Volume 54(1):15-18, 2010
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

ARTICLE

Histological comparison of the rhizome, leafy culm and aerial rhizome of the common reed (*Phragmites australis*)

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ABSTRACT In this study we focused on the morphology of an unusual organ, appeared on the culm of read (named as aerial rhizome), rhizome and leafy culm of the reed plant (*Phragmites australis* (Cav.) Trin. Ex Steudel). The aerial rhizome appeared on young reclined culms or at occasionally broken nodes of reed plants grown in a glasshouse, in perlite and nutrient solution. During the 5 years of cultivation, under greenhouse circumstances the plants remained small if their size is compared to that of the natural reed stands, but the segments developed into an interconnected, well-grown system of roots and leafy culms. According to the histological results, the structure of the aerial rhizome and rhizome revealed a great similarity. The resemblance is not only noticeable in the number of the cell-lines and size of the primary cortex and central cylinder but also in the existence of the lacunae and the central cavity. So far no information concerning the role of the aerial rhizome came to light in the literature; according to our best knowledge, similar structure has not even been mentioned. It is tempting to speculate that the structure's main role may be the vegetative reproduction, horizontal spread of the clone, since histologically greatly resembles to the rhizome. We hypothesize that the structure develops under extreme conditions, e.g. continuous stress such as growing the plants in spatial constrains which cannot be found in natural conditions. Consequently, the cause of the formation of the structure can originate from the extremism of the living-conditions.
Acta Biol Szeged 54(1):15-18 (2010)

KEY WORDS

aerial rhizome
culm type
reed
Phragmites australis

On the horizontal or in some cases broken reclining culms of reed plants, grown under greenhouse conditions in nutrient solution, at the nodes, certain organs named by us as aerial rhizomes, evolved. According to the best of our knowledge the literature did not even mention such an organ. In one of the studies of Haslam (Haslam 1968) in case of reed 4 fundamental culm types are described: the horizontal and vertical rhizome, the aboveground culm and the infrequent runner (*legehalme, long runner).*

In woody monocotyledonous species, e.g. in case of *Cordyline* the appearance of aerial rhizomes was observed (Bell 1991), which under certain circumstances could evolve into an independent plant.

The organs evolved on plants kept under artificial circumstances comprised of several nodes and internodes developing into leafy shoots exhibited negative geotropism. In our study we histologically compared the culm, rhizome and aerial rhizome. According to the results the structure of the aerial rhizome morphologically is highly similar to that of the rhizome. There is no primary cortex in the culm, the multicell-layered epidermis directly reaches the vessels. The aerial rhizome and rhizome are differentiated into a wide multicell-layered primary cortex and a central cylinder. In the parenchyma of the primary cortex lacunae, in the central cylinder three collateral closed vessels are found. The size of the bundles gradually decrease from inside to outward.

Materials and Methods

Morphological observations were carried out on the rhizome, aerial rhizome and culm of common reed (*Phragmites australis* (Cav.) Trin. ex Steudel). The rhizome segments were collected from a natural reed stand, Csukásér canal near Gátér (Csongrád County, Hungary). Two-bud rhizome segments were placed into plastic vessels filled up with perlite, supplied with complete Hoagland nutrient solution. From the initials during 5 years a full-grown rhizome network was developed with 10-15 ramets (Bankó et al. 2002). In the present experiment we worked with 3-3 samples coming from 3 plants. After de-aeration and dehydration we applied paraffin embedment instead of the carcinogenic xylol and benzol. Bioclear was used as an intermediate compound.

The samples placed in 100% ethyl alcohol were transferred in 70-30, 50-50 and 30-70 alcohol-Bioclear rate solution and this was transferred into the Bioclear compound. During the paraffin impregnation the samples were placed into a 35-40°C thermostat, since at a lower temperature the

Accepted November 20, 2009
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Figure 1. The „aerial rhizome", developed on an aerial stem of reed. Bar = 1 cm.

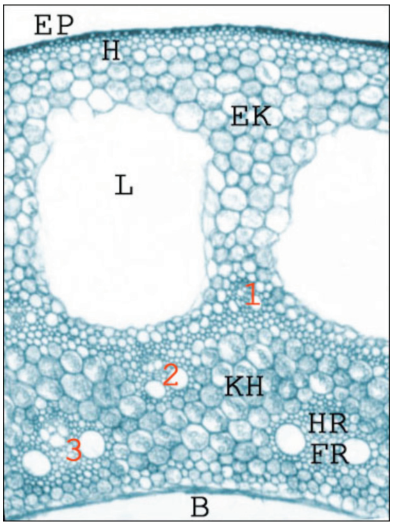

Figure 2. Microscopic photograph on the cross-section of rhizome. EP: epidermis, H: hypodermis, EK: primary cortex, L: aerenchyme, KH: central cylinder, HR: phloem, FR: xylem, B: central cavity. 1: The outer small; 2: the middle, 3: and the inner large-sized vascular bundle circles.

solution would have been become saturated soon. At 60°C the paraffin remained molten and continuously paraffin was added to the solution. The impregnation took 3 weeks then the volume of the paraffin was two-fold of that of the solution. Depending on the samples the evaporation of the Bioclear took 1 week and the material was ready for cutting sections. The samples were set in aluminium blocks, the blocks were filled up with paraffin. Before cutting the foil was removed, the paraffin was cut in the form of a dice then the dices were glued with few molten paraffin onto a wood base for fixing it to the platen.

The samples were cut with microtome equipped with steel blade in large quantity between 12 and 17 μm in thickness. The photograph of the CCD camera joined with the light microscope was digitalized with computer and were evaluated with Image Pro Plus software. The dataset made from the numerous photos were analyzed with Microsoft Excel.

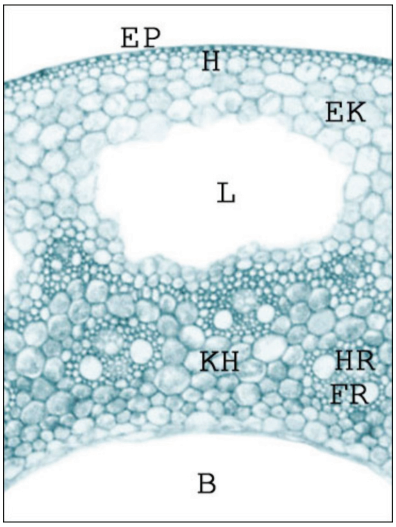

Figure 3. Microscopic photograph of the cross-section of aerial rhizome. The primary cortex and the central cylinder are formed of fewer cell-lines than those of the rhizome. In the structure of the aerenchyme further differences can be discovered; the cavities are flat, narrow, rather resembling an oblong form with rounded sides. EP: epidermis, H: hypodermis, EK: primary cortex, L: aerenchyme, KH: central cylinder, HR: phloem, FR: xylem, B: central cavity.

Results and Discussion

The reed plants gorwn according to the above written circumstances had typical 3-15-noded structures ending in bud on their thin reclined culms (Fig. 1). These mostly resembled the horizontal, underground rhizomes (Fiala 1976). Although variations in soil conditions (Kudo and Ito 1997) sulphide levels (Fogli et al. 2002) or C/N metabolic malfunctions (Erdei et al. 2001) altered rhizome and shoot development, similar aerial organ shown by Figure 1, was not seen in the open nature.

As the histological results revealed, the structure of the rhizome and aerial rhizome showed a high degree of similarity. In both cases under the epidermis consisting of one cell-layer, the hypodermis's wall is slightly thickened. The primary cortex is thick, consisting of 14 and 8 cell-layers for the rhizome and aerial rhizome, respectively.

In the parenchyma substance of the primary cortex large-sized air passages evolved. Their function is oxygen-supply

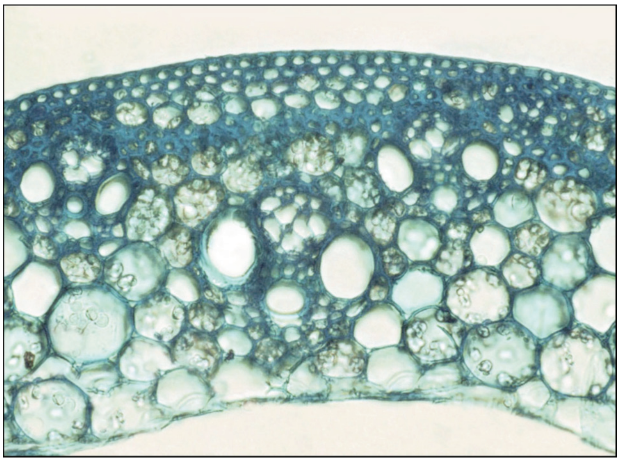

Figure 4. Cross-section of *Phragmites* stem (culm). Under the one-layered epidermis, hypodermis and the primary cortex's two cell-lines the three circles of vascular bundles are embedded in the tissue of the parenchyma of the central cylinder.

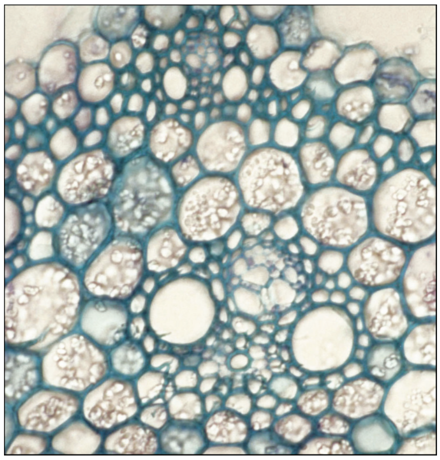

Figure 5. Leucoplasts were found in smallest quantity in the aerial rhizomes. As seen on the picture in rhizome and stem the parenchyma cells – which build up the central cylinder – abundantly contain leucoplasts.

for subterraneous organs under hypoxia. Between the air passages, on their inward sides, small-sized bundles were observed, occupying a place in the tissue of the central cylinder which wave-likely protrudes between the air passages. The collateral closed vessels form three circles. The outer smallest bundles are followed by a medium-sized, then a large-sized thin-walled bundle-circle. Both in case of the rhizome and the aerial rhizome large thin walled cells build up the central part of the cortex, whereas the cells adjutant to the cortex's outer and inner surface hardly reach half of the diameter of the former cells. The lacuna system has a well expressed sharp border. The lacunae are placed in the large parenchyma substance, in turn, along its inner wall facing the central cylinder small-sized, one cell-layer wide cortex parenchyma forms the boundary (Figs 2 and 3).

The parenchyma of the central cylinder exhibits a structure similar to the primary cortex. The cylinder's outer and inner walls are accompanied by cell-lines of smaller cells. The two inner bundles are placed in the large-diametered cell-substance in such a way that these small-sized cells shut them in. The outer smallest bundles are located between the small cells that are intruding wavely into the interspace among the lacunae. The circle of the bundle is separated by one cell-layer from outside from the cortex-parenchyma. The central cavity's border is sharp and due to intensive cell-elongation flat cells border.

Beside the significant morphological similarities between the rhizome and aerial rhizome the different structure of the lacunae is a great difference. The lacunae in case of the rhizome are nearly rounds with rounded sides whereas in

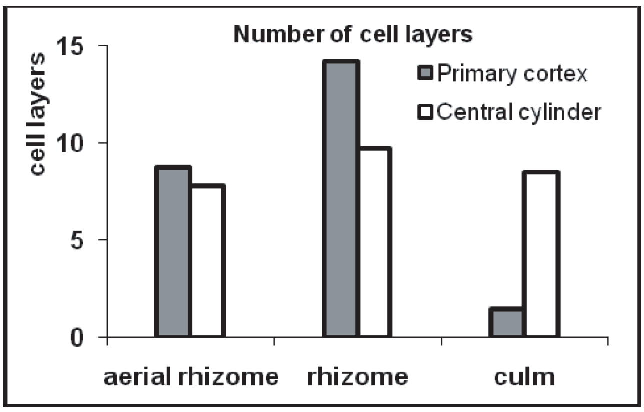

Figure 6. Numbers of cell-lines building up the primary cortex and central cylinder of reed aerial rhizome, rhizome and culm.

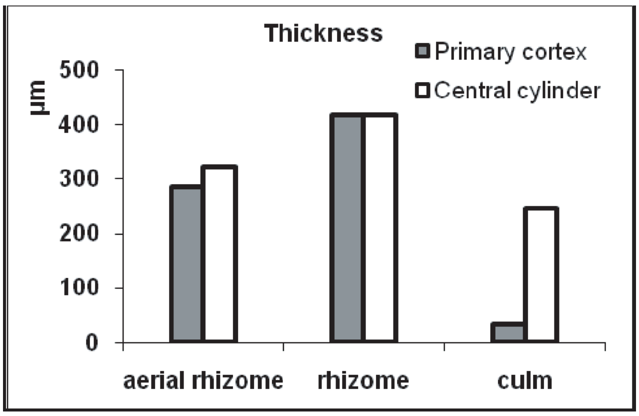

Figure 7. The thickness of the primary cortex and central cylinder of reed aerial rhizome, rhizome and culm.

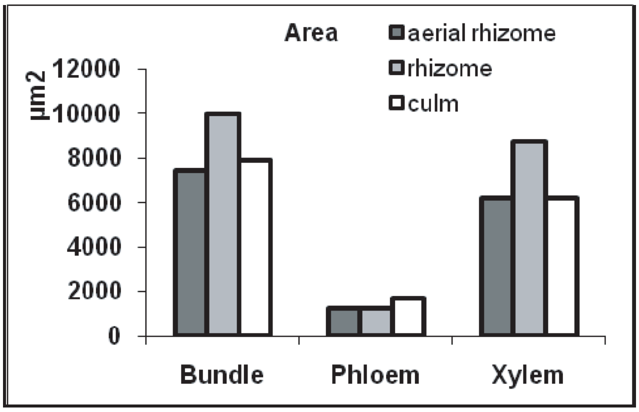

Figure 8. The area of the vascular bundles, xylem and phloem of reed aerial rhizome, rhizome and culm.

case of the aerial rhizome they are oblong-shaped with better expressed sides.

The mechanical strengthening of the culm is provided by sclerenchyma. The supporting tissue is localized in several places: thus under the hypodermis, between the vessels and along the central cylinder and the cortex. In the central cylinder of the leafy culm three bundle-circles evolved similarly to the underground shoot (Fig. 4).

Leucoplasts were found in the least amount in the aerial rhizomes. The parenchyma cells enclosing the vessels in the culm and the rhizome contained a great quantity of starchy granules (Fig. 5).

After evaluating statistically the dataset of the numerous sections, the similarity of the aerial rhizome and rhizome became obvious. The statistical analyses correlated with the results of the light microscopic observations. The primary cortex of the culm and the aerial rhizome are thick which in case of the aerial rhizome and the rhizome consist of 8 and 14 cell layer, respectively. Their central cylinder is also wide, 8 and 10 cell-layered. The cortex of the culm between the most exterior bundles is represented by a thin parenchyma of 1-2 cell layers, which are unobservable above the bundles, while the 9 cell-layered central cortex is comprised of several tissues (Fig. 6).

The width of the primary cortex and the central cylinder in case of the aerial rhizome and rhizome are 600 and 800 µm, respectively. This quite exceeds the shoot's value which is under 300 µm, although the central cylinder here is also thick, having a 250 µm average value (Fig. 7).

In the rhizome and aerial rhizome the phloem to vessel ratio is higher in the outer smallest vessel than in case of the two larger inner vessels. In the culm, the area of the phloem is slightly larger as compared to those of the rhizome and aerial rhizome (Fig. 8).

In conclusion it can be stated that in case of reed plant the characteristics of the aerial rhizome can be described as a new culm type. We only have vague assumptions concerning its function in nature – if it would be found in nature at all.

The aerial rhizome may contribute to the vegetative reproduction of the clone, similarly to the function of the rarely found runner. It is the future's task to elucidate the physiological and hormonal background of this formation. The results may contribute to a better understanding of the reproductive strategy of this clonal species.

Acknowledgements

This work was supported by the Hungarian Sientific Research Fund grant No. OTKA T 032524.

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