Annals of R.S.C.B., Vol. XXII, Issue 2, 2018, pp. 1 - 10
Received 28 June 2018; accepted 26 November 2018.
doi: 10.ANN/RSCB-2018-00015:RSCB

Laser confocal microscopic study of callose in plants at nature submergence

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Key words: callose, submergence, leaf, cell wall, laser scanning microscopy

Summary

The distribution and relative content of callose in cell walls of epidermis, mesophyll and vessels of conductive bundles of Potamogeton perfoliatus, Potamogeton pectinatus and Myriophyllum spicatum leaves with the laser confocal microscopy (LSM 5, Germany) and Pascal program analyzed and compared to leaf anatomical characteristics. Nature submergence stimulates callose production in leaf cells of the epidermis and mesophyll. The dependence on content callose in cell walls on species, tissue and plant stage development established. It is revealed that callose content of mesophyll cells of plants during vegetative stage is much more in comparison with that in leaves at the flowering stage of plants. Callose revealed in cell walls of complexes, which consisted of the epidermis and mesophyll cells of hydrophytes' leaves at vegetative stage. Whereas, the size of cellular callose-restricted complex reduced at the expense of going out photosynthesizing parenchyma cells from complex at the plant flowering stage. The obtained results suggest the effect of nature submergence on the involved of callose in cellular and functional mechanisms of tolerance to submergence, regulation of water transport and transport of assimilates in submerged leaves regardless of anatomical structure.

Introduction

Flooding of soil and submergence is one of the most important environmental factors that, due to its long duration of activity, lead to significant changes in the growth and development of plants, and sometimes even to their deaths (Clarke & Stone, 1963; Chen & Kin, 2009). Under natural conditions, hydrophytes in which submerged leaves grow and develop under water produced protective ways and adaptation mechanisms at the level of tissues and cells (Jackson & Amstrong, 1999) to the action of the physical conditions of the water environment, which differs of the content and rate of transport of gases in water, and also the reduced intensity of light, and its spectral composition compared to those in normal (air) conditions (Sand-Jensen et al., 1992; Doronin, 2000). In the endogenous cellular mechanisms of plant adaptation to submergence an important role is given to the structure of the epidermis and its cell walls, as the cell walls of epidermis are the first to react to the effects of the aquatic environment (Wisserman et al., 1995; Sivaguru et al., 2000). Protecting terrestrial plants and optimizing its vital functions largely depends on the presence of wax, the functioning of the stomata and the composition of the polysaccharides of epidermis cell walls (Albersheim, 1976). Since water plants do not have wax, and the submerged leaves deprived of the system of stomata, the presence and ratio of different polysaccharides can play a key adaptive role for aquatic plants (Nedukha, 2011).

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Callose is main polysaccharide of cell walls, which is involved in the regulation of water transport with apoplast. Callose is a linear amorphous polysaccharide of a cell wall, whose molecule is formed by several hundred glucose residues. Callose is mainly linked with β-1-3-glucosidic bonds and β-1-6 bonds, which can form small branches (Clarke & Stone, 1963). Calloses synthesize only in certain cells; its synthesis is tissue-specific in leaves, roots, and in stems of plants (Wissemeier & Horst, 1995; Sivaguru et al., 2000). Most often, regardless of the cell and tissue, normal or stress, the callose begins to synthesize at the "definite point" of the surface of the cytoplasm membrane, and form small sites, which are greatly enhanced under ideal conditions (Vaughnet et al., 2007). Callose plays a key role in intercellular water transport, this polysaccharide forms into stomata walls and participate in the mechanism of stomata pore opening and closure (Galatis & Apostolakos, 2010). Besides, callose is also formed into wall plasmodesmata (Rinne et al., 2001). Callose can to compress the plasmodesma and thereby it can to drop water transport along tissue (Van der Schoot et al., 2011). Such event is a signal for a change of cell growth. As plasmodesmata close with callose, a symplastic cell isolation occurs. This leads to increased turgor pressure and the promotion of longitudinal cell growth, which shown on the cells of fibril stems of cotton (Ruan et al., 2004). The formation of callose in plasmodesma is similar to the callose biogenesis in the pores of sieve-tubes, where callose reserve in the autumn, when the pores closed, and in the spring at the opening of pores callose begins to lose (Krabel et al., 1993).

The regulation of the synthesis of callose attracts the attention of many scientists, because this polysaccharide makes a significant contribution to the protection of the plant under the influence of various stressors. It established that the mechanism of accumulation of callose controlled by a variety of endogenous and exogenous signaling pathways depending on the surrounding conditions, or from the action of biogenic or abiogenic factors (Luna et al., 2011; Voight, 2014). An important component of the signaling of the synthesis of callose is calcium ions that change the conformation of 1.3-β-glucan synthetase and promote its activation (Kauss, 1987; Kauss & Jeblick, 1991). Like phenomenon was shown in the study of the suspension cells of Hordum bulbosum (Ohara et al., 1993), Glycine max and Catharanthus roseus (Waldmann et al., 1988). We assumed that plants that constantly grow in water are characterized by a rather intense synthesis of callose, not only in the epidermis and in the vessels of vascular system, but also in the cells that come in contact with the epidermis. Because the aim of our study was to carry out of the investigation the presence and distribution of callose in cell walls of leaf different tissues and dependent of callose content from leaf anatomy, tissue type and plant growth stage.

Materials and methods

A research objects were submerged leaves of Potamogeton perfoliatus, Potamogeton pectinatus, and Myriophyllum spicatum that grew a depth of 50 to 80 cm on the bank of the Venetian Strait (left Shore of Dnepr River, in Kiev). The plant samples selected in the stage of vegetative growth (12-14 June) and flowering stage (24-27 July) of 2017. Photosynthetic photon fluency rate (PPFR) was measured by means of the light Meter LI-250 (USA, LI-COR) on the submerged leaf surface (15-17 μmol quantum·m-2·s-1 in June and 25-28 μmol quantum·m-2·s-1 - in July), because the sun illumination above of water surface (over plants) was 1130 and 1350 μmol quantum·m-2·s-1, accordingly. The water temperature in the Venetian Strait was +19o C in June and in July was + 22o C.

The middle part of Potamogeton perfoliatus, P. pectinatus and M. spicatum leaves were used for research. The sections of samples selected from five plants, which had close identifiable size and close morphology; and then they were used for the light optical microscopy and laser-confocal microscopy. Immediately after removal from water, samples fixed on birch in mixture of 3 %

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solution of paraformaldehyde in a 0.5 M phosphate buffer, for 24 h, pH 7.2. Then the fixed material in laboratory terms washed in the buffer, dehydrated in ethanol and aceton, embedded in mixture an epon/araldite resin according to the standard method. Semi thin sections (about 12 µm thickness) stained with Schiff solution and by solution of safranine in accord with standard (Furst, 1979) and studied on the light microscope (Axioscope, Carl Zeiss). The size cells in leaflets were measured with using ImageJ programs. The values of results expressed at the mean and standard errors, using Student's test ($P < 0.05$).

The cytochemical method for the study the presence and content of callose with laser-confocal microscopy carried out according the protocol of Subbaiah and Sachs (Subbaiah & Sachs, 2001). The samples of leaves (0.7-0.9 cm long) of three species plants incubated in the specific fluorescent dye - 0.1% aniline blue (Sigma) dissolved in 0.5 M solution of K2HPO4. The incubation for 20-30 min carried out directly on bank, then samples carefully washed in the solution of K2HPO4 and 0.5 M phosphate buffer, fixed in a solution of 3% paraformaldehyde and 1% glutaraldehyde (1:1, volume) in a 0.5 M phosphate buffer (pH 7.2) and delivered to laboratory (at +4o C) for the investigation. The samples in laboratory samples washed with such buffer and investigated on the laser (confocal) scanning microscope LSM 5 Pascal (Carl Zeiss, Germany). Complex aniline blue-callose excited at 405 nm (by the argon laser), and fluorescence emission detected at 461 nm using x 10, x 20, x 40 objectives. Chlorophyll auto fluorescence excited at 440 nm and fluorescent emission detected at 662 nm. Fluorescence intensity of callose in cell walls and chlorophyll in chloroplasts measured as a function of emissions wave length using Pascal program (LSM 5, Carl Zeiss). From five to seven plants of each species used for cytochemical investigations. We present the average fluorescence intensity in 30-40 cells epidermis, in 30-40 cells of mesophyll, and 10-13 vessel cells of each sample. Values of results expressed at the mean and standard errors, using Student's test ($P < 0.05$).

Results and discussions

Anatomical analysis of leaves. To identify the places of localization of callose in the cell walls of leaf tissues, we first had to study the anatomical structure of the leaves of the studied species of hydrophytes. For description of leaf blade morphology and leaf anatomy of *Potamogeton perfoliatus, Potamogeton pectinatus* and *Myriophyllum spicatum* used standardizing "keys" according of the Indentifier of Higher Plants in Ukraine (1999).

Potamogeton perfoliatus (Potamogetona-ceae) is a water plant. The complete cycle of this plant ontogenesis survive about four months (May-August) in Ukraine. Plant *P. perfoliatus* characterized by the developed submergenced entire leaves (Fig. 1A), situated on short shoots. The third and fourth leaves from the shoot apex selected for the study; selected leaves finished the elongation. Leaves of *P. perfoliatus* are simple, elliptic, oval-shaped, and wide at base. Light-microscopic analysis of the cross sections of leaf exposed isolateral mesophyll, including one layer mesophyll between adaxial and abaxial epidermis (Fig. 1B, left part of section), which were closely joined. The number of mesophyll layers increased to 9-11 in zone of situation of the vascular bundle.

The size of leaf and cells was the next: long axis of leaf is 25 ± 5 mm; short axis - 17± 2 mm; average thickness of leaf blade in vessel zone is 400 ± 37 µm; average size of epidermis cell height and width is 18 ± 2.3 and 27 ± 3.0 µm; average size of mesophyll cell height and width is 21 ± 2.4 and 29 ± 2.3 µm; the number of mesophyll layers per middle section of short axis of leaf varied from one to six. Stomata are absent, cells of epidermis with chloroplasts and mesophyll are small and situated very closely. Whereas mesophyll cells around vascular bundle is very big, and big air space can to see between mesophll's cells.

Potamogeton pectinatus (Potamogetona-ceae) is a water plant too; this plant called fennel pondweed is a cosmopolitan water plant species. The complete cycle of *P. pectinatus* ontogenesis survive also from May to August in Ukraine. Its stems very strongly branched,

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straight, upward. Plant characterized by the presence of needle shaped, thread like leaves (Fig. 1 C) and like threads, thin elongated leaves, and bright green on underwater shoots. For the light-microscopic study we used the middle parts in the third and fourth leaves from the shoot apex.

Light-microscopic analysis of the cross sections of leaf exposed centric, ubifacial structure and isolateral mesophyll (Fig. 1D, 1E),, one-layered epidermis with chloroplasts, 2-7 layers of almost radial situated mesophyll, big air spaces and poorly vessel bundle. Stomata are absent; cells epidermis and mesophyll situated very closely. Air spaces situated almost in central part of sections.

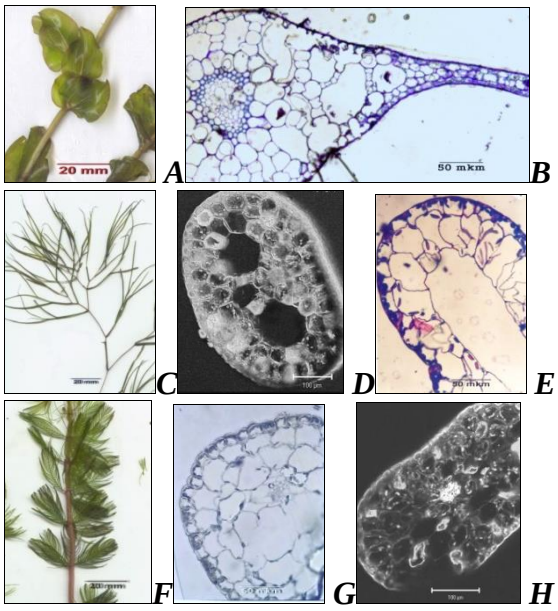

Fig. 1. General view of *Potamogeton perfoliatus* (A), *Potamogeton pectinatus* (C) and *Myriophyllum spicatum* (F) leaves and the cross sections through central plane of *P. perfoliatus* (B), *P. pectinatus* (D, E) and *M. spicatum* (G, H) leaves. Bars: A, C, F = 20 mm; B, E, G = 50 μm; D, H = 100 μm.

The size of leaf and cells was the next: long axis of leaf is 82 ± 7.3 mm; short axis – 2.1 ± 0.6 mm; average thickness of leaf blade in vessel zone is 295 ± 31 µm; average size of epidermis cell height and width is 18 ± 2.5 and 20 ± 1.7 µm; average size of mesophyll cell height and width is 55 ± 5.7 and 41 ± 3.9 µm; the number of mesophyll layers per middle section of short axis of leaf varied from two to seven.

Myriophyllum spicatum (Haloragaceae), spiked water-milifoil, is aquatic plant, which has slender stems (from 20 to 30 cm), on which submerged green four leaves (from 15 to 25 mm long) are situated by a ring around stem; leaf is dissected, the shape of leaf is feather-like with needle like leaflets (**Fig. 1F**). Leaves branched near the water surface. The complete cycle of this plant ontogenesis survive about four months (from May to August) in Ukraine. Light-microscopic analysis of the transverse sections of almost round leaf exposed near centric structure (Fig. 1G), including isolateral mesophyll, one-layered epidermis with chloroplasts, 4-5 layers of almost radial situated big cells of photosynthesizing parenchyma, and small vascular bundle (Fig. 1H). The size of leaf and cells was the next: long axis of leaf is 30 ± 3.2 mm; short axis - 18± 2.5 mm; average thickness of leaf blade in vessel zone is 280 ± 31 µm; average size of epidermis cell height and width is 17 ± 2.3 and 20 ± 4.3 µm; average size of mesophyll cell height and width is 50 ± 3.7 and 51 ± 5.4 µm; the number of mesophyll layers per middle section of short axis of leaf varied from four to five.

Cytochemical study of callose. Laser-confocal microscopy of surface and cross sections of *Potamogeton perfoliatus, Potamogeton pectinatus* and *Myriophyllum spicatum* leaves showed that, regardless of the species under study, the fluorescence of the callose detected in the cell walls of the epidermis, mesophyll (photosynthesizing parenchyma) and vessels of vascular bundles. Callose fluoresce bright green. The dependence of the fluorescence intensity of the callose on the species and tissue type established.

Potamogeton perfoliatus. The cytochemical analysis of the fluorescence of the callose in *P. perfoliatus* the leaves in the vegetative growth stage (Fig. 2A) and the flowering stage (Fig. 2B) showed uneven green color luminescence: with high intensity in the upper epidermis and vessels of the vascular bundle and weak intensity in the cell walls of photosynthetic parenchyma and lower epidermis. Comparison of the callose fluorescence intensity using the Pascal program revealed differences in the content of callose in the cell walls of the epidermis,

doi: 10.ANN/RSCB-2018-00015:RSCB

photosynthetic parenchyma and vessels, that presented in the figure 2C, 2C' (diagram), 3A. The following sequence of intensity of fluorescence of the callose established: the cell walls of the vessels > the adaxial epidermal walls > the abaxial epidermal walls > the cell walls of the photosynthetic parenchyma.

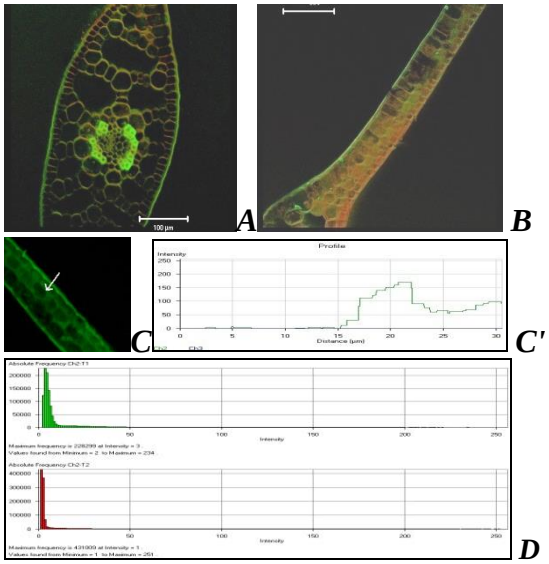

Fig. 2. Micrographs of cytochemical fluorescence of callose in submerged leaf cells of *Potamogeton perfoliatus* (*A, B*). Localization of callose has green fluorescence. *C* and *C'* - histogram of intensity of callose (green line). Ordinate – Fluorescence intensity, relative units (pixels). Abscissa – Distance (µm), which was scanned on the figure *C*. This distance is shown as white line on the figure *C*. Figure *A* and *B* - 3D structure. On figure *D* – absolute frequency of pixels for callose (green graph) and auto fluorescence of chlorophyll (red graph). Bar = 100 µm.

With Pascal set (program) was established high absolute value of the frequency of pixels in the fluorescence callose and auto fluorescence in chlorophyll in cells, particularly fluorescence callose in the first layer of the epidermis was 228299 pixels (Fig. 2D, the green line top of the graph), the greatest frequency of pixels of chlorophyll in the first parenchyma layer was 431009 pixels, respectively (red bottom of the graph). The study shown that during flowering plants callose is in the same tissues of leaves as in the vegetative growth phase, but the intensity of fluorescence increases 1.3-1.8 times in epidermis walls and reduced in 1.7 times - in the walls of photosynthetic parenchyma, and practically are not changed in the walls of the vessels (Fig. 3A).

Potamogeton pectinatus. Analysis of the localization of the callose on the transverse sections and on the surface of the leaf blade of *P. pectinatus* showed a irregular green color of the luminescence of the callose (Fig. 4A): weak in the walls of the photosynthetic parenchyma and intensive - in the walls of epidermis and vessels of the vascular bundle. On the surface of the needle-shaped particles leaves *Potamogeton* is revealed callose continuous layer covering periclinal walls of epidermis (Fig. 4B). The following sequence of callose fluorescence established: walls of vesselsof of vascular bundles > walls epidermis > walls of photosynthetic parenchyma (Fig. 3B**).**

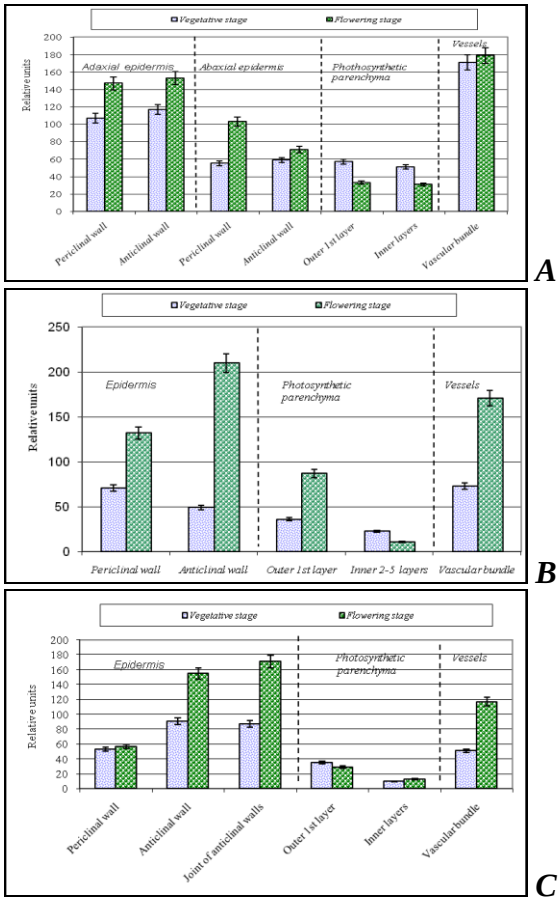

Fig. 3. The effect of cell differentiation and plant development stage on the relative content of callose in the leaf cell walls of *Potamogeton perfoliatus (A), Potamogeton pectinatus (B)* and *Myriophyllum spicatum* (*C*).

The fluorescence intensity of the callose and chlorophyll in the cells of the epidermis and

doi: 10.ANN/RSCB-2018-00015:RSCB

parenchyma was quite high, the greatest intensity of the callose was 193834 pixels (Fig. 4C, 4C′, 4 D: upper green part of the graph); the greatest frequency of chlorophylls in the cells of the epidermis and parenchyma was also high and amounted to 336724 pixels (Fig. 4D: lower part of the graph). The study has shown that during flowering stage callose is revealed in the same tissue of leaves as in the vegetative growth stage, but the fluorescence intensity rises two to four times a periclinal and anticlinal walls of epidermis, 2.7 times - in vessels and 2.4 times - in the walls of the first external layer of parenchyma, accordingly (Fig. 3B).

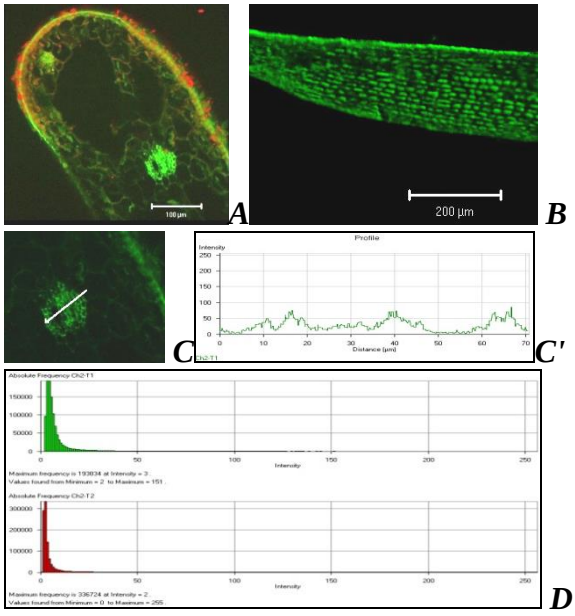

Fig. 4. Micrographs of cytochemical fluorescence of callose in submerged leaf cells of *Potamogeton pectinatus* (*A, B*). Localization of callose has green fluorescence, auto fluorescence of chlorophyll has red. *C* and *C'* - histogram of intensity of callose (green line). Ordinate – Fluorescence intensity, relative units (pixels). Abscissa – Distance (µm), which was scanned on the figure *B*. This distance is shown as white line on the figure C. Figure *A* and *B* - 3D structure. On figure *D* – absolute frequency of pixels for callose (green graph) and auto fluorescence of chlorophyll (red graph). Bars: *A* = 100 µm, *B* – 200 µm.

Myriophullum spicatum. The analysis of localization of the callose showed that the leaves of *M. spicatum* characterized by green luminescence of the callose, which was uneven in the sections of the leaves (Fig. 5A, 5B): weak in the walls of the photosynthetic parenchyma and more intense luminescence - in the anticlinal walls of the epidermis and vessels. The following sequence of callose fluorescence established: epidermal anticlinal walls > walls of zone at the joint of anticlinal walls of epidermis > periclinal epidermis walls > walls of vessels > walls of photosynthetic parenchyma. The intensity of callose fluorescence and auto fluorescence of chlorophyll in the cells was quite high, especially in the joints of the anticlinal and periclinal walls of epidermis (Fig. 5C, 5D, 5D') showed relatively high value.

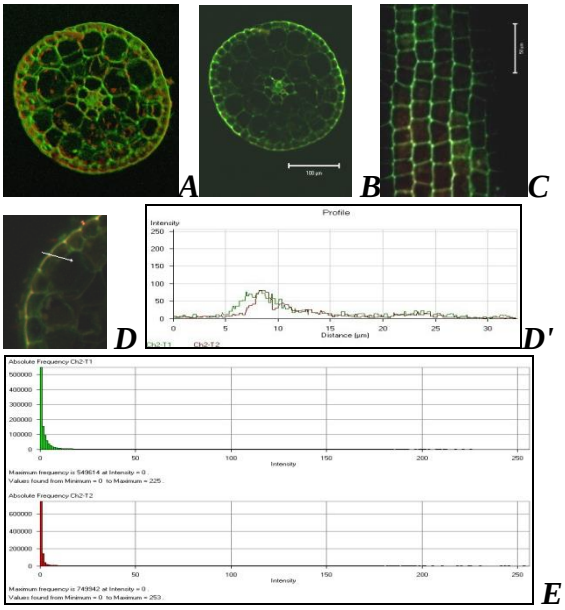

Fig. 5. Micrographs of cytochemical fluorescence of callose in submerged leaf cells of *Myriophyllum spicatum* (*A-C*). Localization of callose has green fluorescence; auto fluorescence of chlorophyll has red. *D* and *D'* - histogram of intensity of callose (green line; auto fluorescence of chlorophyll - red). Ordinate – Fluorescence intensity, relative units (pixels). Abscissa – Distance (µm), which was scanned on the figure *D*. This distance is shown as white line on the figure *D*. Figures *A*, *B* and *C* - 3D structure. On Figure *E* – absolute frequency of pixels for callose (green graph) and auto fluorescence of chlorophyll (red graph). Bar = 50 µm.

This value amounted to 549614 pixels callose fluorescence at the joints of epidermal walls (Fig. 5E, green line of the graph), chlorophyll greatest frequency of pixels in the first outer layer of parenchyma - 749942 pixels (red bottom of the graph). During of the flowering stage callose is revealed in the same tissue of leaves as in the vegetative stage (Fig. 5B, 5C), but the intensity of fluorescence increases at 2.8 times in the place of the joint of anticlinal

doi: 10.ANN/RSCB-2018-00015:RSCB
Received 28 June 2018; accepted 26 November 2018.

walls of adjacent cells of epidermis; 1.7 times - in epidermal anticlinal walls and 2.25 times - in the vessels of vascular bundles (Fig. 5D, 5 D', 3C). In walls of parenchyma callose practically not detected.

Discussion.

Comparison of the anatomical structure of the underwater leaves of **of *Potamogeton perfoliatus, Potamogeton pectinatus* and *Myriophyllum spicatum*** showed common signs, in particular the poorly developed vascular bundles, epidermis without intercellular space, presence of chloroplasts in the epidermis and isolateral mesophyll. A similar type of almost heterogenous mesophyll of the submerged leaves described in *Typha latifolia* and *T. angustifolia* (Black et al., 1981), stem *Trapa natans* (Bercu, 2004), submerged leaves of *Eahinodorus ozelot* (Bercu et al., 2017) and other hydrophytes (Bercu & Fargas, 2002). However, for the floating and surface leaves of many species of hydrophytes the dorsoventral mesophilic type and a large number of chloroplasts per unit leaf area are characteristic (Nekrasova et al., 2003). In addition, we have found a significant decrease in the number of layers of mesophyll of the submerged leaves in the zone, where there is no vessel bundle. As is known, such signs as lowering the thickness of the plate and the localization of a large number of chloroplasts in epidermal cells that is in contact with the external aqueous medium reduce the pathways of carbon dioxide diffusion to chloroplasts. It is known that the adaptation of leaves to underwater conditions occurs by increasing the surface of thin leaves, as is typical of most species of hydrophytes (Bruni et al., 1996; Frost-Christensen et al., 2003). Thin leaves with increased leaf plate area that contributes to the optimal accumulation of CO_2 dioxide in cells, the content of which decreases around the leaves (Madsen & Breinholt, 1995).

The results obtained in this study show that under natural flooding of plants occurs intensified synthesis of callose in cells of the epidermis, photosynthetic parenchyma and vessels of vascular bundles in submerged leaves of *Potamogeton perfoliatus, Potamogeton pectinatus* and *Myriophyllum spicatum*. The data to improve the content of callose in the epidermis of all three species and in vessels of vascular bundles *P. pectinatus* and *M. spicatum* consistent with the data on the increase in the synthesis of callose in sieve tubes of phloem during cells differentiation or action of heat when the cell walls reduce or temporarily stop apoplast water transport (Chen & Kim, 2009; Xie & Hong, 2011). We assume that the large content of callose in cell walls of submerged plants are structural and functional signs for optimal preservation of turgor in submerged leaves, and inhibition apoplast water transport through the epidermis. It is possible that the established by us phenomenon testifies submergence leaves preserve plant from uncontrolled penetration of water from the surrounding aqueous environment inside of cell.

It is shown that an increase of callose occurs in a situation where the cell wall protects the protoplast from penetration of heavy metals, or under conditions limiting their penetration into the cell; while only calcium ions freely penetrate the cell where they are trigger the synthesis of callose (Pirselova & Matusikova, 2013). We do not exclude that increase in callose content in the cells of epidermal tissue layers protects submerged plants from the effects of abiotic and biotic factors, including and the effect of heavy metals and pathogenic fungi and bacteria on plants. Previously, such activation callose synthesis described in the roots of terrestrial plants several researchers (Hofmann et al., 2010).

It is known that increasing the content of callosa in cells of terrestrial plants (Parre & Geitmann, 2005) increases elasticity and flexibility of leaves. Taking into account the above-mentioned data and the results of our experiments, it explain that raised callose content in the epidermis of the underwater leaves increases their elasticity in water and promotes optimal viability of the underwater organs under the influence of strong water currents and its pressure. Obviously, this is due to the interaction of callose-synthetase with calcium ions, which alter the conformation of the callose-synthetase and facilitate its activation (Kauss & Jeblick,

doi: 10.ANN/RSCB-2018-00015:RSCB

1991)**.** Taking into account the literature data obtained at the roots of the *Arabidopsis* mutants, the functions of callose are not only in the regulation of apoplastic water transport (Sjolund, 1997), but also in the regulation of symplast transport through the plasmodesma, in which it deposited under the conditions of activation of β-1-3-glucan-synthase and expression of the *CALS3 / GSL 12* gene (Vaten et al., 2011), we can assume that under the conditions of natural submergence of plants in water in their leaves the gene of *CALS3 / GSL 12* activated.

The presence of callose in the walls of photosynthesizing leaf parenchyma *P. perfoliatus*, and the first (outer) layers of mesophyll *P. pectinatus* and *M. spicatum*, which are adjacent with the epidermis, obviously, explain by the fact that epidermis with photosynthesizing parenchyma formed the conglomerates that communicated by callose. The size of such cellular conglomerates decreased at flowering stage of used plants. We suggest that like conglomerates not only regulates water transport in these cells but it is possible that callose in the stage of vegetative growth can take part in the regulation of transport of assimilates. Our suggestion agree with the idea of Sjolund (1997) an Eun-Ji Koh et al. (2012), according of which callose can prevent of leak of assimilates along phloem in consequence of callose formation in leaves of citrus plants in usual conditions and during infection of plant (Lukas et al., 1993; Kobayashi et al., 2005). These authors consider that callose promotes specific cellular domains that limit the transport of substances for which size less 1 kDa as through plasmodesmata and through the same wall. Provided that the transported substances are less than 1 kDa or even smaller. Found almost no practical callose in the inner layers of walls of photosynthesizing parenchyma in the leaves of plants in the flowering stage obviously contributes active transport assimilates of these cells leaves to developing generative organs. It is possible that at the flowering stage of hydrophytes the decrease of the size of cellular callose-restricted conglomerates it is necessary for the enhance of apoplast and symplast transport of assimilates and phytohormones, which possible are necessary for the formation of flowers, further embryogenesis and seed formation, as it established for terrestrial plants (Kursanov, 1976).

Conclusions

Cytochemical study noticed suggest that polysaccharide callose can be structural-functional marker of plant during of submergence.

Callose revealed in cell walls of epidermis, photosynthetic parenchyma and vessels in submergence leaves of Potamogeton perfoliatus, Potamogeton pectinatus and Myriophyllum spicatum at vegetative and flowering stage by cytochemical method with scanning laser microscopy. Relative content of callose depended on species, tissues and stage of development plants.

Callose form cellular conglomerates from the epidermis and mesophyll cells in the leaves of hydrophytes at the vegetative stage of growth. At the flowering stage, the size of cellular callose-restricted conglomerates reduced due to the release of conglomerate cells of the photosynthetic parenchyma.

Acknowledgements

This work has been supported by Department of Cell Biology and Anatomy of Institute of Botany of National Academy Sciences of Ukraine.

The author wish to thank Ms Victoria Ivacha for expert technical help during the study of the plant samples with the laser confocal scanning microscope.

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doi: 10.ANN/RSCB-2018-00015:RSCB

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