Effects of exogenous bacterial quorum sensing signal molecule (messenger) \(N\)-hexanoyl-L-homoserine lactone (\(C_6\)-HSL) on morphological and physiological responses of winter wheat under simulated acid rain

This research aims to investigate the influence of foliar treatment by bacterial quorum sensing signal molecule/messenger \(N\)-hexanoyl-L-homoserine lactone (\(C_6\)-HSL, 100 ng/ml) on morphological and functional characteristics of \(T\)riticum aestivum \(L\). cv. \(Y\)atran 60 leaves under simulated acid rain (SAR). Acid rain is one of the most dangerous abiotic stressor, which negatively affects the plant growth and development. It changes the permeability and photochemical activity of membranes, leads to the disruption of a chloroplast ultrastructure, stomata system functioning, reduces \(CO_2\) photofixation. Using the scanning electron microscopy, we identified that, after the foliar treatment by \(C_6\)-HSL, the thickness of the leaf cell wall along with the cuticle layer in 20-day-old plants increased by 15 %. Under SAR condition, the plants have suffered the disruption of the cuticular wax layer and uneven wax plates on the epidermis surface, whereas the plants treated with \(C_6\)-HSL showed only a partial cracking of the cuticular wax layer, slight disruption of wax plates, and formation of a wax shell. The \(C_6\)-HSL-treated plants showed the normal functioning of stomata guard cells and the stabilization in the content of photosynthetic pigments. The protective effect of the foliar treatment with \(C_6\)-HSL solution in winter wheat plants under SAR and perspective of its using for the induction of stress resistance has been discussed.

Keywords: \(T\)riticum aestivum, acid rain, \(N\)-hexanoyl-L-homoserine lactone, cell wall, epidermis microstructure, photosynthetic pigments.

Acid rain is one of the most dangerous abiotic stressors that adversely affects the growth and development of a plant, as plants and soil are their main sorbents. Acid rain is typical of countries with a high level of urbanization [1]. The acidity (pH) of pure rainwater is 6.6, while that of acid rain is below 5.5. Acid rains are formed as a result of the reaction between water and sulfur (\(SO_2\))
and nitrogen ($\text{NO}_x$) oxides, which are converted into sulfuric, sulfurous, nitrous, and nitric acid solutions and get into the ground as meteorological precipitation. Rainfall is substantially (up to 60 %) oxidized with anion $\text{SO}_4^{2-}$, which is involved in the oxidation processes that are characterized by free radicals involvement. By oxidizing polyunsaturated fatty acids of cell membranes, free radicals change their permeability, adversely affect the physiological and biochemical processes, and damage the guard cells of stomata. As a result, the stomata remain open or half open, transpiration intensifies, the flow of toxins increases, and photosynthetic cells are damaged [2, 3]. At pH 2.0, plant damage becomes noticeable, dry biomass, photosynthetic fixation, and photochemical activity are decreased [4]. Acid rains cause significant changes in the membrane system of chloroplasts: inhomogeneous allocation of thylakoids inside grana, increasing the distance between thylakoids, thickening of grana thylakoids, disrupted connection between thylakoids and stroma grana. Disturbances in the functioning of individual parts of the photosynthetic apparatus are observed, and the carboanhydrase activity is reduced [5]. Acid rain was reported to reduce the net intensity of photosynthesis of *Cucumis sativus* L. plants, which was associated with a decrease in the maximum potential and effective quantum yield of the photosystem II reaction center. Under these conditions, the activity of peroxidase and superoxide dismutase increased, and the activity of catalase with an increased malondialdehyde content decreased. These results indicate an indirect effect of acid rain on the photosystem II reaction center, which is realized through the peroxidation of lipids and proteins in the thylakoid membrane [6].

*N*-acyl homoserine lactones (AHLs) belong to the class of bacterial quorum sensing signal molecules involved in a distance signal transduction between bacteria-colonizers of plants and bacteria and plant [7]. In recent years, special attention of researchers is paid to the participation of AHLs in the regulation of plant growth and development. Last studies have demonstrated direct (on plants) and indirect (on rhizosphere microflora) effects of AHLs, which offer the challenge for using these compounds in biotechnology for seed priming, and for foliar treatment of plants, in modeling of protective reactions, and induction of genetic resistance [8, 9]. The aim of our work was to study the protective effects of exogenous N-hexanoyl-L-homoserine lactone ($C_6$-HSL) on the microstructure of the leaf lamina surface and on the photosynthetic pigment content in winter wheat plants under the simulated acid rain.

**Materials and methods.** The plants of short-stemmed, mid-early bare, soft wheat cv. Yatran 60, recommended for cultivation in the Forest-Steppe, Polesie, and Steppe regions of Ukraine were studied. The grains were obtained from the collection of the Institute of Plant Physiology and Genetics of the NAS of Ukraine. The calibrated grains were sterilized in 80 % ethanol solution, washed with distilled water, placed in a cuvette with water for 3 h, and then germinated in a thermostat on filter paper moistened with distilled water at $+24$ °C for 21 h.

The germinated grains were planted in 2-liter containers. Calcinated river sand was used as the substrate. The plants were grown under controlled conditions at $+20/17$ °C (day/night), light intensity 690 μmol / (m$^2 \cdot $s), photoperiod 16/8 h (day/night), relative humidity of 65 ± 5 %, the substrate humidity was maintained at 60 % of the total humidity. Irrigation was performed daily using a Knop solution (50 ml per container).

On day 14, the plants were subjected to the foliar treatment with an aqueous solution of $C_6$-HSL, 100 ng/ml, and then kept for 4 days under the above-mentioned temperature and light conditions. To prepare the mixture for simulated acid rain (SAR) $\text{NH}_4\text{NO}_3$ (1.3 g/l),...
MgSO$_4$ $\cdot$ 7H$_2$O (3.1 g/l), Na$_2$SO$_4$ (2.5 g/l), KHCO$_3$ (1.3 g/l), CaCl $\cdot$ 2H$_2$O (3.1 g/l) were used. Obtained solution was diluted with 1 : 100 water and brought pH to 2.8, using 0.3 M H$_3$PO$_4$ and 0.2 M H$_2$SO$_4$, and tween 80 (0.5 % v/v) was added as a surfactant [4].

A mixture of SAR salts (pH 5.6) was used as a control. On day 18, C$_6$-HSL treated and control plants were sprayed with an aqueous solution of a mixture of SAR (pH 2.8) and an aqueous solution of a mixture of salts (pH 5.6) for three minutes and then kept for 2 days under the above-mentioned temperature and light conditions.

Leaf samples were examined using a scanning electron microscope (SEM) JEOL JSM-6060 LA (Japan). The material was dehydrated in solutions of ethyl alcohol of increasing concentration. After the treatment with absolute alcohol, it was transferred and glued to the object tables with an adhesive tape, frozen in liquid nitrogen and dried to air-dry state in a freeze-dryer. In order to give them conductivity, they were coated with a layer of gold in an ion pollinator. The dimensions of the structures in the microphotographs were determined using the UTHSCSA Image Tool 3.0 with a scale bar set on the image.

Photosynthetic pigments were extracted with 80 % acetone and detected by the method of Wellburn [10]. The experiments were performed in two biological and three analytical replicates. The significance of the difference was assessed by Student’s t-test using a 5 % significance level ($P \leq 0.05$, $P \leq 0.001$). The values presented correspond to the mean and their standard errors.

**Results and discussion.** Adaptation of plants is controlled by a molecular genetic system that provides support for homeostasis and protects cellular components from destruction under

![Fig. 1. Cross-section of leaf *Triticum aestivum* L. cv. Yatran 60 under SEM: cell walls of leaf epidermis with cuticle layer in control (a); after foliar treatment with C$_6$-HSL (b); SAR (c); pre-treatment with C$_6$-HSL followed by SAR (d)](image-url)
Effects of exogenous bacterial quorum sensing signal molecule (messenger) N-hexanoyl-L-homoserine lactone...

Extreme external conditions. Leaf, as an organ that exhibits phenotypic plasticity, is one of the universal models for studying the effect of stressors. Our studies have shown that the thickness of the cell wall of the leaf epidermis, along with the cuticle layer of 20-day-old wheat plants, varies depending on the type of foliar treatment (Fig. 1). It is noted that the cell wall thickness of 20-day-old plants treated solely with C₆-HSL was increased by 15% relative to the control. On the contrary, the treatment with SAR caused a significant thinning of the cell wall by 33%, while the pretreatment with C₆-HSL followed by SAR reduced wall thickness of cells only by 9% (Fig. 2).

The epidermal tissue of the cereal leaves is formed by the epidermal cells, trichomes, and stomata. Paracytic stomata are present on both epidermises; to every dumbbell-like guard cell, one semilunar near stomatal cells is connected. We found that both the adaxial and abaxial epidermises in leaf lamina of 20-day winter wheat control plants are characterized by the presence of a well-developed layer of wax (Fig. 3, a). It is formed by multidirectional waxy uneven plates and is observed on all epidermal cells covering basal part of trichomes and stomata guard cells. A well-developed layer of wax is also observed on outer periclinal walls of cells of both epidermises after the foliar treatment with a solution of C₆-HSL (Fig. 3, b). The type of wax deposits on the leaf blade did not change compared with the control (Fig. 3, b). In all specimens subjected to the foliar treatment with SAR, the significant destruction of the epicuticular wax layer on both surfaces of the leaf till its complete disappearance is observed (Fig. 3, c, indicated by arrows). Because of a damage to stomata guard cells, up to 30% of stomata on the surface of the leaf lamina, affected by acid rain, remained open. At the same time, epicuticular wax was not significantly damaged in wheat leaves pre-treated with C₆-HSL after the SAR treatment (Fig. 3, d). There is a slight destruction of the wax plates and the formation of wax crusts on the adaxial surface of the leaves (Fig. 3, d, indicated by arrows). Guard cells of stomata were not damaged, stomata pore was closed. In general, no major destructive changes have occurred.
Cuticular wax plays a vital role for plants, protecting them from abiotic and biotic stresses [11]. Waxes create a continuous hydrophobic barrier to reduce water loss from plant organs and function as the first protective line to reduce the loss of “non stomata” moisture. Reflecting capacity of epicuticular wax regulates temperature and reduces water loss of plants. In addition, epicuticular wax reflects a drop of water, which often carries fungal spores and dust particles [12]. It has been already established that the waxes degrade under the action of atmospheric pollutants: their chemical composition changes, the aging of the cuticle accelerates, which leads to its destruction. It has been found that there are two ways of pollutant action: 1) direct interaction of the wax components with atmospheric toxicants and 2) indirect disruption of the development of cuticle and epicuticular waxes. Erosion of epicuticular waxes increases cuticular transpiration, which in turn, increases the negative effect of atmospheric pollutants and reduces the viability of leaves [13].

It was reported earlier [8] that, after the exogenous treatment of Arabidopsis leaves with a solution of N-3-oxo-tetradecanoyl-L-homoserlinactone (oxo-C14-HSL), stomata closed, and the cell wall thickened. State of stomata guard cells is known to be mainly controlled by abscisic acid (ABA). It was found that, after the treatment with oxo-C14-HSL, components of oxylipin/salicylate-dependent signaling pathway but not ABA-dependent one were involved in regulation of stomata apparatus activity [8].

One of the negative effects of acid rain is a decrease in the content of photosynthetic pigments. The pigment complex actively responds to the environmental signals, and changes in the
Effects of exogenous bacterial quorum sensing signal molecule (messenger) N-hexanoyl-L-homoserine lactone...  

content/ ratio of the pigments serve as a test evaluating the influence of a certain factor on the plant condition. We found that, after the foliar treatment with C₆-HSL solution, the content of photosynthetic pigments was increased significantly, especially, chlorophyll \( a \) – by 35 % and chlorophyll \( b \) – by 37 % (Fig. 4). On the other hand, in the experiment with SAR, the content of photosynthetic pigments on winter wheat leaves was significantly reduced. Chlorophyll \( a \), as the antenna and the reaction center core in both photosystem I (PS I) and photosystem II (PS II), plays a crucial role. Chlorophylls were more sensitive to the SAR treatment: the content of chlorophyll \( a \) decreased by 29 %, chlorophyll \( b \) – by 35 % (Fig. 4). Foliar treatment of wheat leaves with C₆-HSL solution significantly mitigated the negative effect of SAR. Thus, the content of chlorophyll \( a \) decreased by 15 %, chlorophyll \( b \) – by 11 %. Other researchers [14] reported that

![Graph showing the content of photosynthetic pigments in leaves of 20-day-old *Triticum aestivum* cv. Yatran 60 variety in control (1) and after the foliar treatment with C₆-HSL (2), SAR (3) and the pre-treatment with C₆-HSL with subsequent SAR treatment (4). * *, **, *** – \( P \leq 0.05 \) (\( n = 16 \)) compared with the corresponding control.]

**Fig. 4.** The content of photosynthetic pigments in leaves of 20-day-old *Triticum aestivum* cv. Yatran 60 variety in control (1) and after the foliar treatment with C₆-HSL (2), SAR (3) and the pre-treatment with C₆-HSL with subsequent SAR treatment (4). * *, **, *** – \( P \leq 0.05 \) (\( n = 16 \)) compared with the corresponding control.

### Influence of simulated acid rain on the ratio of photosynthetic pigments in leaves of 20-day-old *Triticum aestivum* cv. Yatran 60

<table>
<thead>
<tr>
<th>Sample</th>
<th>Chl ((a + b)) mg/g of fresh weight</th>
<th>Chl (a/b)</th>
<th>Chl ((a + b)/)car</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.798 ± 0.113</td>
<td>1.98</td>
<td>5.87</td>
</tr>
<tr>
<td>SAR</td>
<td>1.255 ± 0.081*</td>
<td>2.12</td>
<td>4.56</td>
</tr>
<tr>
<td>C₆-HSL+SAR</td>
<td>1.555 ± 0.102*</td>
<td>1.9</td>
<td>5.45</td>
</tr>
<tr>
<td>C₆-HSL</td>
<td>2.444 ± 0.122*</td>
<td>1.96</td>
<td>6.85</td>
</tr>
</tbody>
</table>

* Significant differences between the control and experimental groups for \( P \leq 0.05 \) (\( n = 16 \)).
overall, as the acidity of precipitation per unit pH was increased, the chlorophyll content in leaves of trees, shrubs, and grasses was decreased by 6.71%. At the same time, no significant difference in the fluctuation of the chlorophyll content under acid rain was found between the species of calcephyles, calcephybes, and ubiquists, indicating that there is no correlation between soil acid sensitivity and leaf damage by acid rain [14]. Carotenoids (Table) were the least sensitive to SAR, which, as components of the antioxidant system, are involved in the detoxification of oxidative stress products. Similar effects of acid rain on the content of photosynthetic pigments in *Lolium perenne* L. plants have been reported in [15].

The total number of chlorophylls $a+b$ in the leaves of wheat under the action of SAR was decreased by 1.4 times relative to the control (Table), indicating a decrease in photochemical activity. In the experiment with the $C_6$-HSL pretreatment and subsequent SAR, changes in the amount of chlorophylls $a+b$ are insignificant (by 1.16 times, see Table), which indirectly indicates the protective effect of $C_6$-HSL on the photosynthetic apparatus of cells under the conditions of such aggressive stressor as SAR. It should be noted that the sum of chlorophylls $a+b$ in wheat leaves treated exclusively with $C_6$-HSL was increased by 1.36 times, indicating a positive effect of $C_6$-HSL on the photochemical activity. The degree of formation and functioning of the photosynthetic apparatus under the action of unfavorable environmental factors characterizes the indicator of the ratio between chlorophylls $a$ and $b$. Due to the action of SAR, the ratio $a/b$ is the highest (Table) as the content of chlorophyll $b$ is reduced, which, in turn, negatively affects the functioning of light-harvesting complexes of FS I and FS II. The ratio of chlorophylls $a+b/car$ is an informative indicator of the intensity of damage caused on photosynthetic apparatus. The lower the ratio, the greater the damage caused by aging or stress. We found that, under the SAR conditions, the ratio of chlorophylls $a+b/car$ was decreased by 23%, whereas, after treatment with $C_6$-HSL with subsequent SAR, the ration is decreased by 7% (Table). In the experiment with only the foliar treatment with $C_6$-HSL, the $a/b$ index differs significantly from the control; the value of $a+b/car$ was increased by 16% (see Table), which indirectly evidences the stabilization and strengthening of the photosynthetic apparatus under the influence of $C_6$-HSL.

Acid rain is a serious problem for the environmental balance in the world that interferes with plant growth and developments. Various physiological and morphological characteristics of plants have been negatively affected by acid rain.

The results of our study indicate that simulated acid rain (pH 2.8) caused the damage to stomata guard cells, complete destruction of the wax plates on the epidermis of leaf surface, and significantly suppresses chlorophyll content. The foliar treatment of wheat with an aqueous solution of bacterial quorum sensing signal $N$-hexanoyl-$L$-homoserine lactone resulted in a partial cracking of the cuticular wax layer, slight destruction of the wax plates, and formation of wax crusts. The stomata were closed and the content of photosynthetic pigments was stabilized.

Thus, our results demonstrate the protective effect of the foliar treatment with $C_6$-HSL solution of winter wheat plants under SAR and perspective of its using for the induction of a stress resistance.

*The paper is based on the results of the project № 0120U102936 “Development of innovative biotechnology for increasing the stability and productivity of cereals based on a complex of signaling molecules of plant and bacterial origin for environmental protection and restoration” (2020—2024) funded by the National Academy of Sciences of Ukraine.*
Effects of exogenous bacterial quorum sensing signal molecule (messenger) N-hexanoyl-L-homoserine lactone...

REFERENCES


Received 27.04.2020
І.В. Косаківська, Л.М. Бабенко, 
К.О. Романенко, О.А. Футорна
Інститут ботаніки ім. М.Г. Холодного НАН України, Київ
E-mail: irynakosakivska@gmail.com, lilia.babenko@gmail.com,
katerinaromanenko4@gmail.com, oksana_drofa@ukr.net

ВПЛИВ ЕКЗОГЕННОЇ ОБРОБКИ ВОДНИМ РОЗЧИНОМ 
СИГНАЛЬНОЇ МОЛЕКУЛИ-МЕДІАТОРА БАКТЕРІАЛЬНОГО 
ПОХОЖЕННЯ N-ГЕКСАНОЇЛ-L-ГОМОСЕРИНЛАКТОНУ (C6-ГГЛ) 
НА МОРФОЛОГІЧНІ ТА ФІЗІОЛОГІЧНІ РЕАКЦІЇ ОЗИМОЇ ПШЕНИЦІ 
ЗА УМОВ ДІЇ МОДЕЛЬОВАНОГО КИСЛОТНОГО ДОЩУ

Досліджено вплив фоліарної обробки водним розчином сигнальної молекули-медіатора бактеріального 
походження N-гексаноїл-L-гомосеринлактону (C6-ГГЛ, 100 нг/мл) на структурно-функціональні харак-
теристики Triticum aestivum L. сорту Ятрань 60 за умов дії моделюваного кислотного дощу (МКД). Кис-
лотні дощі належать до найнебезпечніших абіотичних стресорів, які негативно впливають на ріст і розви-
tок рослин. Наслідком кислотного дощу є зміна проникності мембран, порушення в ультраструктурі хло-
ропластів, функціонування продихової системи, зниження фотофіксації СО₂ і фотохімічної активності.
Методом сканувальної електронної мікроскопії встановлено, що товщина клітинної стінки разом із шаром 
кутикули у 20-добових рослин, оброблених C6-ГГЛ, збільшилась на 15 %. Під дією МКД у рослин руйну-
вався шар кутикулярного воску і нерівнокраї воскові пластинки на поверхні епідермісу, тоді як у рослин, 
що були оброблені C6-ГГЛ, спостерігалося лише часткове розтріскування шару кутикулярного воску, не-
значне руйнування воскових пластинок і формування воскових кірок. У рослин, оброблених C6-ГГЛ, за-
фіксоване нормальне функціонування заливаючих клітин продихів і стабілізація у вмісті фотосинтетич-
них пігментів. Обговорюється захисний ефект фоліарної обробки розчином C6-ГГЛ рослин озимої пше-
ниці в умовах МКД та перспективи використання цієї речовини для підвищення стресостійкості.

Ключові слова: Triticum aestivum, кислотний дощ, N-гексаноїл-L-гомосеринлактон, клітинна стінка, мікро-
структура епідермісу, фотосинтетичні пігменти.