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INFLUENCE OF GROWTH CONDITIONS ON CONTENT OF MONOLIGNOLS IN *PHRAGMITES AUSTRALIS'* LEAVES

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Using a cytochemical method and laser confocal microscopy, a sensitivity of content and distribution of monolignols in the leaves of *Phragmites australis*, grown in different natural conditions, was established. The leaves at stage of vegetative growth of two ecotypes of *Ph. australis*, which grew in water and on land, were studied. We present results obtained by comparing the data on the leaves of *Ph. australis* of air-water and terrestrial plants growing in natural conditions (Kyiv, Ukraine). It was found that the decrease in soil moisture leads to an increase in ratio of syringyl monolignol to guaiacyl (S/G) and to an increase in total content of monolignols (S+G) in epidermis and tissues of vessel bundles of the terrestrial plants. It was assumed that changing the ratio of monolignols and changing their content in the epidermis of leaves of terrestrial reed plants is one of the mechanisms of plant adaptation to lower soil moisture, which reduces transpiration and maintains optimal water potential in leaves of *Ph. australis* growing on land. Based on the obtained experimental data, we believe that high content of syringyl monolignol, which gives high strength to leaves and stems of terrestrial reeds, can serve as a marker for commercial use of these plants in various sectors of economy.

Key words: *Phragmites australis, lignin, soil moisture, leaf, laser confocal microscopy*

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Fluctuations in soil water balance in biotopes, such as flooding or soil drought, cause changes in the structural and functional organization of plants, leading to the activation of adaptive mechanisms in response to stress (Tyree & Cheung, 1977; Vartapetian & Jackson, 1997; De Micco & Aronne, 2012). Therefore, for survival in changed conditions, plants developed numerous mechanisms of tolerance at tissue, cellular and molecular-genetic levels. Main problem during flood is the shortage of oxygen and CO_2, so flooding causes formation of an aerenchyme in plant roots to store oxygen and CO_2 (Armstrong et al., 1994; Jackson &, Colmer, 2005). In conditions of soil drought, reduction of water supply by plant root system leads to significant changes in structure of roots and leaves, functioning of stomata and change of composition of cell walls in epidermis leaves to maintain an optimal water balance in plants in dry areas (Granier & Tardie, 1999; De Micco & Aronne, 2012). Drought stress is considered to be a condition in which water potential and turgor are reduced, as a result, a plant potentially has to stop or inhibit photosynthesis

and metabolism, which can even lead to cell death (Chaves et al., 2009). Analysis of complex drought responses, ranging from stress perception to transcriptional and physiological responses, involves numerous interrelated processes in which lignin can maintain water potential in tissue by regulating cuticular and stomata transpiration, reducing water consumption (Hu et al., 2009).

Lignin and its constituents monolignols are one of the main structural polymers in cell wall of higher plants, fulfils a number of functions, among which mention should be made of their remarkable mechanical strength properties, and wall impassability for water. Lignin is a complex biopolymer of aromatic alcohols, which are synthesized in cell walls. Lignin is highly branched and composed of cross-linked units of three monolignols: *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S)

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phenylpropanoid units (Boerjan et al., 2003). Lignin synthesis depends on tissue type, organ and species (Fengel, & Wegener, 1984; Monties, 1998). This biopolymer can reduce speed of cell growth and participate in plant adaptation to stress**,** changing structure of cellular wall matrix, providing impassability of water and water solutions through walls, and forming in epidermis or other tissues a barrier to pathogens. It is known that wall lignification intensify plant resistance to pathogens invasion (Moura et al., 2010). It is possible that phenomenon occurs because of monolignols are characterized by hydrophobic feature, forming covalent and hydrogen bonds between polysaccharides (Boerjan et al., 2003).

In nature, there are known plant species that can grow normally in water and in terrestrial soil. One of such species is *Phragmites australis* (Cav.) Trin. ex Steud, helophyte, which is characterized by a wide geographical distribution amplitude (Clevering & Lissner, 1999; Packer et al., 2017), and by using in agriculture, building industry and energy technologies. Vegetative organs (leaves, roots) and seeds of *Ph. australis* are also used in medicine at treatment of colds and bacterial-viral diseases (Zhang et al., 2016; Tewksbury et al., 2002). This species grows both on banks of rivers and lakes, and far from water, in field or in mountains.

Considering the above-noted data on lignin properties we supposed that in the mechanism of tolerance of *Ph. australis* plants growing in water and on drought soil the lignin and its monolignols can participate, regulating transport of water through apoplast. Therefore, the aim of our work was to carry out a comparative analysis of the presence, distribution and content of lignin monomers guaiacyl and syringyl in the leaves of *Ph. australis* plants growing in water on the river bank and in moderate drought soil away from the shore.

METHODS

The object of study was the leaves of *Phragmites australis* plants that grow in water and on terrestrial soil. Plants were harvested at the vegetative phase of plant growth on June 2019. Air-water plants grew in water along-shore of the Venetian Strait (left shore of Dnipro River, in Kiev, Ukraine) on the depth of 40-50 cm. Terrestrial plants grew near 10-15 meters far from the shore in a sandy soil. The temperature of water was + 24°C and of air – + 27°C. Leaves from 12-13 plants of each ecotype were used for the biochemical and the microscopic investigations. To study the localization and relative content of monolignols (syringyl and guaiacyl) in leaf cells, a cytochemical method was used according to the protocol of Wuyts and co-authors (2003). Leaf sections (near 20 μm of thickness) were hand cut from middle part of fresh leaf samples. For the detection of monolignols, samples sections were stained within 2 min with 0.25% (w/v) diphenyl boric acid-2-aminoethyl ester (DPBA) (Sigma) in H_2O, then washed and fixed with 0.5% solution of paraformaldehyde in phosphate buffer (pH 7.2). The leaf sections were visualized with a laser scanning microscope LSM 5 Pascal (Carl Zeiss, Germany). For detection of complex DPBA-syringyl fluorescence a laser was excited at 340-380 nm, and the fluorescence emission detected at 430 nm wavelength; and for detection of complex DPBA-guaiacyl fluorescence a laser was excited at 450-490 nm, and the fluorescence emission detected at 520 nm wavelength, using an ×10, ×20 and ×40 objectives. Chlorophyll auto fluorescence was excited at 440 nm and fluorescent emission detected at 660 nm. Fluorescence intensity of monolignols was measured in the cell walls as a function of emissions' wavelength using the PASCAL program. For statistical treatment we took for three leaves from each plant, in every leaf took at least 30 different cells (epidermis, photosynthesizing parenchyma, and tissue of vascular bundle). Values of cytochemical results were expressed as the mean and standard error. Statistical significance of relative content of monolignols in cell wall was determined using a Student's test ($p < 0.05$) and Origin 6.1 program.

To determine a moisture content of the soil on which the terrestrial and air-water plants grew, the soil samples were taken at depth of approximately 35-40 cm from the surface. Standard biochemical method was used to determine the relative water content of the soil. This method based on drying the soil specimens in a thermostat at temperature of 105°C to constant weight (Arasimovich, 1987). The soil humidity of air-aquatic plants was 79.3 ± 2.1%, and the soil humidity of terrestrial plants was 43.3 ± 1.7 %.

RESULTS AND DISCUSSION

Leaves of air-water and terrestrial *Phragmites australis* plants were characterized by a similar linear shape, but were different in size (Fig. 1, A-D). The height of air-water plants of reed in phase of vegetative growth ranged from 90 to 130 cm. The height of terrestrial plants of reed ranged from 120 to 165 cm.

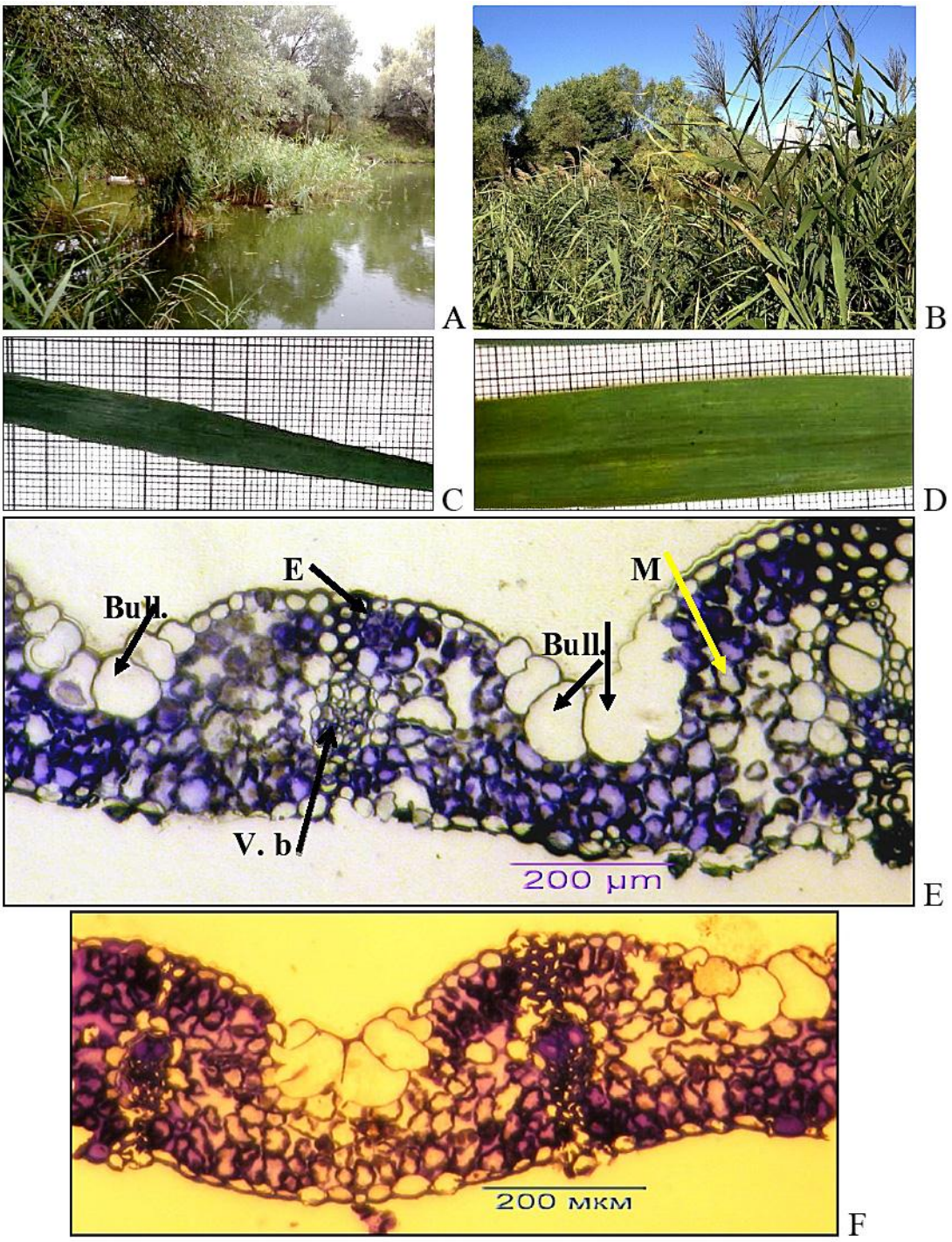

Fig. 1. General view of *Phragmites australis* grown in water (A) and on terrestrial soil (B). The appearance of leaves of air-water plants (C) and terrestrial plants (D), located on graph paper (one division is 1 mm). Figure E and F are transverse sections of leaves of plants grown in water (E) and in terrestrial soil (F); Signs: Bull – bulliform cells, E – epidermis, M – mesophyll, V.b – vascular bundle. Bar = 200 µm.

The leaves of reed grown on shallow water were shorter and narrower than the leaves of plants grown on terrestrial soil. The average leaf size of reeds grown in water was 51 ± 7 cm at long axis and 1.3 ± 0.5 cm—at short axis (Fig. 1, C). The water content of the leaves was 62.8 ± 0.5%. The structure of leaf was isolateral (Fig. 1, E). The thickness of the leaf blade in area of recess or depression ranged from 200 to 380 microns, in the area of combs, where conductive bundles lies, ranged from 300 to 600 microns. The adaxial epidermis was characterized by the presence of six to seven large bulliform cells in depression area. The number of mesophyll layers in recess zone ranged from four to six, while in conduction zone there were eleven to twelve layers. In cross sections, the

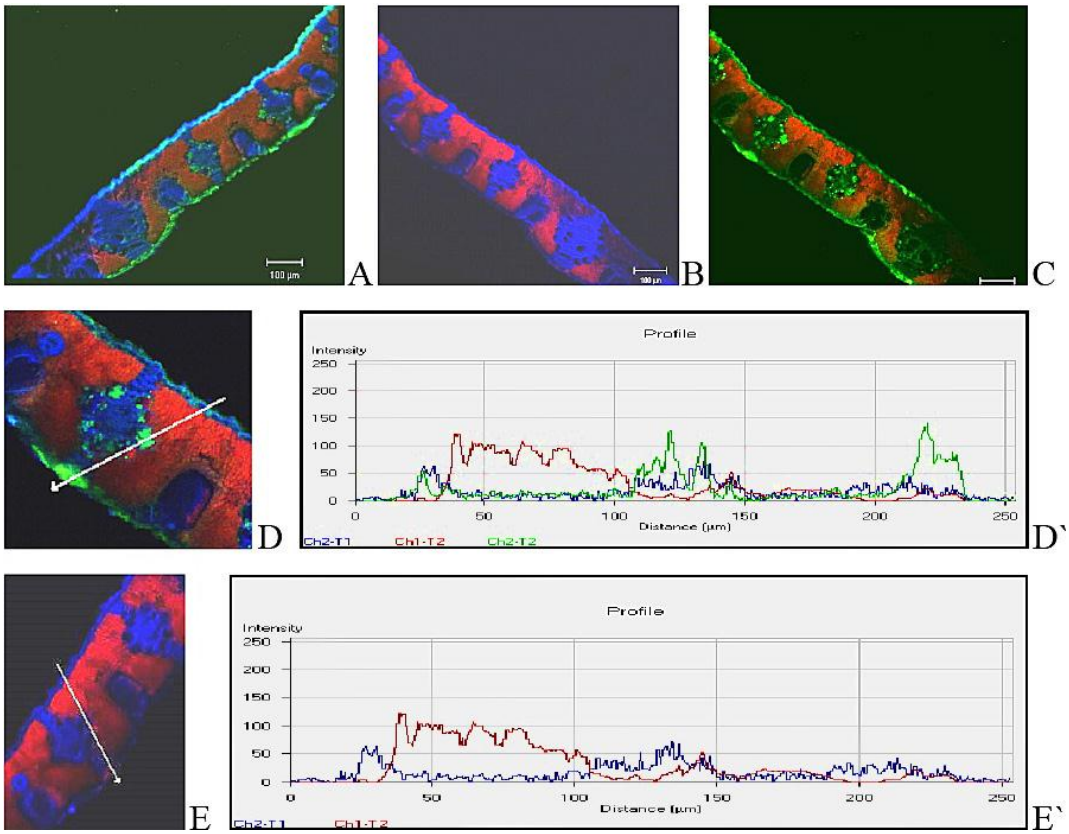

Fig. 2. Micrographs of cytochemical fluorescence of monolignols in the leaf cells of *Ph. australis* grown in water. Localization of syringyl - blue fluorescence, guaiacyl – green, chlorophyll – red. Monolignols are shown: A - syringyl+guaiacyl; B - syringyl, C - guaiacyl, D, E, D' and E' - histograms of fluorescence intensity of syringyl (blue line), guaiacyl (green line) and chlorophyll (red line). Bars = 100 µm.

shape of epidermal cells is oval, of mesophyll cells - almost round, and rarely elongated - in the area of conduction bundles. The average size of cells of the upper epidermis was (height x width) 33 ± 1,4 x 63 ± 2,1 µm, the lower epidermis - 38 ± 2,1 x 52 ± 1,7 µm, mesophyll cells - 56 ± 4,3 x 59 ± 2.7 µm, respectively. The average number of chloroplasts per section of one mesophyll cell was 5.82 ± 0.40.

The anatomical and morphological features of terrestrial plants of reed were similar to those that grew in water; the structure of leaf plate was isolateral (Fig. 1, F**)**. Differences were manifested in increasing the length and width of leaf blade. The average size of leaf blade was 71 ± 8.9 cm at long axis and 2.5 ± 0.2 cm - at short axis (Fig. 1, D). The water content of leaves of terrestrial reed was 57.2 ± 0.7%. On the transverse sections of leaves, the upper surface of plate was almost flat, the lower - wavy with the formation of combs in areas of vascular bundle. The area of hollow contains four layers of mesophyll, the area of ridges, near the leading beams - from eight to ten layers. The thickness of leaf in the area of depression was 180-320 microns, the thickness in area of combs, where conductive bundles are, ranged from 300 to 320 µm. The shape of cells of epidermis and mesophyll was similar to that of aquatic plants. The average size of cells (height x width) of the upper epidermis was 11 ± 0.7 x 14 ± 1.1 µm, the lower epidermis - 11 ± 1.7 x 19 ± 1.3 µm, mesophyll cells - 20 ± 1.7 x 17 ± 0.9 µm. The average number of chloroplasts per cut of mesophyll cells was 6.3 ± 0.28.

Laser confocal microscopy

Air-water plants. Cytochemical analysis of leaf monolignols of *Ph. australis* grown in water are shown as blue fluorescence for syringyl and as green fluorescence for guaiacyl in cell walls of epidermis, tissues of vascular bundle (outer parenchyma sheath, inner parenchyma sheath and vessels), sclerenchyma and bulliform cells (Fig. 2, A-C). But the fluorescence intensity of DPBA-syringyl and DPBA-guaiacyl complex was different in the tissues (Fig. 2, D, D', E, E' and Table 1). The fluorescence intensity of syringyl (blue line), guaiacyl (green line) and chlorophyll auto fluorescence (red line) is shown on the histograms (Fig. 2, D' and E'), where the ordinate is fluorescence intensity (in relative units), abscissa—distance (µm), which was scanned on the (D and

Table 1. The intensity of complex DPBA-syringyl and DPBA-guaiacyl fluorescence in leaves of air-water *Phragmites australis* (data are means ± SD of 3 replicates; 30-35 cells of epidermis, 30-35 parenchyma cells of sheath in vascular bundle, 15-20 bulliform cells and 12-15 cells of vessels 30-35 cells of mesophyll in each replicate)

Tissue/cell wall	Intensity of monolignols fluorescence, relative units			S/G
	Syringyl	Guaiacyl	S+G	
Adaxial epidermis				
Periclinal wall	82 ± 11	162 ± 3.7	244 ± 14	0.506
Anticlinal wall	71 ± 3.9	47 ± 1.3	118 ± 5.1	1.511
Abaxial epidermis				
Periclinal wall	62 ± 2.1	130 ± 10	192 ± 12	0.476
Anticlinal wall	33 ± 1.9	71 ± 4.3	104 ± 6.2	0.464
Mesophyll	No observed	17 ± 2.3	17 ± 2.3	0
Vascular bundle				
Vessels	83 ± 12	41 ± 3.7	124 ± 15.3	2.024
Cells of outer sheath	50 ± 3.3	40 ± 3.1	90 ± 6.1	1.250
Cells of inner sheath	80 ± 5.7	39 ± 2.7	119 ± 8.4	2.051
Sclerenchyma	100 ± 9.1	50 ± 4.1	150 ± 13.2	2.000
Bulliform cell	47 ± 3.3	50 ± 4.7	90 ± 8.0	0.940

E) and is shown as white line. Cell walls of sclerenchyma, vessels, inner sheath of bundle and periclinal walls of epidermis had the greatest fluorescence intensity of DPBA-syringyl. DPBA-guaiacyl complex was more only in the periclinal walls of adaxial and abaxial epidermis. It should be noted that in the walls of mesophyll syringyl and guaiacyl were almost not observed, and only single mesophyll cells were characterized by very weak fluorescence. The size of S/G ratio in cells took place in the next order: vessels, inner sheath of vascular bundle, sclerenchyma > walls of outer sheath and anticlinal walls of adaxial epidermis > bulliform cells and anticlinal walls of abaxial epidermis (Tab. 1**).** It was revealed that maximum frequency for syringyl in the epidermal cell was 221040 pixels, the maximum frequency for guaiacyl was 748706 pixels, and the maximum frequency for auto fluorescence of chlorophyll was 887503 pixels. Thus, maximum fluorescence for guaiacyl was almost in three times more than that for syringyl.

Terrestrial plants. The fluorescence of monolignols in leaves of *Ph. australis* grown in terrestrial soil was like to that in leaves of plants grown in water along-shore of the Venetian Strait. Cytochemical analysis of the DPBA-syringyl and DPBA-guaiacyl complex in leaves was revealed blue fluorescence of syringyl and green fluorescence of guaiacyl in cell walls of epidermis, all types' cells of vascular bundle, sclerenchyma and bulliform cells (Fig. 3, A-C). There were some differences in fluorescence intensity of monolignols in cell walls of epidermis, and in walls of bundle sheath. The level of fluorescence intensity of monolignols is presented in Fig. 3 (D, D', E, E') and Tab. 2.

The fluorescence intensity of syringyl (blue line), guaiacyl (green line) and chlorophyll auto fluorescence (red line) is shown on the histograms (Fig. 3, D' and E'), where the ordinate is fluorescence intensity (in relative units), abscissa—distance (μm), which was scanned on the (D and E) and is shown as white line.

It is necessary to note that syringyl was not at all finded in mesophyll of terrestrial plants' leaf, and only weak fluorescence of guaiacyl was observed in some mesophyll cells near adaxial epidermis. The values of the ratio of syringyl to guaiacyl (S/G) in cell walls of terrestrial *Ph. australis'* leaves were situated in such order: cells of outer sheath of vascular bundle > vessels, cells of inner sheath of vascular bundle, sclerenchyma > walls of adaxial epidermis and bulliform cells > walls of abaxial epidermis (Table 2). It is revealed that maximum frequency for monolignols in leaves of terrestrial plants also was more than that in leaves of air-water plants. It was determined that maximum of fluorescence of syringyl in the epidermal cell was 464110 pixels, the maximum of fluorescence of guaiacyl was 519348 pixels, and the maximum of auto fluorescence of chlorophyll was 703855 pixels. Thus, maximum fluorescence of syringyl was in two times more than that in leaves of air-water plants, while the maximum fluorescence of guaiacyl was almost in 1.44 times less than that in leaves of air-water plants.

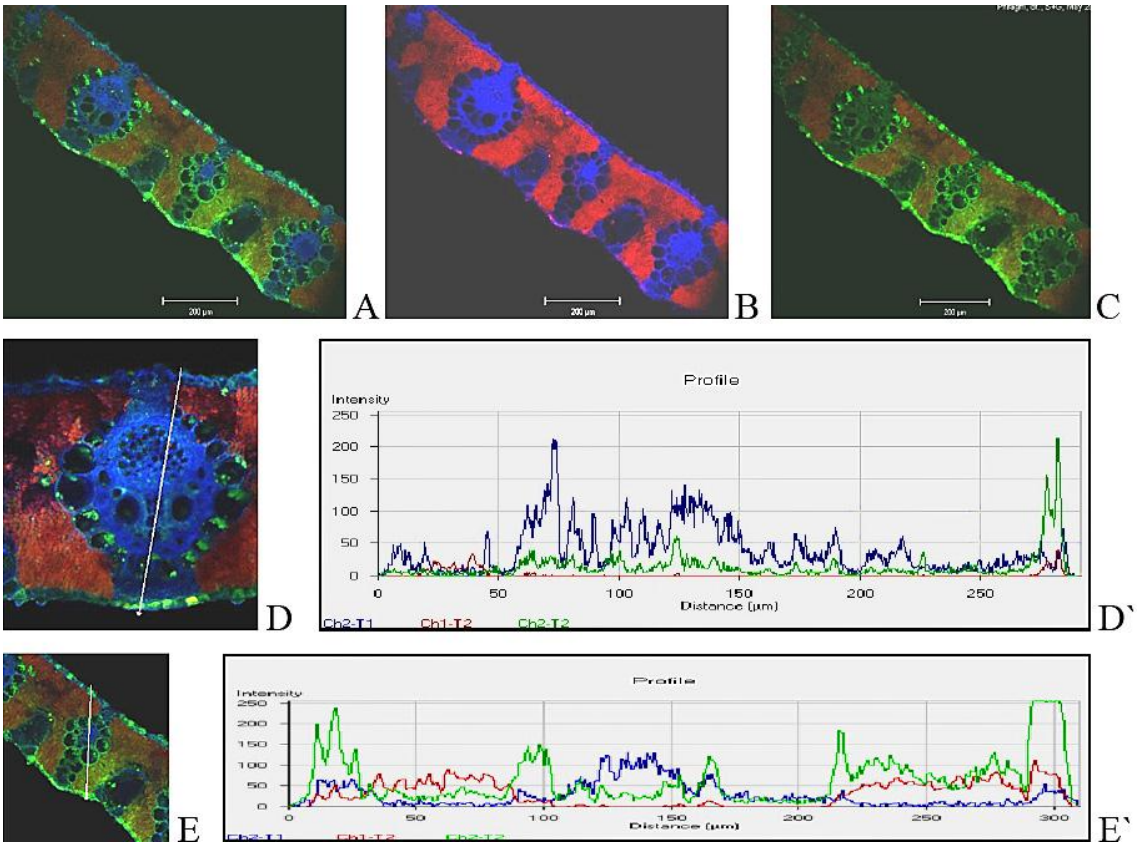

Fig. 3. Micrographs of cytochemical fluorescence of monolignols in the leaf cells of *Ph. australis* grown in terrestrial soil. Localization of syringyl - blue fluorescence, guaiacyl – green; chlorophyll – red. Monolignols are shown: A - syringyl + guaiacyl; B – syringyl; C – guaiacyl, D, D', E and E' - histograms of fluorescence intensity of syringyl (blue line), guaiacyl (green line) and chlorophyll (red line). Bars = 200 µm.

Thus, we have shown that in the phase of vegetative growth of *Ph. australis'* plants grown on the canal bank in the water and terrestrial plants germinating far from the shore had leaves of different size. Terrestrial plants had larger leaves, but the thickness of the leaf blade and cell sizes were smaller than those of air-water plants. It is known that this index is very sensitive to change of moisture in leaves and moisture of soil. It is known that even in case of weak soil drought (-2.2 MPa) in *Zea mays* cells there was observed inhibition of S-phase of cell cycle (Setter & Flannigan, 2001), and in *Helianthus annuus'* leaves there was observed inhibition of G0 and G1 phases (Granier & Tardie, 1999). Besides, it is possible that two ecotypes *Ph. australis* studied by us can be of different ploidy, which is known to influence the size of leaves and cells. There was early established that the octoploid, hexaploid, and decaploid plants of this species (Pauca-Comanescu et al., 1999) differ in cell size and morphological signs. It is shown that the species with smaller ploidy is characterized by the smaller size of cells and nuclei (Robinson et al., 2018).

The early investigations of structural-functional indices of leaves of *Alisma plantago-aquatica* growing in water and on terrestrial soil shown clear differences in structure of cells and some differences of biochemical indices (Nedukha et al., 1998a; 1998b; Kordyum et al., 2003). In these works using the method of light microscopy, electronic microscopy and electronic cytochemistry it was established clear signs of difference in structural and functional indicators of the impact of moderate drought of soil on plants of *Alisma plantago-aquatica* in the phase of vegetative growth and the phase of budding-flowering. They showed that in the leaves of terrestrial plants the cell sizes were smaller, the thickness of cuticle and wax increased 2-5 times, the content of cellulose, carotenoids and chlorophyll (*a*+*b*) increased too, whereas the content of callose in epidermis and mesophyll decreased. Based on the results of our experimental investigations and above-noted data, the following conclusions could be made: the differences in cell size and leaf thickness of *Ph. australis* can be attributed to signs of adaptation to environment and to phenotypic plasticity of studied species that is manifested under the change of both endogenous

Table 2. The intensity of complex DPBA-syringyl and DPBA-guaiacyl fluorescence in leaves of terrestrial *Phragmites australis* (data are means ± SD of 3 replicates, 30-35 cells of epidermis, 30-35 parenchyma cells of sheath in vascular bundle, 15-20 bulliform cells and 12-15 cells of vessels 30-35 cells of mesophyll in each replicate)

Tissue/cell wall	Intensity of monolignols fluorescence, relative units			S/G
	Syringyl	Guaiacyl	S+G	
Adaxial epidermis				
Periclinal wall	135 ± 12	142 ± 2.7	277 ± 14.7	0.950
Anticlinal wall	87 ± 3.7	84 ± 12	171 ± 15,7	1.035
Abaxial epidermis				
Periclinal wall	77 ± 2.2	150 ± 12	227 ± 14.2	0.513
Anticlinal wall	73 ± 2.9	124 ± 10	197 ± 12.9	0.588
Mesophyll	No observed	47 ± 2.7	47 ± 2.7	0
Vascular bundle				
Vessels	137 ± 11	51 ± 2.7	188 ± 13.7	2.686
Cells of outer sheath	200 ± 19	47 ± 2.1	247 ± 21	4.255
Cells of inner sheath	125 ± 10	53 ± 2.5	178 ± 12.5	2.358
Sclerenchyma	125 ± 10	62 ± 3.1	182 ± 13.1	2.016
Bulliform cell	55 ± 3.5	57 ± 4.1	112 ± 7.7	0.965

and exogenous factors. This question remains open and studies to be continued.

The use of cytochemical method and laser confocal microscopy allowed us to reveal the presence of syringyl and guaiacyl monolignols in leaves of *Phragmites australis* grown in different natural conditions. There were the general and different signs concerning these monolignols in leaves of common reed plants in depend on moisture of soil on which these plants grown. General signs were: 1) presence of syringyl and guaiacyl in the investigated leaves regardless of conditions of growth of plants; exceptions were cell walls of mesophyll, in which syringyl was not detected, and guaiacyl - in the form of trace amounts; 2) high values of syringyl in the cell walls of vessel, the cells of sheath of vascular bundle and sclerenchyma. Whereas, different cytochemical signs were next: the increased total content of syringyl and guaiacyl (S+G) and increased ratio of S/G in leaves of terrestrial plants in comparison with that in leaves of air-water plants. We observed the presence of monolignols in epidermis and vascular bundles, whereas it was very poor in single mesophyll cells. Presence of lignin and its monomers in tissues of vascular bundle and epidermis is a known fact and typical sign for many plant species, including *Populus sieboldii* × *P. grandidentata* vessels (Sato et al., 2004), sclerenchyma fibrils and sclereids of *Arundo donax* and *Phragmites karka* leaf (Shakoor et al., 2016**),** in trichoma's and in epidermis internode of *Hordeum vulgare* (Begovici et al., 2015**)**, and other numerous species of Monocotyledons and Dicotyledons (Lourenco, Pereira, 2017).

The increased content of the studied monolignols in the leaves of terrestrial *Phragmites australis'* plants compared with the content of these monolignols in the leaves of air-water plants can be explained as follows. It is known that epidermis is the one barrier and protection of leaf from action of UV radiation, which is provoke intensified synthesis of lignin (Hilal et al., 2004). Besides, lignin and its monomers are the chemical barrier increasing cell protection from penetration of water and invasion of pathogens (Menden et al., 2007)**.** Except it, it is known that this sign (S/G) testifies to the increase of mechanical durability of cells (Christiernin, 2006). Taking into account the above and the results of our experiments about the greatest fluorescence of monolignols in the epidermis cells of terrestrial *Ph. australis* we can suppose that terrestrial plants' leaves of reed receive a higher dose of ultraviolet radiation than leaves of reed in plants growing in water. Based on our experimental data, we believe that the high content of syringyl, which gives high strength to the cells and tissues of terrestrial plants of reed, can serve as a marker for the use of reed plants in various industries.

In addition we see that the presence of these monolignols, and also their ratio in the cell walls of epidermis and vessels correlates with the data received for angiosperms dicotyledons terrestrial species (Baucher et al., 1998). It is known that lignin is the most important secondary metabolite synthesized by phenylalanine/tyrosine metabolism during the differentiation of distinct cell types. Lignin and its monomers participates in adaptation of plant to water regime changes, altering structure of cell wall matrix, providing passage of water and aqueous solutions through cell walls of vessels

(Barros et al., 2015)**.** It is known that many cell types can to synthesize lignin, including elements of conductive bundles, where lignin protects the hydro-mineral composition of cell juice from leaking and counteracts the effects of gravity in sclerenchyma and vascular tissue. Lignin also maintains a mechanical strength of an central axis of organ from wind and rain, and participates in mechanical anti-gravity support of vegetative organs, and also is capable to reinforcement tensile strength of cell wall (Gibson, 2012).

Lignin present in cells of bundle sheath of vascular bundle is like to that in differentiated tissues of xylem and phloem. As a complex phenolic polymer, lignin increases cell wall stiffness of conductive bundles, increasing hydrophobicity of cell walls, it protects plants from invasion of various pathogenic fungi and microorganisms into leaf and stem cells (Schuetz et al., 2014). The differences of relative content of lignin in different epidermal cells which we revealed in *Ph. australis'* leaves can be explained by the next. It is known that peroxidase and laccase are involved in lignin synthesis. These are key enzymes, involved in polymerization of lignin monomers (Berthet et al., 2011). Besides, plant protection during drought occurs not only at the cell level, but also at the molecular one. Researchers noted the increased activity of CoA reductase (cinnamyl-CoA reductase, CCR) enzyme and cinnamyl alcohol dehydrogenase, and the expression of relevant genes (Fan et al., 2006). Taking above-noted and our results, we can suppose that enhanced synthesis of monolignols (S and G) in the leaves of *Phragmites australis* terrestrial plants is due to activation of the respective enzymes.

The phenomenon of an increase in content of lignin in the leaves of terrestrial reed, which grew under conditions of relative drought of soil, is noteworthy. Extreme drought, as well as a high salt stress, usually occurs at the same time and causes osmotic stress, which causes plant cells to lose water, significantly inhibiting plant growth and development, or even can to die (Chaves et al., 2009). Lignin exactly can decrease the water flow off cells, which occurs with complete or partial cessation of transpiration, which helps to support the osmotic balance and cell integrity (Monties, 1998). Taking into account the above mentioned data and the results of our experiments on increasing the ratio S/G in cells of conductive vessels and cells of the upper epidermis in leaves of *Ph. australis* terrestrial ecotype, we assume that the main factor that influenced on this indicator is decrease of soil moisture.

Conclusions

The cytological study of leaves of *Ph. australis* at vegetative stage of growth of plants grown in water and in moderate drought in nature conditions was shown the differences in leaf size, thickness of leaf blade and cell size. Smaller sizes of cells and thickness of the leaf plate of terrestrial plants of reed testify to phenotypic plasticity of the investigated species, which contributes to its growth in moderate drought.

The presence of monolignols syringyl (S) and guaiacyl (G) was detected in the leaves of plants grown on river bank and in plants on moderate drought soil with the cytochemical method and laser confocal microscopy. It was found that the decrease of soil moisture lead to increase of syringyl and guaiacyl content and S/G ratio of in cell walls of the adaxial epidermis and tissues of vessels. We assume that the increase of lignin content in the cell walls of leaf epidermis of the terrestrial plants lead to reduce a cuticular and stomatal transpiration and to optimization of water balance of plants grown in moderate soil drought.

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ВПЛИВ УМОВ ЗРОСТАННЯ НА ВМІСТ МОНОЛІГНІНІВ У ЛИСТКАХ *PHRAGMITES AUSTRALIS*

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За допомогою цитохімічного методу та лазерної конфокальної мікроскопії встановлені відмінності у розподілі та вмісті монолігнінів у листках рослин *Phragmites australis,* що зростали у різних природних умовах. Листки досліджували на стадії вегетативного росту двох екотипів *Ph. australis*. Представлені результати, отримані шляхом порівняння даних для листків *Ph. australis* повітряно-водних та суходільних рослин, що зростали в природних умовах (Київ, Україна). Встановлено, що зменшення вологості ґрунту призводить до збільшення співвідношення монолігнінів сирингілу до гваяцилу та підвищення загального вмісту монолігнінів в епідермісі та тканинах провідних пучків листків суходільних рослин. Висловлено припущення, що зміна співвідношення монолігнінів та зміна їх вмісту в епідермісі листків суходільних рослин очерету є одним з механізмів адаптації рослини до зниження вологості ґрунту, що сприяє зменшенню транспірації та підтриманню оптимального водного потенціалу в листках рослин *Ph. australis*, що зростали на суходолі. На підставі отриманих експериментальних даних зроблено висновок, що високий вміст монолігніну сирингілу, який надає високої міцності листкам та стеблам суходільного очерету, може служити маркером для комерційного використання цих рослин у різних галузях народного господарства.

Ключові слова: *Phragmites australis, лігнін, вологість ґрунту, листки, лазерно-конфокальна мікроскопія*

ВЛИЯНИЕ УСЛОВИЙ ПРОИЗРАСТАНИЯ НА СОДЕРЖАНИЕ МОНОЛИГНИНОВ В ЛИСТЬЯХ *PHRAGMITES AUSTRALIS*

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С помощью цитохимического метода и лазерной конфокальной микроскопии обнаружены отличия в распределении и содержании монолигнинов в листьях растений *Phragmites australis*, которые произрастали в различных природных условиях. Листья исследовали на стадии вегетативного роста двух экотипов *Ph. australis*. В работе представлены результаты, полученные путем сравнения данных для листьев *Ph. australis* воздушно-водных и сухопутных растений, которые росли в естественных условиях (Киев, Украина). Установлено, что уменьшение влажности почвы приводит к увеличению соотношения монолигнинов сирингила к гваяцилу и повышению общего содержания монолигнинов в эпидермисе и тканях проводящих пучков листьев сухопутных растений. Предполагается, что изменение соотношения монолигнинов и их содержания в эпидермисе листьев сухопутных растений тростника является одним из механизмов адаптации растений к снижению влажности почвы, что способствует уменьшению транспирации и поддержанию оптимального водного потенциала в листьях *Ph. australis*, растущего на суше. На основе полученных экспериментальных данных сделано заключение, что высокое содержание монолигнина сирингила, который придает высокую прочность листьям и стеблям сухопутных растений, может служить маркером для их коммерческого использования в различных отраслях народного хозяйства.

Ключевые слова: *Phragmites australis, лигнин, влажность почвы, листья, лазерно-конфокальная микроскопия*