PLANT SCIENCE

Aerenchyma formation in seminal roots of *Hordeum vulgare* in hydroponic conditions

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Abstract

Hypoxia is a severe stress factor which negatively affects plant growth. One of the adaptations to hypoxia is formation of aerenchyma. Lysigenous aerenchyma was found in many species, including barley (*Hordeum vulgare* L.). At what moment of root development and in which part of it the lysigenous cavities are formed is not completely clear. For this, barley plants were grown hydroponically with and without aeration for 30 and 40 days, respectively. Every 5 days seminal roots from barley plants were detached from the seedlings, and were divided into 4 equal parts characterizing the age gradient of cells from the apical (1) to its base (4) part. Under hypoxic conditions, aerenchyma in barley roots was formed in two stages — to the 10th day of growth (1st stage) and to the 30th day of growth. Lysigenous cavities were mostly formed in the parts with the most mature cells (in the 3rd and 4th parts). Accumulation of H_2O_2 in basal part of roots could be considered as a trigger for the aerenchyma formation at the first stage, but not at the second one. Aerenchymal lacunae were absent in seminal roots of aerated plants.

Keywords: seminal roots, primary cortex, aerenchyma, lacunae, *Hordeum vulgare*.

Introduction

During growth and development, plants are affected by various environmental factors including negative ones. One of these factors is waterlogging. It causes crop yield losses more than 50 % (de San Celedonio, Abeledo, and Miralles, 2014; Liu et al., 2020). Waterlogging leads to hypoxia in plant roots, and one of the mechanisms to survive under hypoxia is the formation of aerenchyma in roots and shoots (Takahashi, Yamauchi, Colmer, and Nakazono, 2014). Usually in aquatic species lysigenous aerenchyma is formed under both hypoxia and normoxia (Takahashi, Yamauchi, Colmer, and Nakazono, 2014; Yamauchi and Nakazono, 2021; Yamauchi, Noshita, and Tsutsumi, 2021), and such aerenchyma type is called constitutive (Seago et al., 2005; Takahashi, Yamauchi, Colmer, and Nakazono, 2014). In many other plants, which normally do not grow under oxygen deficiency, lysigenous aerenchyma can also be formed in response to hypoxia and osmotic stress (Basu et al., 2021; Yamauchi and Nakazono, 2021). That aerenchyma type is an induced aerenchyma (Takahashi, Yamauchi, Colmer, and Nakazono, 2014). Lysigenous aerenchyma is also found in some species that are exposed to drought and lack of nutrients (Hu, Henry, Brown, and Lynch, 2014; Oyiga et al., 2020). One of the processes involved in the formation of lysigenous aerenchyma is a programmed cell death (PCD) (Drew, He, and Morgan, 2000; Takahashi, Yamauchi, Colmer, and Nakazono, 2014).

The formation of lysigenous aerenchyma is a highly regulated process and includes several common stages. According to the Evans's hypothesis (Evans, 2004), the initiating stage is characterized by ethylene synthesis and its accumulation in roots. This stage is critical, as if ethylene synthesis is suppressed,

Citation: Malygin, M. and Kiseleva, I. 2024. Aerenchyma formation in seminal roots of *Hordeum vulgare* in hydroponic conditions. Bio. Comm. 69(3): 136–148. https://doi.org/10.21638/spbu03.2024.302

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Manuscript Editor: Evgeny Abakumov, Department of Applied Ecology, Faculty of Biology, Saint Petersburg State University, Saint Petersburg, Russia

Received: December 27, 2023;

Revised: February 19, 2024;

Accepted: March 26, 2024.

Funding: The research was supported by Russian Science Foundation, project No. 22-24-00817, https://rscf.ru/project/22-24-00817/.

Ethics statement: This paper does not contain any studies involving human participants or animals performed by any of the authors.

Competing interests: The authors have declared that no competing interests exist.

lysigenous aerenchyma does not appear under hypoxia (Rajhi et al., 2011). Development of aerenchyma is also controlled by auxin (Nguyen et al., 2018; Yamauchi et al., 2020), reactive nitrogen species (RNS) (Wany, Kumari, and Gupta, 2017; Wany and Gupta, 2018; Basu et al., 2021) and reactive oxygen species (ROS) (Steffens, Steffen-Heins, and Sauter, 2013; Xu et al., 2013; Yamauchi et al., 2014; Wany and Gupta, 2018; Tong et al., 2021). Followed by hormone signal perception in roots, concentration of ROS and RNS in root cortical cells rises and activates processes, which are responsible for cell death (Drew, He, and Morgan, 2000; Takahashi, Yamauchi, Colmer, and Nakazono, 2014), resulting in the appearance of lacunae. Further development of lacunae lead to a formation of aerenchyma (Takahashi, Yamauchi, Colmer, and Nakazono, 2014; Yamauchi, Abe, Tsutsumi, and Nakazono, 2019). Thus, the hormonal regulation of aerenchyma formation is well studied. Other aspects of aerenchyma formation, e. g. ontogenetic changes in different root parts during prolonged hypoxia, are less studied.

It is known that the main and undisputable function of aerenchyma is to provide the normal respiration in tissues. Oxygen is supposed to be transported through aerenchyma lacunae from plant organs that are not exposed to hypoxia (Takahashi, Yamauchi, Colmer, and Nakazono, 2014). It is assumed that oxygen translocation through the lacunae ensures the functioning of the root apical meristem (Nishiuchi et al., 2012; Yamauchi, Abe, Tsutsumi, and Nakazono, 2019) and the absorption of nutrients (Herzog, Striker, Colmer, and Pedersen, 2016). Nevertheless, previously published data indicated that during aerenchyma formation radial and xylem transport in roots was limited (Hu, Henry, Brown, and Lynch, 2014). This mechanism could be compensated by adventitious and lateral root formation to increase the uptake and transport of nutrients (York, Nord, and Lynch, 2013; Saengwilai et al., 2014).

Lysigenous aerenchyma was found in many plants, including cultivated species, for example, *Hordeum vulgare* L. (Tong et al., 2021). This species is a widely used laboratory plant and an important crop culture. It is highly sensitive to unfavourable environmental conditions, for example, hypoxia by waterlogging (de San Celedonio, Abeledo, and Miralles, 2014; Liu et al., 2020). Most of the previous research on aerenchyma formation in barley was focused on mature plants and adventitious roots (De Castro, Hill, Stasolla, and Badea, 2022), while studies of aerenchyma formation in seminal roots of barley seedlings under hypoxia are limited (Colmer and Greenway, 2011). The development of aerenchyma during long-term growth under oxygen deficiency remains poorly understood. The present work aimed to study the development of aerenchyma in the cortex of seminal roots of barley (*Hordeum vulgare* L.) at different stages of their growth under hypoxic conditions.

Materials and methods

Growing conditions

Barley plants (*Hordeum vulgare* L, cv. Pamyati Chepeleva), were grown in hydroponic cultivators. Barley seeds were randomly selected for an experiment and were carefully placed on plastic plates 20 × 20 cm with perforation in tap water for 2 days until germination (20–23 °C, day light). After the germination, 35 plants were displaced at a distance of 2 cm from each other in each of 10 plates and transferred in 1 L cultivators without aeration (hypoxic plants without aeration) for further growth up to 40 days (21–23 °C, photoperiod 16 h (light)/8 h (dark), PAR flux 200 μM/m^2 × s). Control plants were transferred with the same procedure at the same growing conditions in 3 L cultivators with aeration for 30 days to provide normal growth of aerated plant roots (control aerated plants). During the first 10 days after germination, when seedlings used organic compounds and ions reserved in kernel, the cultivator was filled with tap water, which was then replaced with Hoagland's nutrient medium for further growing (Schneider et al., 2017). Water or nutrient solution were renewed every two 2 days for hypoxic and every 5 days for aerated plants.

Winckler test was used to confirm the formation of hypoxic condition during plant growth. Fresh tap water or Hoagland medium contained 6.4–7.5 mg/l dissolved O_2. After filling the cultivators with the solutions, the concentration of dissolved oxygen decreased to 3.5–4.5 mg/l up to 8 h of growth and the concentration remained no higher than 4.5 mg/l until the renewing of solutions. The O_2 content of 4.5 mg/l or less is considered as hypoxic concentration (Ruperti et al., 2019; Ma et al., 2022). The solution of control plants contained from 8.86 mg/l to 10.36 mg/l of oxygen, which confirmed normal oxygen conditions for aerated plants.

Anatomy and morphology studies

Seven randomly selected hypoxic plants were harvested every 5 days after germination, while aerated plants were taken at 5, 15 and 30 days after germination in 5 individuals for each date. The selected plants were scanned (resolution 600 dpi) and the resulting images were used to measure the morphological characteristics of root system (the number and the length of seminal roots and the number of adventitious ones) in the ImageJ program (US National Institutes of Health, Bethesda, Maryland,

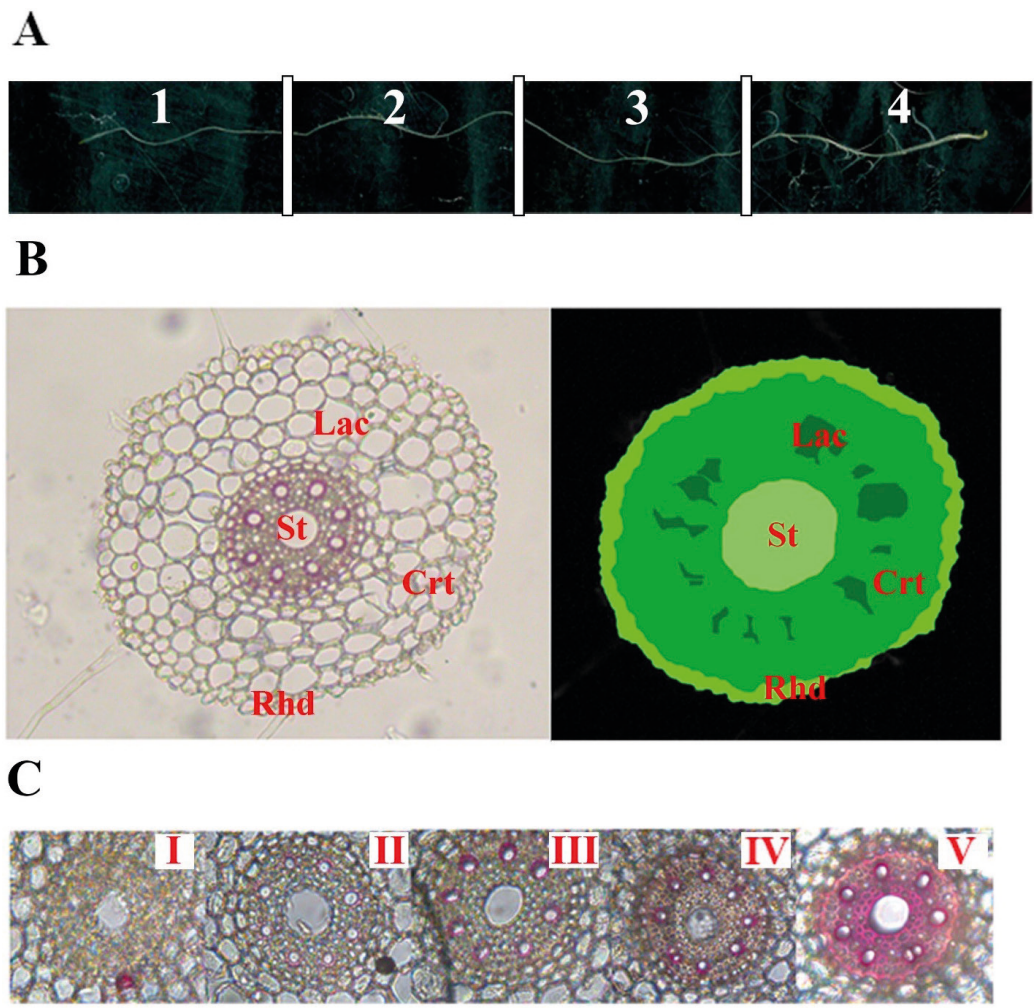

Fig. 1. A — The scheme of root division into parts (1–4: from the apex to the base); B — root tissues and parts (Rhd — rhizodermis, Crt — cortex, Lac — lacunae, St — stele) — photo by the optical microscope (left) and after processing in Adobe Photoshop (right); C — the scale for lignification assessment (1–5: from absence to high staining intensity).

USA). After scanning, plants were fixed in 2.5 % glutaraldehyde in phosphate buffer (pH 7.0) for further study of anatomy and morphology.

Tree seminal roots of each plant, both aerated and hypoxic, were detached for studying the anatomical characteristics. Roots were selected according to the average length. Each root was divided into 4 equal parts: parts 1, 2, 3, 4 (numbered from apex to the base; Fig. 1A), which corresponds to cells from the youngest less differentiated in the 1st part to the oldest most differentiated in the 4th part. In the middle of each part, cross-sections were done by razor blade and stained with Wizner phloroglucinol solution to determine the intensity of lignification (Fig. 1C) as a marker of the cell differentiation and specialization. Cross-sections from every root part were incubated for 2 minutes in 2 % pholoroglucinol ethanol solution and then rinsed in droplets of concentrated HCl for 20 seconds. After that, HCl were carefully removed from cross-sections using filter paper, and cross-sections were covered in 70 % glycerol for further analysis.

After staining, cross-sections from each part were viewed on a Leica DM 5000B microscope (Leica Microsystems, Germany; magnification ×100) and photographed (Leica DFC295 camera, Leica Microsystems, Germany). Anatomical parts and tissues were highlighted as rhizodermis, cortex, aerenchyma (if it existed) and stele using the software Adobe Photoshop 2021 (version 22.4.2, Adobe, San Jose, California, USA).

The projective area of the highlighted structures was measured by the ImageJ program (Fig. 1B). For each section, the projection area (μm^2) of rhisodermis, cortex, lacunae (if existed), and stele were determined. Also, the percent (%) of the lacunae area in the cortex was estimated. The presence of lacunae in the cortex was ranked as: 0 — absence, 1 — presence. The lacunae frequency of occurrence (%) for every root part was calculated as the percent of cross-sections with lacunae

to the total number of 21 cross-sections (3 from each root of 7 plants). The intensity of lignification in stele elements was assessed (ranked from 1 to 5, where "1" is the absence of the staining, and "5" is the most intensive staining (Fig. 1C)).

Hydrogen peroxide content

The concentration of hydrogen peroxide was determined according to Bellincampi et al. (2000) test, based on the oxidation of xylenol orange iron (III) chelates by peroxide. 5–6 individuals of hypoxic and aerated plants were harvested at 8, 10, 27 and 30 days after germination in 5 replicates. Root segments of the basal part (2–2,5 cm, 3–4 part) of the roots from each replicate were cut, homogenized in liquid nitrogen and centrifuged (10000 × g, 10 min, 4 °C). Then supernatant was mixed with xylenol orange reagent for 1 hour, and the optical density of the mixture was measured with a plate reader Infinite 200 Pro (Tecan Group Ltd., Männedorf, Switzerland). Data were expressed as μmol H_2O_2 to g fresh weight (FW) of the root segments.

Statistics

The normality of the data was checked using the Shapiro-Wilk test, the equality of variances — using the Leuven test. Statistical processing of the obtained data included one- or two-way analysis of variance (ANOVA) using Tukey's test or Unequal HSD test (Statistica 13 software, Statsoft, USA). In the case of sample abnormality and inequality of variances, the Kruskal-Wallace ANOVA was used. The data for each part from each root were averaged over individuals at every plant age.

Results

Control plants developed longer seminal roots in contrast to hypoxic plants (Fig. 2). However, hypoxic and aerated plants demonstrated the same tendencies in seminal root growth. Both hypoxic and control plants started to form roots from the second day after seed soaking. The 5-day-old plants had the maximum number of seminal roots equal to 6 (Table 1). The length of seminal roots in the individuals of each age varied from 20 to 50 % related to the mean length, which is confirmed by variation coefficient (Table 1, Fig. 2). Seminal roots growth continued until the plants reached the age of 25 days, and then an intensive development of adventitious roots was observed, but more intensive origin was observed for aerated plants (Table 1). Adventitious roots appeared already in the 10-day-old plants for both hypoxic and control plants, however, the most intensive increase in the number of adventitious roots was observed after the end of the seminal root growth (30–40-day-old plants, Table 1).

The total cross-section area of seminal roots during hypoxia was bigger in part 4 > part 3 > part 2 > part 1 during the experiment (Fig. 3), while in control plants the same tendency was observed only for 5-day old plants (Fig. 4). By time the cross-section area in hypoxic conditions decreased in parts 1–3 up to the 15th day but did not practically change in part 4 performed by mature cells. In control plants, a tendency to a decrease of the cross-section area was observed for all parts with an expection of part 1, that slightly increased up to 30 days. The same tendencies were observed for root anatomical parts and tissues in control and hypoxic plants, respectively (Fig. 3A, B, Fig. 4). In addition, cross-section

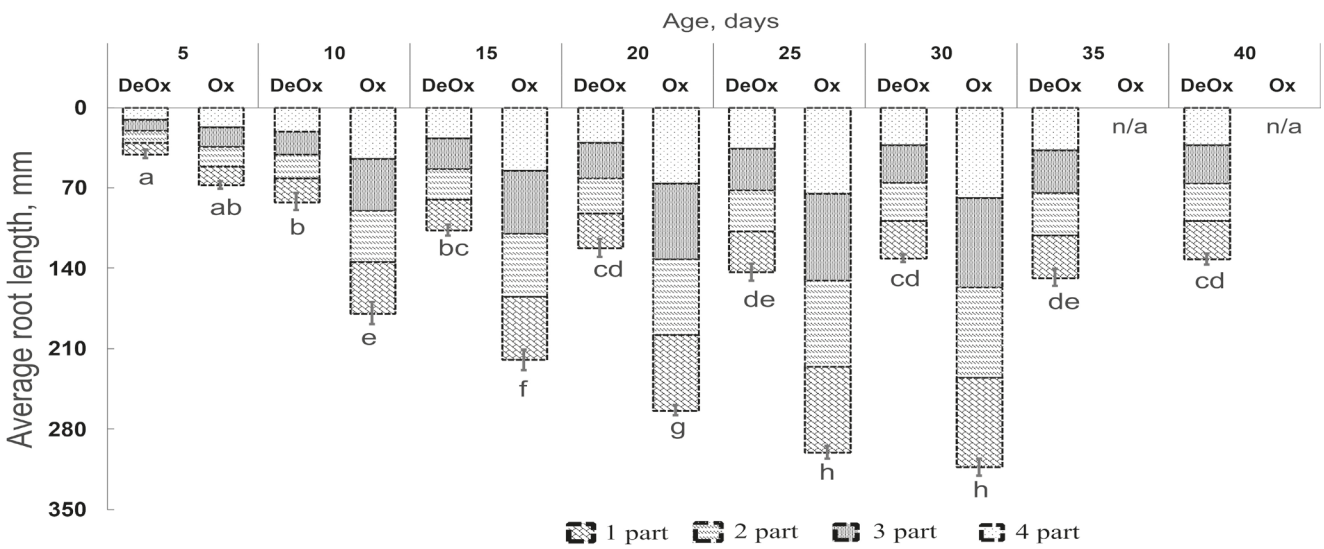

Fig. 2. The average root length in barley plants of different age and conditions. Parts 1–4 perform their localization in seminal roots. Results are expressed as mean seminal root length ± standard error (n = 7). DeOx — conditions without aeration (hypoxic), Ox — conditions with aeration (control). Letters represent significant differences between mean seminal root length in plants of different age ($p < 0.05$, Unequal HSD test). n/a — no available data.

Table 1. Root development in hypoxic and aerated barley plants during 40 and 30 days of growing, respectively, in hydroponic system

Age, days	Number of seminal roots		Number of adventitious roots		Seminal root length		
					mean±standard error, mm		CV, %
	HP	AP	HP	AP	HP	AP	HP
5	6	6	0	0	40.6 ± 3.66	67.7 ± 3.35	44.68
10	6	6	1	1	81.9 ± 7.39	179.03 ± 9.72	48.77
15	6	6	1	1	107 ± 7.79	219.78 ± 9.04	52.44
20	6	6	2	2	122.51 ± 7.79	263.58 ± 4.51	47.97
25	6	6	2	2–3	143.40 ± 7.69	300.47 ± 5.5	48.68
30	6	6	2	5–7	131.05 ± 3.53	313.13 ± 7.54	50.66
35	6	6	2–3	n/a	147.96 ± 7.50	n/a	42.50
40	6	6	2–4	n/a	131.67 ± 4.83	n/a	48.6

HP — hypoxic plants (without aeration), AP — aerated plants (with aeration); n/a — no available data.

and anatomical tissues areas of 15- and 30-day old plants were higher in all root parts with an exception of part 4 in aerated conditions.

Staining with phloroglucinol revealed that lignification took place in the stele and endodermis in both aerated and hypoxic plant roots. The highest color intensity was observed in part 4, gradually decreasing to part 1 in roots of all ages and under both conditions. At the same time, in each part, the color intensity increased by the 10th day, and then it remained relatively constant in hypoxic plants (Fig. 5A, B, Fig. 6), while in aerated conditions the most intensive staining was observed in 30-day old plants. No other elements were stained by phloroglucinol with an exception of endodermis in part 4.

Lacunae were absent in 5-day old plants and in aerated roots (Fig. 7). On the 10th day of growth, they were found in parts 2–4 with a maximum frequency of occurrence in part 3. Further, the frequency of occurrence increased in each part, reaching a maximum after the end of root growth. Part 1 was characterized by the lowest percentage of lacunae occurrence or even their absence (Table 2).

In parts 2–4, aerenchyma lacunae started to form on the 10th day. They occupied approximately equal partial areas in the primary cortex of seminal roots (2–4 %) and remained constant in part 2 until the end of the experiment (Fig. 8). However, after the root growth stopped on the 25th day, the total projective lacunae area in the cortex increased in parts 3 and 4, rising to 10–12 % from the 30th day (Figs 7, 8). In part 1 aerenchyma lacunae were absent until the plants reached the age of 40 days and then occupied 0.5 % of the total cross-section area (Fig. 6).

Table 2. Lacunae frequency of occurrence in hypoxic barley root parts at different age

Age, days	Lacunae frequency of occurrence, %							
	Part 1		Part 2		Part 3		Part 4	
5	0	a	0	a	0	a	0	a
10	4.76 ± 4.76	a	28.57 ± 11.34	ab	80.95 ± 6.73	bc	28.57 ± 8.69	a
15	0	a	71.43 ± 13.47	b	85.71 ± 6.73	bc	66.67 ± 10.29	bc
20	4.76 ± 4.76	a	76.19 ± 9.52	c	61.90 ± 13.47	b	52.38 ± 9.91	ac
25	14.29 ± 9.91	a	76.19 ± 14.02	c	76.19 ± 9.52	bc	71.43 ± 11.34	bc
30	9.52 ± 6.15	ab	90.48 ± 6.15	c	100	c	85.71 ± 6.73	bc
35	4.76 ± 4.76	a	100	c	100	c	95.24 ± 4.76	b
40	50 ± 14.09	b	95.24 ± 4.76	c	90.48 ± 6.15	bc	95.24 ± 4.76	b

Results are expressed as mean ± standard error (n = 7). Letters represent significant differences between the parts in plants of different age ($p < 0.05$, Tukey's test).

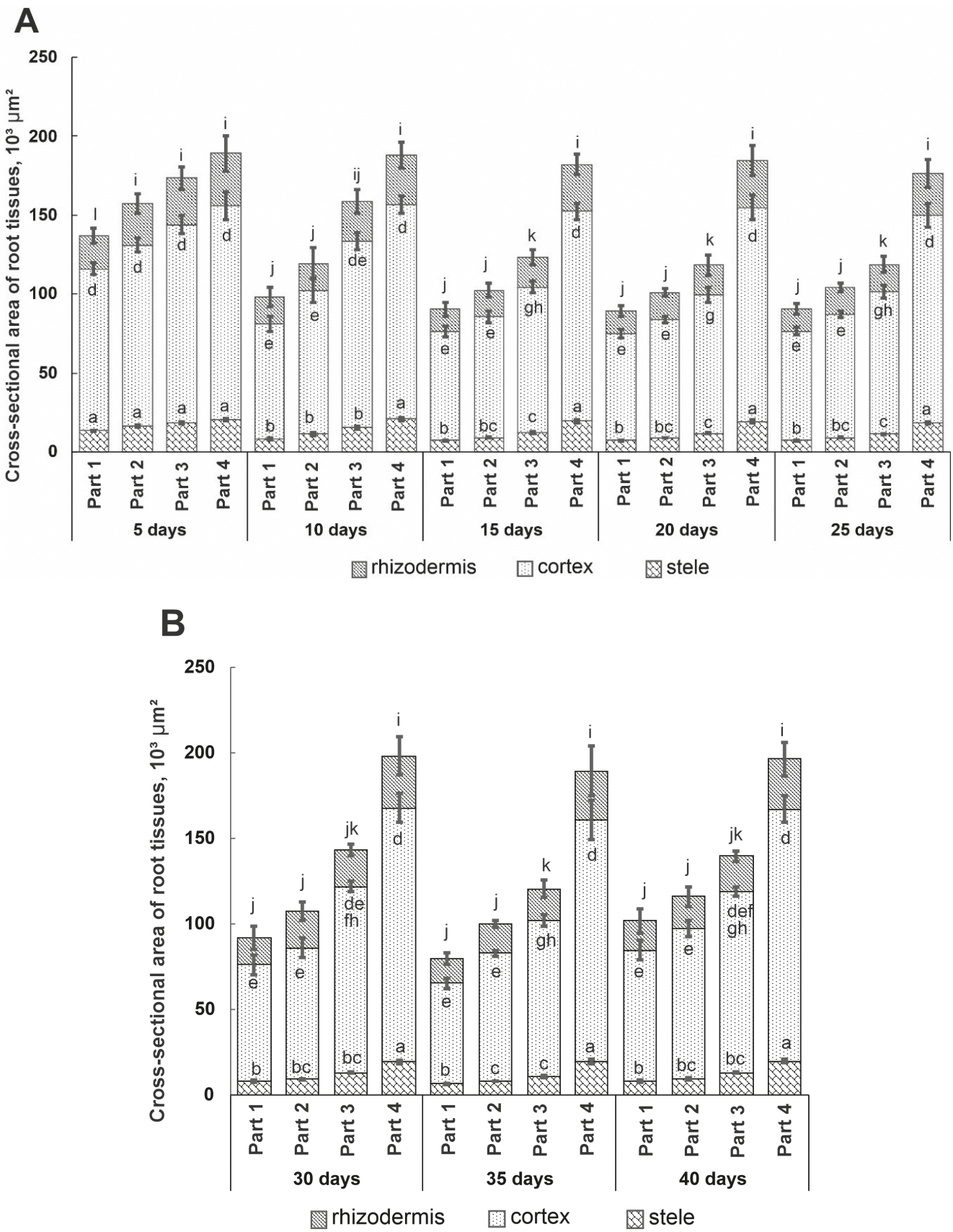

Fig. 3. Anatomy characteristics of hypoxic barley roots. Time laps from A — 5 to 25 days; B — 30 to 40 days. Results are expressed as mean ± standard error (n = 7). Different letters represent significant differences between the stele (letters a–c), cortex (letters d–h) and the whole cross-section area (letters i–k) of different age ($p < 0.05$, Tukey's test).

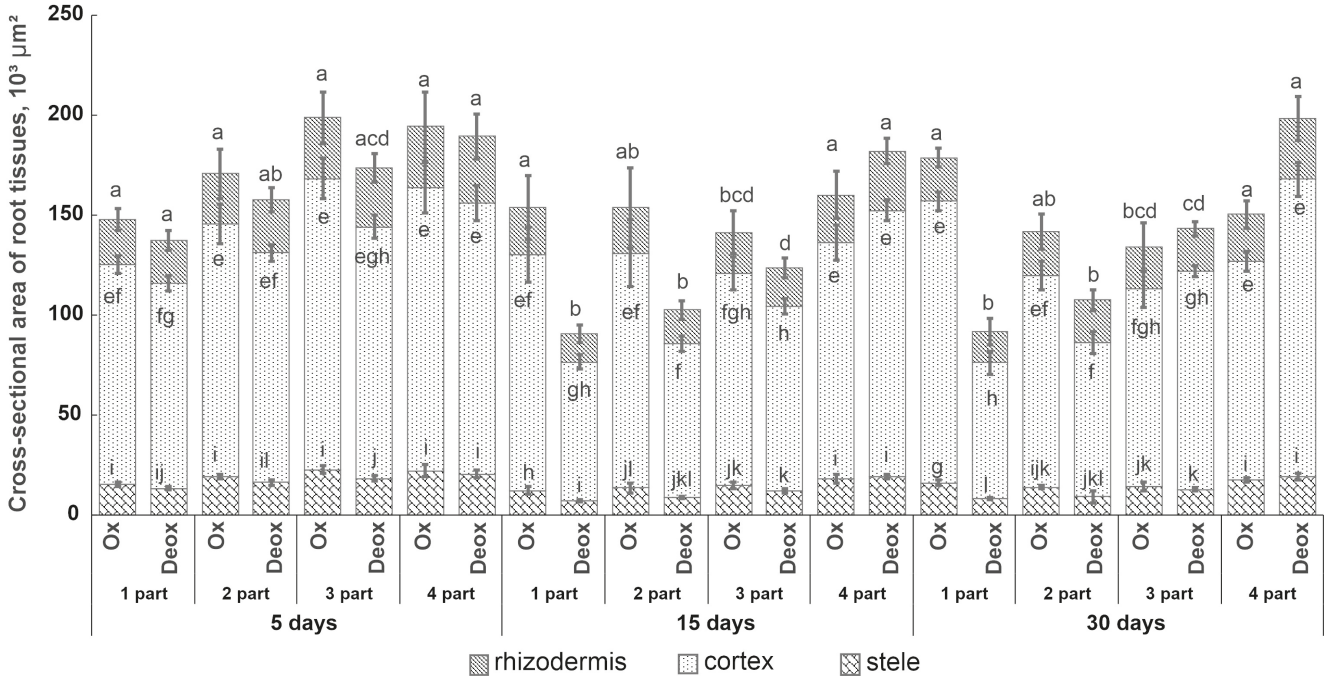

Fig. 4. Anatomy characteristics of aerated and hypoxic barley roots. Different letters represent significant differences between the stele (letters i–l), cortex (letters e–h) and the whole cross-section area (letters a–d) of different age — 5, 15 and 30 days ($p < 0.05$, Unequal HSD test).

Table 3. H_2O_2 content in the basal part of seminal roots in hypoxic and aerated plants of different age

Age, days	8	10	27	30
H_2O_2 content, µmol/g fresh weight	7.55 ± 0.65 a	1.17 ± 0.17 b	2.02 ± 0.37 b	7.16 ± 0.45 a
	3.16 ± 0.35 c	n/a	n/a	3.76±0.19 c

Represented data is expressed as mean ± standard error. Different letters marked significant differences according to Tukey's test ($p < 0.05$).

In the basal part of the root the concentration of hydrogen peroxide as a marker of stress and a proposed trigger of aerenchyma formation was the highest on the 8th day of growth — 7.5 µmol/g, and decreased to 1.17 µmol/g on the 10th day. It was 2.01 µmol/g on the 27th day and increased up to 7,16 µmol/g_2 in the 30-day plants (Table 3). In the basal part of the aerated plant roots the concentration of hydrogen peroxide was twice lower on the 8th day and on the 30th day — 3.16 µmol/g and 3.76 µmol/g, respectively.

Discussion

Seminal barley roots, both hypoxic and aerated, continued their growth up to the 25th day after germination (Table 1, Fig. 2), but the root system in whole continued to develop, increasing the number of adventitious roots (Table 1). It was shown previously that seminal roots, when they reached a maximum of their length, still continued to increase their biomass, and when the root biomass reached the maximum, root senescence began (Schneider et al., 2017). At that time several changes occurred in seminal root morphology, for example, browning (Schneider et al., 2018). In our experiment root browning was detected only in part 4 (basal part) starting from the 25th day after germination as a common trend for hypoxic and aerated plants. Basal browning occurred due to intensive tannins accumulation, which is known for aging organs, e. g., in *Populus trichocarpa* (Wojciechowska et al., 2018). The high number of adventitious roots at the end of seminal root elongation (Table 1) can be related to the decrease of seminal root absorption activity (Hu, Henry, Brown, and Lynch, 2014). It was noted that under waterlogging the development of adventitious roots was stimulated because of their compensative role for nutrients acquisition (York, Nord, and Lynch, 2013; Loades, Bengough, Bransby, and Hallett, 2015; Nguyen et al., 2018; Pedersen, Sauter, Colmer, and Nakazono, 2021). We observed the higher number of adventitious roots in aerated plants in contrast with hypoxic ones (Table 1). This aspect of plant functioning during hypoxia needs additional studies.

The root anatomy of aerated and hypoxic plants showed different traits. The whole cross-section area, cortex and stele areas increased from part 1 to 4 during root growth in all ages (Fig. 3A, B, Fig. 4). The cross-section area was bigger in the aerated plants, because

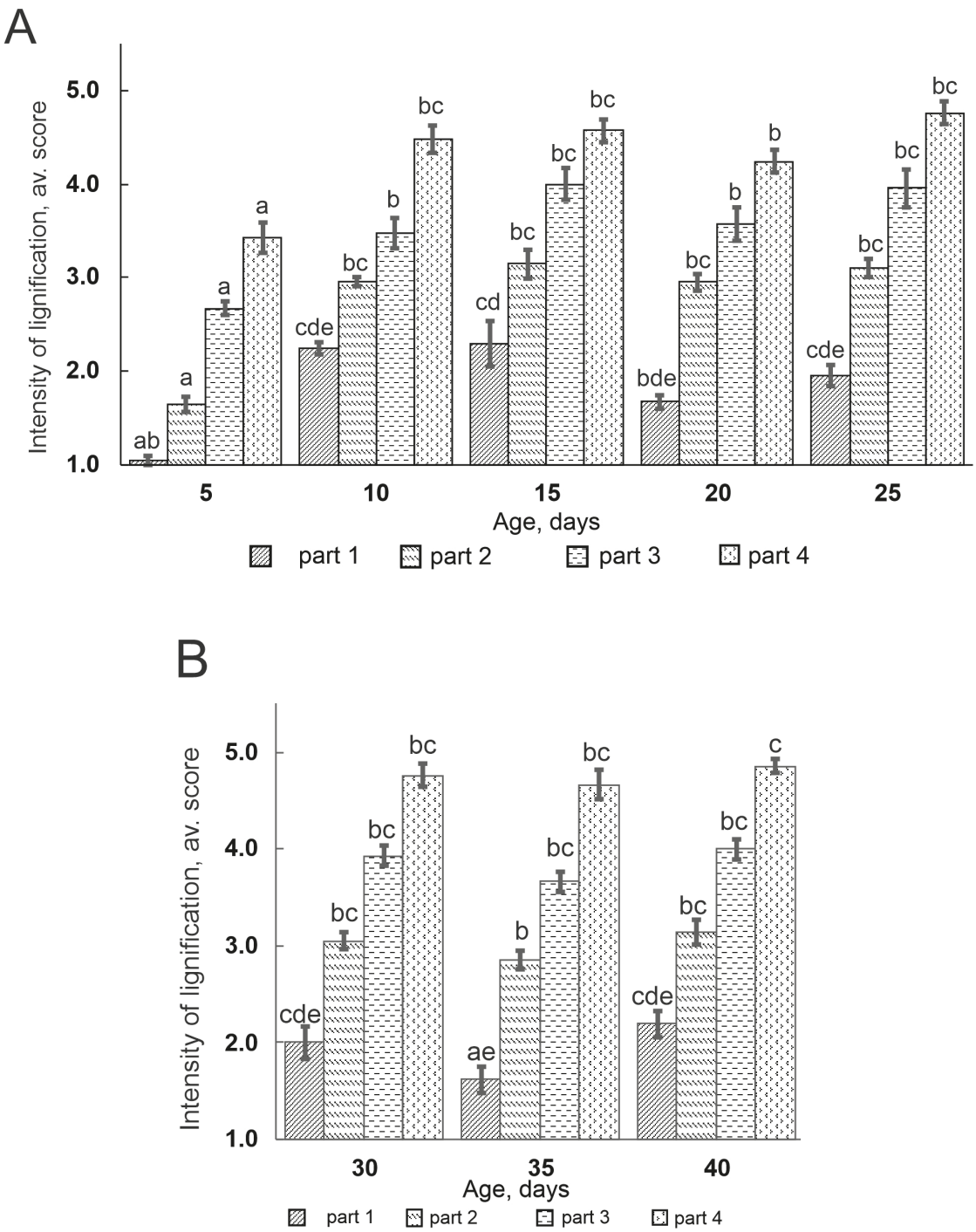

Fig. 5. Lignification in hypoxic barley roots of different age and in different root parts. Time laps from: A — 5 to 25 days; B — 30 to 45 days. Results are expressed as mean ± standard error (n = 7). Letters represent significant differences between the parts in plants of different age ($p < 0.05$, Tukey's test).

radial growth of cortex was not affected by hypoxia. Obviously, seminal roots slow down their growth during hypoxia in cereals (Kırmızı and Bell, 2012; Herzog, Striker, Colmer, and Pedersen, 2016) and can manage to have thinner roots to compensate the elongation cost. A decrease of the root cross-section area and tissue areas observed in parts 1–3 for 15-day plants can be explained by the younger age of these parts. Thicker aerated seminal roots in contrast to hypoxic ones are related to an enlarged apical meristem and cell layers (Kirschner, Stahl, Von Korff, and Simon, 2017). All plant cells originated once remain at the same position during all ontogenetic stages. Seminal roots reached their maximal length on the 25th day of growth. Part 4 in the 25-day

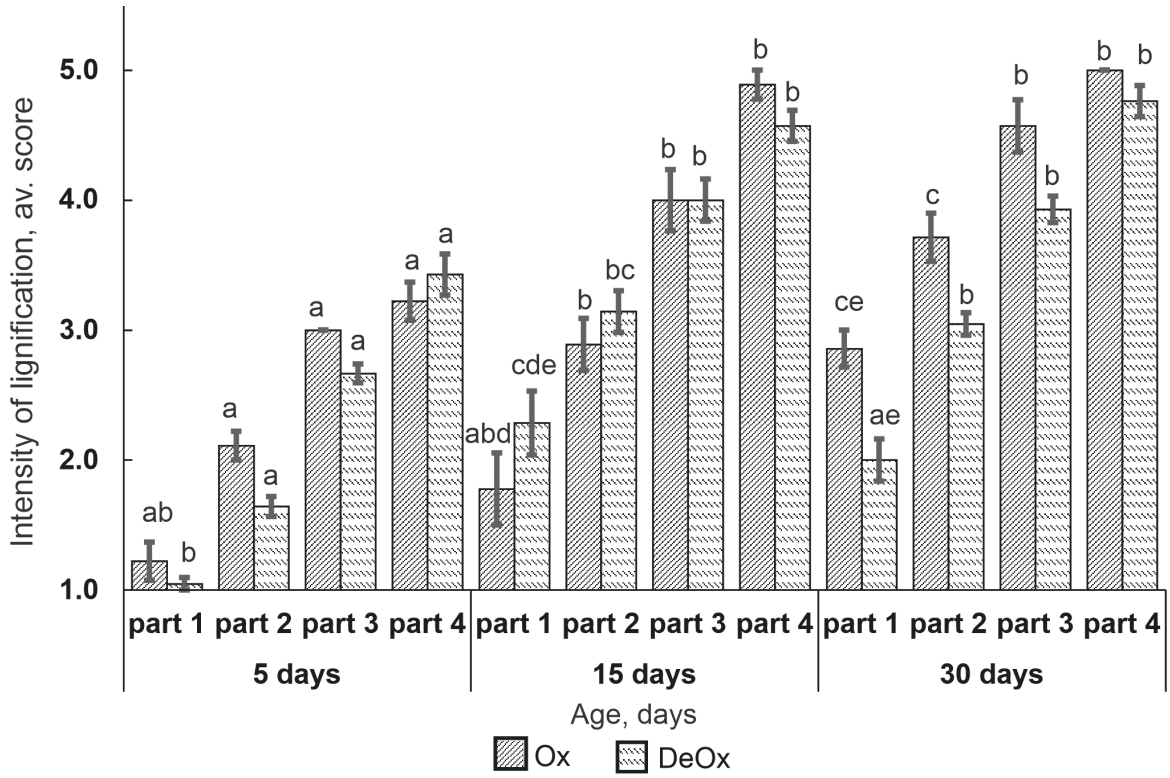

Fig. 6. Lignification in aerated and hypoxic barley roots of different age and in different root parts. Letters represent significant differences between the parts in plants of different age and conditions ($p < 0.05$, Unequal HSD test). Ox — aerated conditions, DeOx — conditions without aeration.

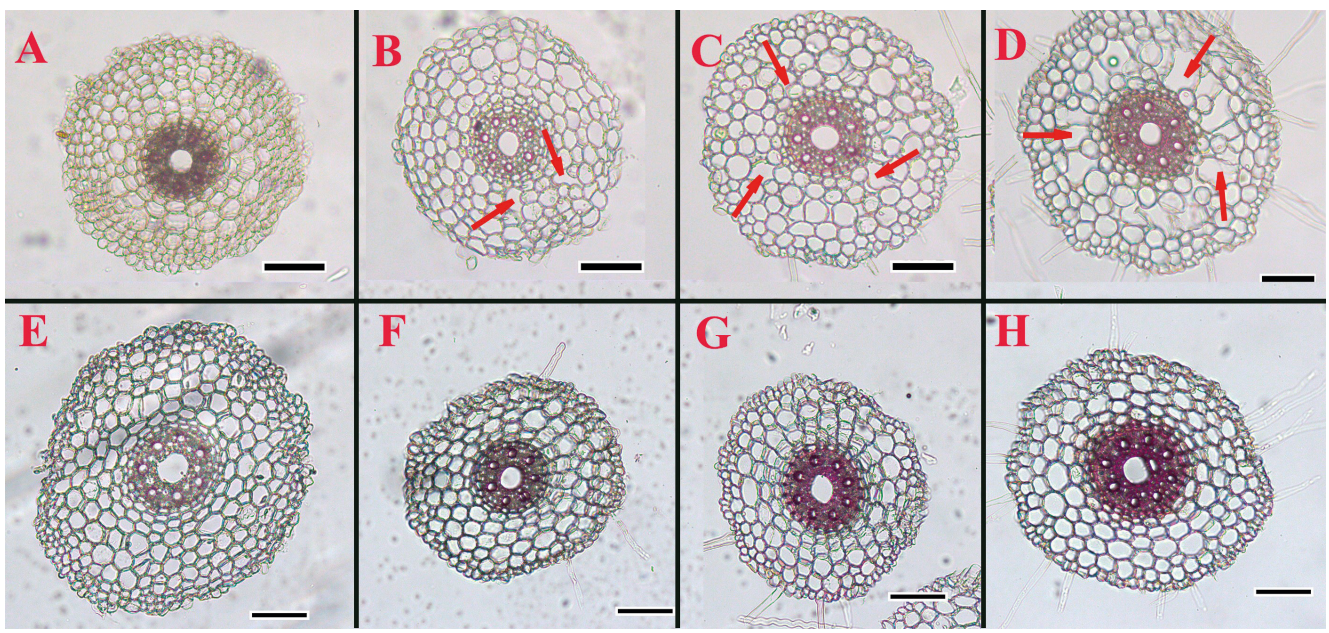

Fig. 7. Seminal root cross-sections of the 30-day *H. vulgare* growing in hypoxic (A–D) and control (E–H) conditions. A, E — part 1, B, F — part 2, C, G — part 3, D, H — part 4. Arrows indicate aerenchymal lacunae. Scale bar is equal to 100 μm.

roots was formed by cells that were already originated in parts 1–4 of the 5-day root; and in part 3 of the 25th day root — from parts 1 and 2 of the 10-day root. Root part 1 in 15-day plants was formed by the newly formed most young cells, and part 2 — by elder cells formed earlier on the 10th day (Fig. 2).

Phloroglucinol staining revealed that the root parts of both aerated and hypoxic plants differed in the degree of lignification. It increased from the apex to the base (Fig. 5A, B, Fig 6). The lowest degree of lignification in hypoxic plants was predictably observed in 5-day plants (Fig. 5A), and the highest degree was reached on

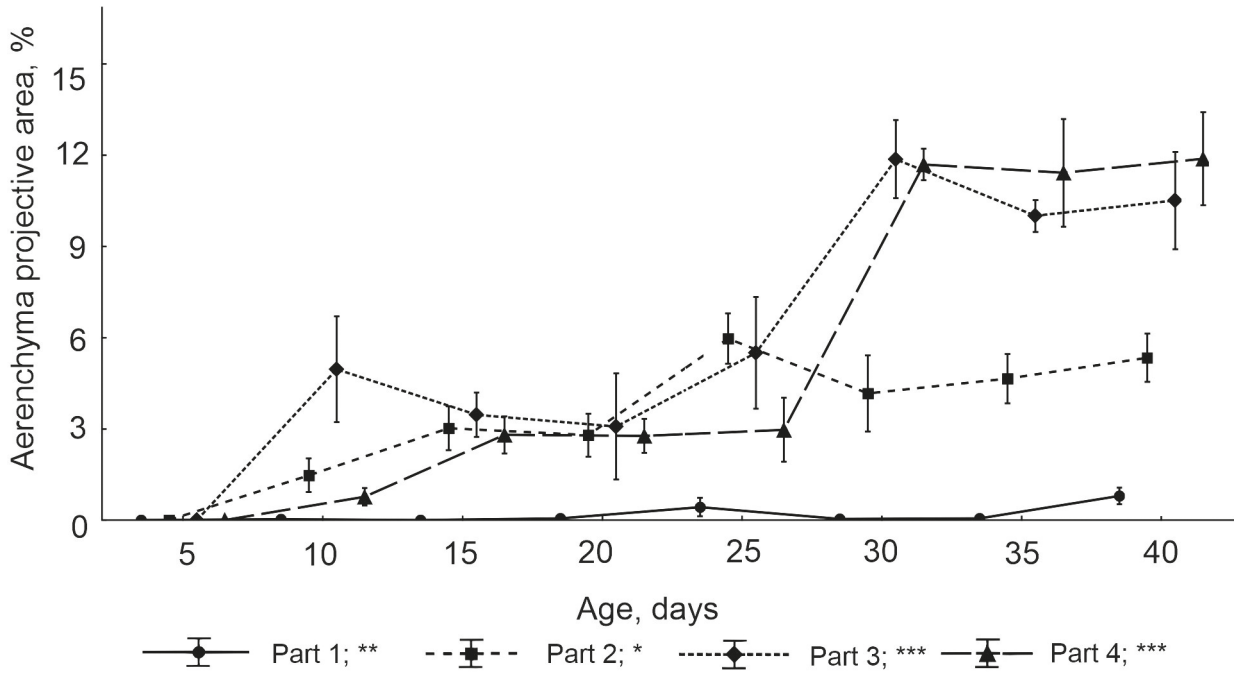

Fig. 8. Aerenchyma projective area (percent of lacunae area in root cortex area) in root parts during barley development. Performed as mean ± standard error. Differences between different ages for every part were calculated using Kruskal-Walles ANOVA. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

the 40th day after germination for all root parts (Fig. 5B), whereas in aerated plants the maximum was reached already on the 30th day. Apparently, it related to a higher root length. A gradual increase in the intensity of lignification reflects the further maturation of the tracheal, mechanical and parenchymal elements of the stele and endodermis (Evert, 2006; Ranathunge et al., 2017) and can be a marker for related age of root parts (Loades, Bengough, Bransby, and Hallett, 2015). It was shown that both mono- and polyphenolics content increased from the apical to the basal part of seminal roots that was associated with the age and the formation of xylem vessels, Casparian bands and mechanical tissues and lignification (Ranathunge et al., 2017). This corresponds to our results on the intensity of phloroglucinol staining (Figs 1, 5, 6). It is also known that the amount of lignin (Evert, 2006) and tannins (Wojciechowska et al., 2018) increased in roots with age, and this effect corresponds to the stimulation of cell degradation (Wojciechowska et al., 2018). In other species like rice and maize lignification during hypoxia occurs in exodermis to prevent radial oxygen loss (ROL) (Ejiri, Fukao, Miyashita, and Shiono, 2021; Hose et al., 2001; Nishiuchi et al., 2012; Tylová, Pecková, Blascheová, and Soukup, 2017), but the identification of this layer in barley seminal roots is a subject of discussion (Lehmann et al., 2000; Enstone, Peterson, and Ma, 2002; Kreszies, Schreiber, and Ranathunge, 2018). In our study no other structures except stele were lignified, so we suppose that there was no ROL barrier that is typical for barley growing in hydroponics (Ranathunge et al., 2017).

In the present study aerenchyma development occurred at two stages: the first — at 5–10 days and the second — at 25–30 days in basal parts. Starting from the 10th day the frequency of lacunae appearance increased in all seminal root parts. In part 1 lacunae were not found at all until the 40th day of growth (Figs 7, 8, Table 2). It is known that lacunae are formed by cell lysis as a result of the programmed cell death (Takahashi, Yamauchi, Colmer, and Nakazono, 2014). This process is known as inducible and is caused by oxygen deficiency (Takahashi, Yamauchi, Colmer, and Nakazono, 2014), which is common for hydroponics. In our study we observed aerenchyma formation in spatiotemporal aspect and found that lacunae frequency and projective area in parts 2–4 peaked to the 30th day (Table 2, Fig. 8). This fact indicates that aerenchyma lacunae in the cortex of seminal roots increased in size with tissue age and the age of the whole plant. One of the possible explanations for this phenomenon is the necessity of oxygen supply for the parts, where lateral roots intensively emerge and grow. Oxygen transportation through lacunae to lateral roots provided a higher absorption surface in a plant (York, Nord, and Lynch, 2013). Moreover, seminal roots gradually reduced the capacity to absorb nutrients with age (Schneider et al., 2017), which caused the increase of cell lysis (Schneider et al., 2017). Cell degradation and consequent lacunae origin in different parts related to the cell age. The highest level of lacunae projective area was observed in the parts close to a root base, which were formed by the most aged cells. In aerated plants aerenchyma was not detected.

Aerenchyma formation coincided with ROS accumulation (Xu et al., 2013; Takahashi, Yamauchi, Colmer, and Nakazono, 2014; van Dongen and Licausi, 2014). In the present study a high amount of H_2O_2 was found in the basal part of the hypoxic roots on the 8th day of growth when the lacunae development started. On the 10th day of growth, H_2O_2 content decreased to the level of control roots and aerenchyma projective area plateaued (Fig. 6, Table 3). This fact agreed with previous research, where H_2O_2 accumulated at the moment of aerenchyma emergence (Yamauchi et al., 2014). It is known that H_2O_2 is a stress marker and its high content can induce PCD. However, at the second stage of the lacunae development H_2O_2 concentration reached the same level as it was in 8-day-old hypoxic plants only to the end of lacunae development (Fig. 8, Table 3). This can be explained as post hypoxic reactions to additional oxygen transportation through enlarged lacunae (Armstrong and Armstrong, 2014), which corresponds to previous data for post anoxia and hypoxia (León, Castillo, and Gayubas, 2021; Shikov et al., 2022).

Conclusion

Our study revealed the changes in the formation of specific root anatomy in barley during hydroponic growing. The analyses of root sections in plants of different age and in different parts of them have shown that aerenchyma formation in hydroponic conditions is stronger expressed in the basal part and is enhanced with the age of plants. In aerated conditions aerenchyma was not emerged in the seminal root tissue. Aerenchymal lacunae development can be divided into two stages: at the first stage lacunae originated in plant roots between the 5th and 10th days of growth, and at the second stage the lacunae enlarged in plants between the 25th and 30th days. Accumulation of H_2O_2 indicates stress conditions in the basal part of roots and could be considered as a trigger of the aerenchyma formation at the first stage, but not at the second one. Thus, the spatial-temporal character of aerenchyma formation was shown in barley roots under hypoxia.

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