated (+O) hydropony. Mean ± SD, $n = 5$. Aerenchyma fraction is the area of lysigenous aerenchymatous channels *versus* the area of cortex, not including endodermis and hypodermis.

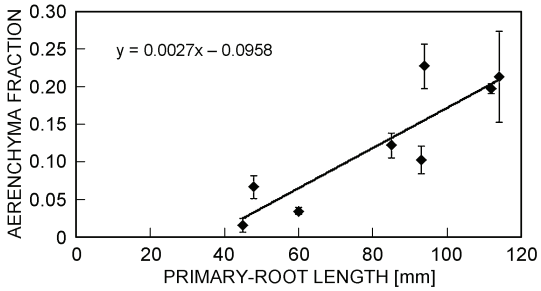

Fig. 2. Correlation of the aerenchyma fraction with the root length. Roots of 7-d-old plants grown in the "wrapped-in-paper". Means of aerenchyma fraction at ¾ of the root length ± SD, $n = 5$, $r^2 = 0.79$.

Table 1. The effect of various concentrations of AOA, darkness and light on the length of root and shoot [mm] and the aerenchyma fraction (at ¾ of the root length) in the "wrapped-in-paper" cultivation. Mean values ± SD, n = 9 - 11 roots.

AOA [μM]		Root length	Shoot length	Aerenchyma fraction
0	light	112.88±39.61	55.00± 7.56	0.13±0.09
0	dark	252.22±43.53	-	0.20±0.06
200	light	25.40±13.95	43.64±11.31	0.01±0.02
200	dark	65.44±28.29	85.78±26.19	0.08±0.05
900	dark	70.60±19.15	41.00±12.73	0.00±0.00

In plants grown at irradiance of 500 μmol m^{-2} s^{-1}, the 200 μM AOA reduced the root length to 22.5 %, but the shoot length only to 79.3 % while in darkness the root length was reduced to 57.8 % and the shoot length increased to 119.6 % . The 900 μM AOA decreased the root length to 62.5 % and the shoot length to 74.5 % .

TUNEL reaction: To determine whether aerenchyma formation is connected with PCD-related nuclear changes, the TUNEL reaction was performed. TUNEL positive nuclei were found at 0 or 200 μM AOA, but their number was affected by AOA treatment ($P < 0.0001$) and by the concentric cell tier position ($P < 0.05$), but not by their interaction. The control roots differed significantly from the AOA-treated roots in tiers 3, 4, 5 and 6 (Fig. 3). The distribution of TUNEL-positive nuclei reveals apparent radial clusters (Fig. 4*B*).

TEM of cortical cells: The most interesting ultrastructural changes in cortical cells were found in the 2-d-old parts (about 1/3 of the root length from the tip) of roots from the aerated treatment and in the 1-d-old parts (about ¼ of the root length from the tip) of roots from the non-aerated one. The first visible changes include plasmalemma and cell-content shrinkage, probably preceded by tonoplast disruption. However, it is hard to claim this unambiguously, as the tonoplast was not clearly visible either in collapsing or in intact cells of this root region (Fig. 5*G*). The nucleus and other organelles degraded gradually in these "vesicles" surrounded by plasma membrane (Fig. 5*A,B,C*). Inside these vesicles, smaller ones were sometimes found, often covered with ribosome-like granules, indicating that their origin might be endoplasmic reticulum (Fig. 5*D,F*). This seems to be supported by their occasional presence near plasmodesmata (Fig. 5*E*). This process resulted in the cell-wall collapse and partial degradation (Fig. 5*H*). Fig. 5*G* shows intact cells from the same region.

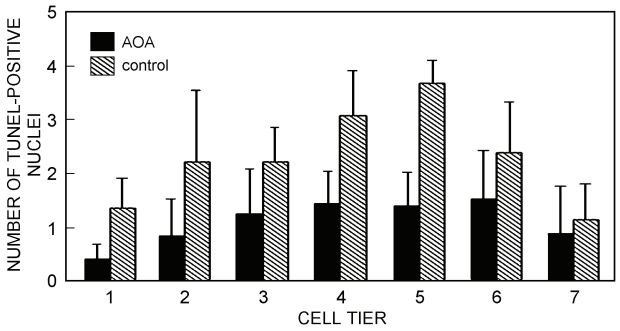

Fig. 3. Differences between the numbers of TUNEL-positive nuclei in roots of plants cultivated in Hoagland solution (control) and in Hoagland solution with 200 μM AOA in the "wrapped-in-paper" cultivation. Mean ± SD, n = 5. The distribution of these nuclei in the middle cortex was described by their number in each concentric cell tier. The first tier was the one neighbouring the endodermis.

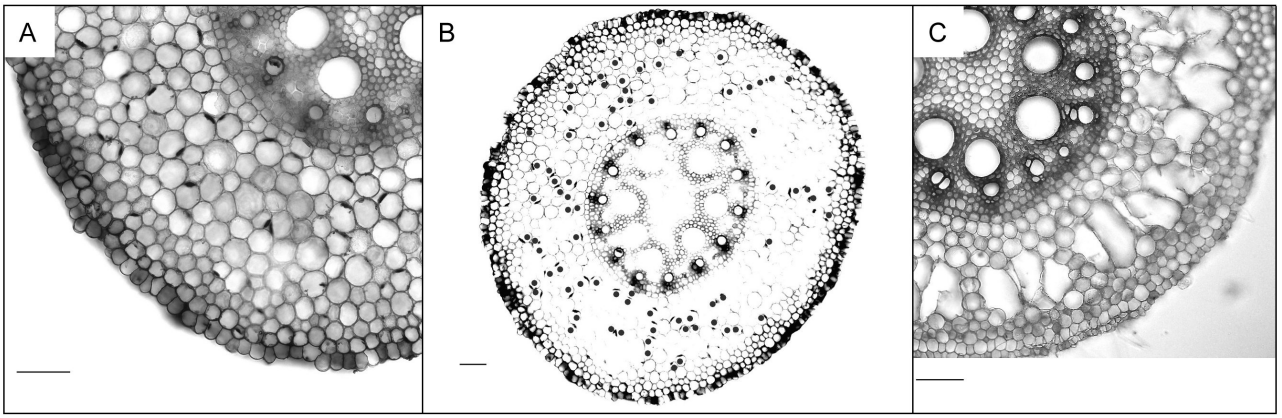

Fig. 4. TUNEL reaction and aerenchyma formation in roots of maize cultivated for 7 d in the "wrapped-in-paper" cultivation, photographs from 2-d-old root sections. Cells with TUNEL-positive nuclei (*A*) were marked with points (*B*), and their number in every layer was counted. The distribution of these cells reveals radial clusters, their localisation corresponds with the localisation of aerenchyma-channels in older root-segments (*C*). *Bar* = 100 μm.

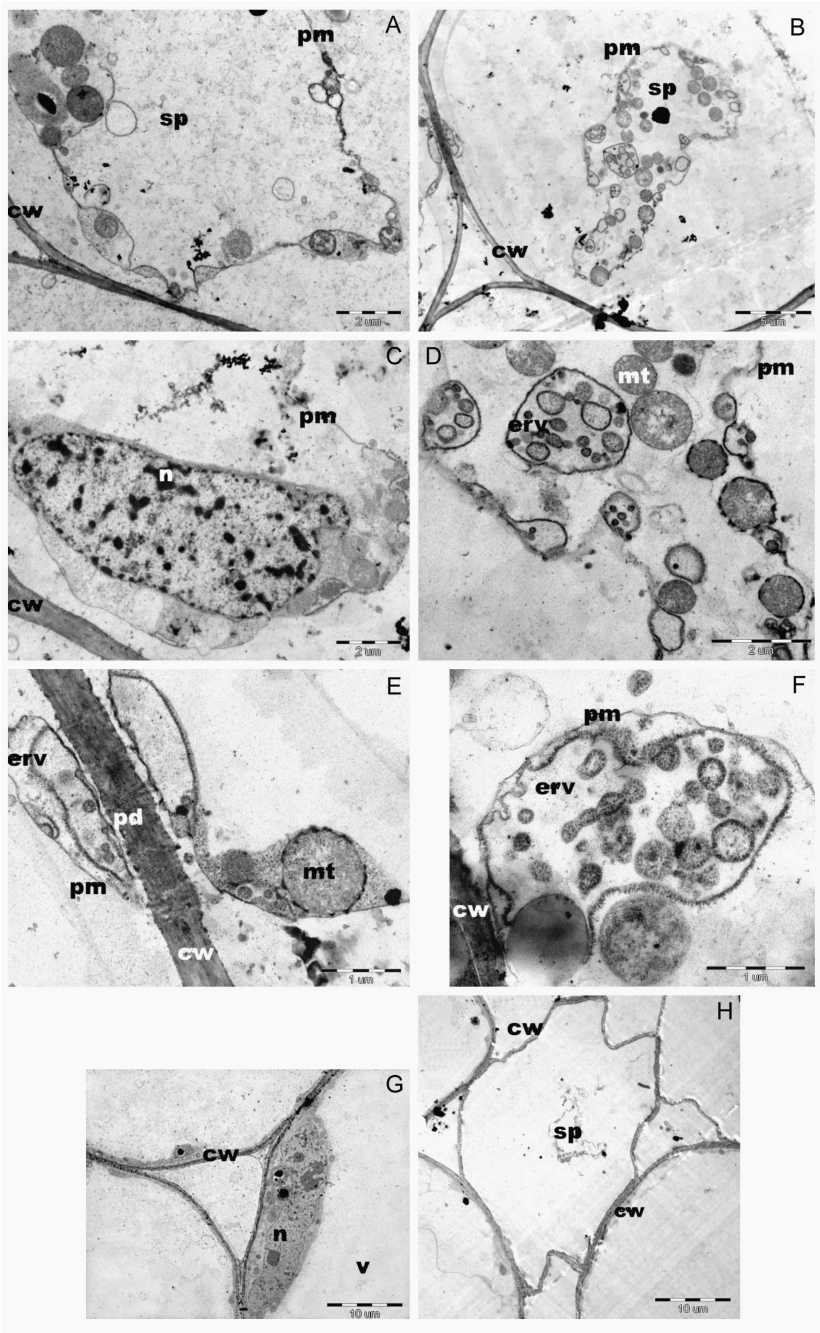

Fig. 5. TEM pictures of ultrastructural changes in cortical cells before the breakdown of cell walls (*A* - *F*), an intact cell (*G*) and the cell-wall breakdown (*H*). 2-d-old region of roots of 8-d-old plants cultivated in aerated hydropony (cw - cell wall, erv - endoplasmic reticulum-derived transport vesicle, mt - mitochondria, pd - plasmodesmata, pm - plasma membrane, n - nucleus, sp - shrunken protoplast, v - vacuole;. *bars*: *A*, *C*, *D* = 2 µm; *B* = 5 µm; *E*, *F* = 1 µm, *G*, *H* = 10 µm).

Discussion

Zea mays ssp. mays is generally considered to be a plant with inducible aerenchyma formation (Drew *et al.* 2000, Gunawardena *et al.* 2001a,b). Nevertheless, we have obtained plants with aerenchymatous roots from aerated hydroponic cultivations with a high oxygen level, with its formation being delayed only by one day in comparison with the non-aerated treatment. Some authors admit the presence of aerenchyma even in these conditions, generally considered as not aerenchyma-inducing, delayed in comparison with a hypoxic treatment, in both seminal or adventitious roots (He *et al.* 1996, Bouranis *et al.* 2003, Enstone and Peterson 2005). This is also in

accord with our earlier results (Votrubová *et al*. 2009).

As the genotype can play an important role in plant behaviour under stress conditions, in our study 12 different maize accessions were grown in damp substrate, and in all of them aerenchyma was found to some degree. Mano *et al*. (2006) discovered that aerenchyma forms in two of fifteen maize accessions cultivated under non-flooded conditions.

Not only hypoxia, but also mechanical impedance of the substrate can induce aerenchyma formation in maize seminal roots (He *et al*. 1996). To avoid these conditions potentially triggering aerenchyma formation, we applied a special ("wrapped-in-paper") cultivation. Nevertheless, aerenchyma was present in all the plants.

In all the maize accessions observed in our studies, there was a clear correlation between root length and the aerenchyma fraction – the longer the root, the greater the aerenchyma fraction in it. Similar correlations are described in other species too. Justin and Armstrong (1987) claim that the rooting depth depends on porosity. In rice and maize, the aerenchyma fractions are related more to the root length than to its age (Justin and Armstrong 1991). The porosity is probably the factor that determines root-length in flooded substrate. Aguilar *et al*. (1999) postulate that an increase in porosity should anable roots to extend their length in situations of low oxygen supply. On the other hand, it was clear that roots of plants cultivated under more stressing conditions (aerated *vs*. non-aerated hydropony, drained *vs*. flooded substrate, comparison not shown) were shorter and with more aerenchymatous tissue than those that were not stressed, which corroborates other results regarding various plant species including maize, rice, *Juncus effusus, Caltha palustris, Carex palustris, Ranunculus sceleratus, Rumex palustris* (Justin and Armstrong 1991, Brailsford *et al*. 1993, Visser *et al*. 2000, Enstone and Peterson 2005, Bouranis *et al*. 2006). It can be seen as a consequence of slower root growth, which is connected with the tissue differentiation localised nearer the root apex (Rost 1994).

In roots of plants of the same treatment, aerenchyma formation was dependent on root length, while the tissue age was not a reliable enough indicator; the roots of the same age varied highly in length. Thus, for further anatomical studies we recommend the distance from the root tip to be the main determinant. The amount of aerenchyma was higher in the middle of the root than at the root base, which corresponds with findings of other authors, not only in maize roots (Bouranis *et al*. 2006), but also *e.g.* in banana roots (Aguilar *et al*. 1999).

The lysigenous aerenchyma formation is known to be connected with ethylene (Justin and Armstrong 1991, He *et al*. 1996, Drew *et al*. 2000, Gunawardena *et al*. 2001a, Colmer *et al*. 2006). Exogenous ethylene can induce or increase aerenchyma formation in plants where it does not occur normally (*e.g*. maize; Gunawardena *et al*. 2001a) or is less developed (*e.g*. rice; Colmer *et al*. 2006), which is an effect similar to decreased oxygen concentration. Likewise, when endogenous ethylene synthesis is blocked, aerenchyma formation is decreased or inhibited, *e.g.* in rice (Justin and Armstrong 1991).

Using AOA, an inhibitor of ACC-synthase, which is a key enzyme of the ethylene synthesis pathway (Peng *et al*. 2001), we confirmed the above-mentioned dependence of the aerenchyma formation on ethylene synthesis, even in conditions previously not considered to be aerenchyma-inducing. The 200 μM AOA has decreased the root growth and reduced the aerenchyma fraction almost to zero values. The decreased length of the roots, connected with the absence of aerenchyma, seems akin to the above-described correlation of the root length and aerenchyma fraction. Similar results with reduced aerenchyma formation were obtained in rice when $AgNO_3$, an ethylene antagonist, was used (Justin and Armstrong 1991).

Diurnal fluctuations of ethylene production were detected in many plants even though the results often differed for different plants. While some authors state that darkness reduces ethylene production, *e.g.* in *Impatiens balsamina* (Michalczuk and Rudnicki 1993), *Chenopodium rubrum* (Macháčková *et al.* 1997) or *Phaseolus sp.* (Van Gronsveld *et al*. 1988), others claim the opposite (Finlayson *et al*. 1998, 2004 in *Sorghum bicolor*). In our study, the aerenchyma formed more excessively in etiolated 200 μM AOA-treated maize plants, which grew more rapidly than similarly treated plants from light cultivations. In darkness, the AOA was fully effective (total inhibition of aerenchyma formation) only when it was used in 900 μM concentration. It is not clear whether the difference between the AOA effect in light and darkness is caused by different ethylene-synthesis rates, as is the case in *Sorghum bicolor* (Finlayson *et al*. 2004), or by darkness-increased sensitivity to ethylene. In maize the later might be possible, judging by N and P deficiency-caused increase in ethylene-sensitivity (Drew *et al*. 1989). The effect of ethylene on organ growth is not uniform, plants adapted to flooding are described as a general exception where ethylene causes enhanced vegetative growth, as in deep-water rice (Metraux and Kende 1983) or *Rumex sp.* (Voesenek and Blom 1989).

In our study, the positive influence of darkness on the effects of ethylene-activity and plant growth was confirmed. Nevertheless, it is not possible to state whether the connection between the ethylene activity and plant growth is dependence or merely a correlation given by the typical characteristics of etiolated plants.

Lysigenous aerenchyma formation is considered to be a case of a programmed cell death (PCD), and DNA fragmentation detectable with the TUNEL reaction is one of the commonly used markers of this event (Gunawardena *et al*. 2001a, 2004). Since ethylene plays a role in aerenchyma formation (as shown above), we tried to connect these two facts using the AOA. We assumed that the aerenchyma formation was associated with the occurrence of TUNEL positive nuclei. The action of AOA was perceptible on the TUNEL reaction as well (Fig. 3); the number of TUNEL-positive nuclei was much

higher in the AOA-untreated roots, where the aerenchyma fraction was higher too (*cf.* Fig. 3 and Table 1). The TUNEL-positive nuclei were found mainly in the cell tiers 3 - 6, *i.e.* in the middle part of the cortex, which corresponds with the localisation of maize aerenchyma-channels (Drew *et al*. 2000, Bouranis *et al*. 2003). It is not possible to state that the cell death starts preferentially in one of these tiers, unlike in rice, which has a more regularly organised inner structure of the cortex (Kawai *et al*. 1998). Nonetheless, it was obvious that the cells with TUNEL-positive nuclei formed a pattern with more or less radial clusters.

Although we are aware of the risk of the data from TUNEL reaction being misinterpreted (microscopy artefacts, possible DNA fragmentation even in necrotic cells (Dong *et al*. 1997), our results suggest that the role of ethylene is connected with aerenchyma formation and also with a specific DNA fragmentation in cortical cells. This corresponds with the data provided by (Gunawardena *et al*. 2001a), who found out that also the exogenously applied ethylene induces aerenchyma formation in maize roots, including the increased TUNEL positivity in the cortex of primary roots. Nevertheless, these authors did not mention any pattern of these nuclei similar to the later distribution of lysigenous aerenchyma. Concerning endogenous ethylene, He *et al*. (1996) measured ethylene production and the activity of enzymes necessary for its synthesis in seminal roots of maize plants subjected to hypoxia and found a connection between them and aerenchyma formation, accompanied by activity of cellulases, *i.e.* enzymes involved in the degradation of cell walls. The aerenchyma formation thus seems to be dependent on ethylene, exogenous or endogenous, and shows characteristics of PCD.

Plant PCD is also characterised by ultrastructural changes observable by electron microscopy (Campbell and Drew 1983, Beers 1997, Schussler and Longstreth 2000, Gunawardena *et al*. 2001a, 2004, Gupta 2007). Our findings correlate with the results of (Campbell and Drew 1983), who described the protoplast condensation and degradation of organelles following the tonoplast rupture in maize cortical cells. Even though Gunawardena *et al*. (2001a) claim that in maize the cortical cells form apoptotic bodies originating from the vacuole, surrounded by tonoplast, we tend to think that these objects are (at least in our case) formed by plasmalemma-surrounded cell contents undergoing degradation caused by vacuolar-lytic-enzyme release and activation. A very similar process is described by Gunawardena *et al*. (2004) in *Aponogeton madagascariensis*, the "lace plant", during its leaf perforation development. In this study, the authors admit, together with the TEM pictures and positive TUNEL reaction, unsatisfactory results regarding the DNA apoptotic ladders, the same as those we obtained (Lenochová *et al*. 2004).

We conclude that, at least in the 12 maize accessions we studied, aerenchyma in primary roots forms under normoxic and non-stressing conditions although to a lesser extent than in hypoxia, and this event is dependent on ethylene synthesis. Darkness applied during the cultivation seems to facilitate the ethylene synthesis or the plants' sensitivity to this phytohormone. We can state that the aerenchyma formation under the described conditions exhibits features of programmed cell death, as shown by the TUNEL reaction and TEM, is correlated with the root length, and is distributed along the root axis similarly to other plant species with aerenchymatous roots.

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