ISSN 0206-5657. Вісник Львівського університету. Серія біологічна. 2013. Випуск 62. С. 331–338
Visnyk of the Lviv University. Series BIology. 2013. Issue 62. P. 331–338

УДК 57.044+58.02

SPECTRAL ANALYSIS OF PHOTOSYNTHETIC PIGMENTS OF PLANTS, EFFECTED BY CADMIUM IONS AND SALICYLIC ACID

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Spectral characteristics of photosynthetic pigments of wheat and maize plants, effected by cadmium ions and salicylic acid, were studied. Cadmium impact initiates pigment absorption maxima shifts, what indicate appearance of insignificant amount of pigment derivatives. Decrease in the number of absorption peak shifts influenced by SA confirmed its protective role in stressful conditions. [Cd]-chlorophyll was not detected in extracts from examined plants, hereby indicating indirect effect of Cd ions on chlorophyll.

Keywords: spectral properties, absorption, photosynthetic pigments, chlorophyll, cadmium, salicylic acid.

Cadmium (Cd) is highly toxic trace element, which enters environment mainly from anthropogenic sources, contaminating the soil, water and air. Plants are able to absorb ions of cadmium and other heavy metals (HM) actively and to accumulate them in tissues, what results in significant disturbs of their metabolism. Specifically, loss of chlorophylls, carbohydrates and soluble proteins, increase in reactive oxygen species content and activity of antioxidant enzymes, accumulation of various low-molecular compounds and secondary metabolites are observed in plants under cadmium stress. In view of inhibition of the growth processes and biomass accumulation in cadmium-stressed plants, changes of photosynthesis and respiration are considered as most important [16, 18].

Effect of cadmium on photosynthetic complex of plants has a wide range of negative reactions at different stages of photosynthesis. Accumulation of cadmium ions in plant tissues causes inhibition of the activity of enzymes, involved in chlorophyll biosynthesis, activation of degradation processes, disruptions of thylakoid membranes and chlorophyll-protein complex stability, decay of photochemical processes and changes in Calvin cycle [17]. Divalent metal cations (Cu^{2+}, Zn^{2+}, Cd^{2+}, Hg^{2+} et al.) are able to form stable complexes with chlorophyll. Chlorophyll with HM-substituted Mg atom ([HM]-chl) has lower fluorescence quantum yield, compared with [Mg]-chlorophyll, it is unstable in the excited state, what is resulting in release of thermal energy, so the transfer of energy from the antenna complexes to the reaction centers of thylakoids becomes impossible. All known [HM]-chls have low ability to release electrons from singlet excited state [13]. Formation of [HM]-chl causes non-reversible loss of chlorophyll functional activity. It results in changes of pigment spectral properties, inducing a blue-shift of absorption maxima. Exactly this is using as diagnostic feature of [HM]-chl presence in pigment extracts from [HM]-stressed plants [5, 14].

Although the existence of [HM]-chl *in vitro* has long been confirmed, *in vivo* it is investigated mainly in phototrophic bacteria and cyanobacteria [9], unicellular algae and water plants [13, 14, 19]. In land plants [HM]-chl was not detected yet, while high concentrations of HMs were established in chloroplasts [5, 23].

Search and investigations of biogenic compounds with protective properties is one of the main topics of current phytophysiology. Salicylic acid (SA) is one of the actively investigated compounds for this role. It is known, that levels of SA in plant tissues rapidly increase under stress conditions. Experiments with mutant plants, which were not able to accumulate SA, showed the importance of it in stress reactions [20]. Its ability to bind catalase, modifying antioxidant activity and involvement in the regulation of gene expression are discussed as possible mechanisms of SA action. Signaling function of SA is important; it participates in NADPH-oxidase and MAPK-signaling systems and cooperates with NO, jasmonic acid and plant hormones [21]. SA influence results in metabolic changes for more effective performance of basic physiological functions in stress conditions. It is known to cause oxidative burst in plant cells even after short-term application, initiating the resistance formation. SA can be convenient for its application for agricultural purposes.

Studying HM effect and SA protection on agricultural plants, such as wheat and maize, is important due to intense emission of xenobiotics in farmland. Our previous studies showed, that rapidly decrease of chlorophyll in Cd-stressed *Triticum aestivum* L. and *Zea mays* L. plants is related mainly to pheophytin formation [2]. So we suggested possibility of [Cd]-chlorophyll formation in such plants. The purpose of our study was to establish spectral changes of photosynthetic pigments in extracts from plant leaves for identifying chlorophyll derivatives. We used wheat and maize plants in our experiments, because both of them have great importance in agriculture, particularly in Ukraine and other countries with temperate climate. Also *Z. mays* L. is known as [Cd/Pb]-hyperaccumulator with strong root/stem barrier mechanisms [12], so it gives us opportunity to compare Cd effect on plants with different ability to accumulate HMs.

Matherials and methods

Wheat (cv Podolianka) and maize (cv Zakarpatska zhovta zubovydna) plants were grown in pots filled with 1.5 kg washed and incidérated sand, artificially contaminated with Cd as $CdCl_2 \cdot 2.5H_20$ in concentration of 0 and 25 mg cadmium chloride in kg^{-1} sand. Pot cultivation was carried out in greenhouse under controlled conditions. SA treatment was performed as pre-sowing soaking of seeds in 0.5 mM salicylate during 5 h, control group of seeds was soaked in distilled water under the same conditions. Then seeds were allowed to germinate on moist filter paper in the dark and transported to pot culture. The pots were watered to 60% water holding capacity of the sand and fertilized twice a week with modified Hoagland's nutrient solution (6.7 mM $CaNO_3 \cdot 4 H_2O$, 5 мM KNO_3, 1.2 mM $MgSO_4$, 1 mM NH_4NO_3, 1 mM KH_2PO_4, 0.4 mM $FeSO_4$, 0.4 mM $Na_2EDTA \cdot 2 H_2O$, 0.046 mM H_3BO_3, 0.009 mM $MnCl_2 \cdot 4 H_2O$, 0.77 μM $ZnSO_4 \cdot 7 H_2O$, 0.32 μM $CuSO_4 \cdot 5 H_2O$, 0.05 μM $(NH_4)_6Mo_7O_{24} \cdot 4 H_2O$). Plants with active pigment system formation (14-, 21- and 28-days-old plants) were analysed for spertral changes with spectrophotometer AnalytikJena Specord 210 Plus in the range 400-700 nm with a step of 0.1 nm. Pigments were extracted with 5 ml of 80% acetone from 50 mg of homogenous middle leaves [5].

[Cd]-chl determination in extracts of studied plants was carried out by looking for blue-shift changes of absorption spectra in 80% acetone extracts of pigments [5, 19].

The data were worked out statistically [4].

Results and discussion

Chlorophyll degradation is known as one of aspects of cadmium stress. There are some possible reasons for this process: inhibition of chlorophyll biosynthesis enzymes, destruction of molecules owing to free radical reactions, activation of natural enzymatic processes of chlorophyll catabolism, imbalance of plant mineral nutrition [17]. Chlorophyll degradation in stressful conditions often is followed by decreasing in carotenoids [6, 22]. In some cases stress-initiated accumulation of carotenoids was accumulated, what is related to their antioxidant properties [8]. HM-effected changes of absorption spectra were detected in plants [5, 19] and bacteria [3]. The result of analysis of pigment electronic absorption spectra confirmed changes of their content (Fig. 1, 2). Cd ions caused decrease in photosynthetic pigments content. This effect was leveled by exogenic SA, whose solely influence had positive effect on assimilatory pigments accumulation (Fig. 1, 2).

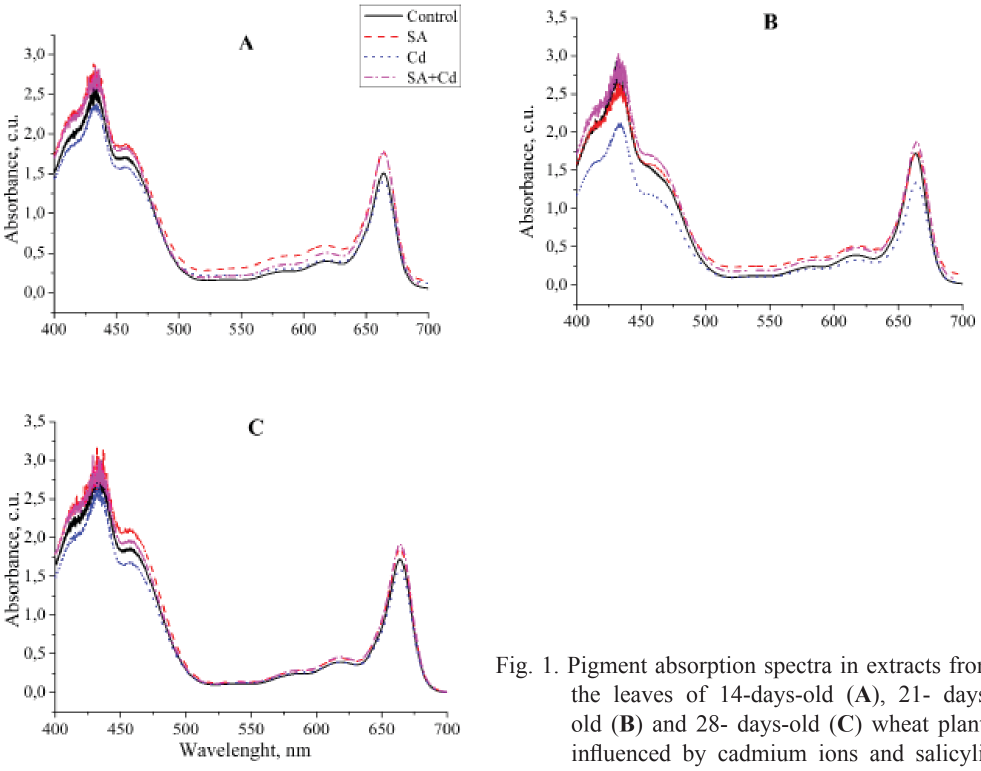

Fig. 1. Pigment absorption spectra in extracts from the leaves of 14-days-old (**A**), 21- days-old (**B**) and 28- days-old (**C**) wheat plants influenced by cadmium ions and salicylic acid.

Registration of absorption peaks in spectral analysis showed the age-related changes in pigment composition of plants and resulted in Cd and SA impact. In the early stage of plant development (14 day) appearance of absorption peaks in the area of 456-458 nm was observed. They were found in both investigated plants and were subjected to some shifting caused by SA. Based on the analysis of shifting absorption peaks of pigments, significant displacements were observed in Cd-stressed plants: in *Z. mays* – shifts in areas 584 nm (21 day-old plants), 541 and 433 nm (28 day-old plants); in *T. aestivum* – shifts in areas 588 nm (14 day-old plants), 617 nm (21 day-old plants) and 540 nm (28 day-old plants). Such changes of absorption peaks suggest appearance of pigment derivatives, but in negligible concentrations. Exogenous treatment of SA showed insignificant changes of pigments spectral properties. It should be noted that matching occurred in most cases of solely SA effect and with Cd ions (tabl. 1, 2). These results confirm the assumption of our previous investigations and research findings of other scientists [5, 7].

Degradation of pigment-protein complexes under Cd stress, which we observed in previous studies [1], creates favorable conditions for chlorophyll pheophytinisation and phytol removal. So, provided sufficient amount of Cd^{2+} in thylakoid environment, [Cd]-chl can be formed.

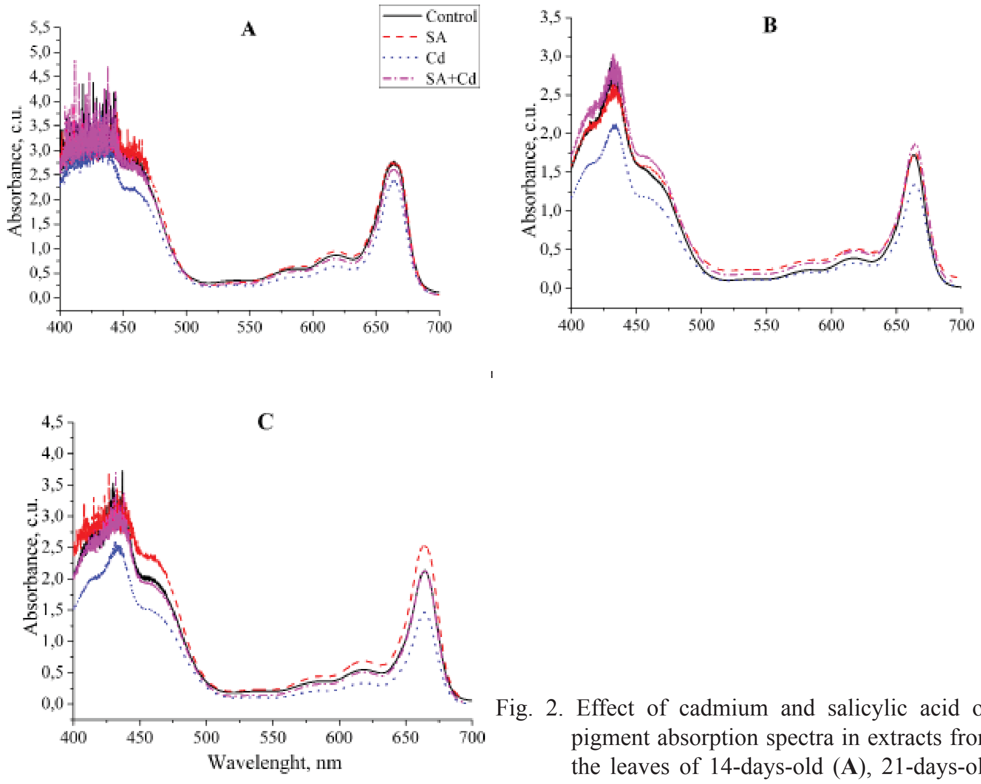

Fig. 2. Effect of cadmium and salicylic acid on pigment absorption spectra in extracts from the leaves of 14-days-old (**A**), 21-days-old (**B**) and 28-days-old (**C**) maize plants.

The results of chlorophyll absorption peaks investigation for identification of [Cd]-chl are presented in table 3. The typical spectral changes (blue shift) were not observed in experimental plants, therefore formation of [Cd]-chl is very unlikely in them. Our data support the idea of other scientists [5], that Cd doesn't have a direct effect on chlorophyll. However, shift in the red area of the spectrum suggest increase in pheophytin content [10] in early stages of the development of Cd-stressed plants, what is consistent with our previous investigations of chlorophyll pheophytinisation in studied plants [2]. In both cases, exogenic SA mitigated pheophytin formation in Cd-stressed plants.

For verification possibility of [Cd]-chl formation in wheat and maize plants, experiments with foliar application of cadmium chloride were carried out. Application of 25 mg/kg $CdCl_2$ by substrate and 25 mg/l $CdCl_2$ by spraying resulted in 40-50% of lethal effect for investigated plants. But, in survived plants [Cd]-chl was also not detected. Less concentrations of sprayed cadmium chloride (15 mg/l) resulted only in chlorophyll and biomass loss. So, we suggest that in higher plants the appearance of [Cd]-chls in stressful condition doesn't occur. [Cd]-chl is considered to be formed and detected difficultly than other [HM]-chls [15].

According to the obtained data and previous results [1, 2, 7], Cd ions have significant impact on composition and functioning of photosynthetic pigments. Method of spectral analysis makes it possible to estimate rapidly and reliably the effects of HM stress on plant pigments. The results can be used for developing rapid method of assessing pigment system of plants under stress conditions.

Studying SA impact on spectral properties of plants in Cd-initiated stressful conditions is important, because such experiments are conducted for the first time. Positive effect of SA is evident, it manifests itself as quantitative (Fig. 1, 2) and qualitative (Tab. 1–3) characteristics of pigments. A. Krantev with coauthors suggested, that protective effect of short-term SA application on photosynthesis of Cd-stressed plants is based on increase in endogenous SA level and activation of specific and non-specific defensive mechanisms [11].

Table 1

Pigment absorption peaks in extracts from *Triticum aestivum* leaves, effected by cadmium and salicylic acid

Control		SA		Cadmium		Cadmium + SA	
λ_{max}	Absorbance	λ_{max}	Absorbance	λ_{max}	Absorbance	λ_{max}	Absorbance
14-days-old plants							
664	1.505±0.069	*664*	1.781±0.093	*664*	1.403±0.072	*664*	1.781±0.075
619	0.395±0.020	*619*	0.596±0.026	*619*	0.411±0.019	*619*	0.504±0.021
588	0.273±0.011	*588*	0.468±0.026	*586*	0.304±0.009	*587*	0.359±0.017
538	0.165±0.005	--	--	*538*	0.218±0.012	--	--
456	1.710±0.089	*456*	1.886±0.055	*456*	1.582±0.073	*457*	1.834±0.079
432	2.575±0.124	*432*	2.899±0.162	*433*	2.405±0.125	*431*	2.734±0.126
21-days-old plants							
663	1.726±0.078	*664*	1.726±0.088	*664*	1.343±0.058	*664*	1.874±0.043
617	0.393±0.018	*618*	0.511±0.021	*619*	0.327±0.013	*618*	0.487±0.014
584	0.243±0.012	*586*	0.371±0.016	*586*	0.214±0.009	*586*	0.331±0.013
538	0.119±0.005	*541*	0.246±0.005	*540*	0.110±0.003	*540*	0.191±0.003
433	2.931±0.164	*432*	2.672±0.130	*434*	2.126±0.091	*432*	3.030±0.139
28-days-old plants							
664	1.719±0.091	*665*	1.855±0.096	*664*	1.592±0.057	*664*	1.912±0.082
619	0.394±0.014	*618*	0.443±0.012	*619*	0.392±0.007	*619*	0.456±0.025
587	0.245±0.010	*588*	0.283±0.012	*587*	0.257±0.011	*588*	0.288±0.012
540	0.110±0.006	*540*	0.136±0.005	*544*	0.124±0.006	*540*	0.130±0.004
433	2.990±0.126	*437*	3.138±0.132	*432*	2.701±0.113	*433*	3.031±0.157

Table 2

Effect of cadmium ions and salicylate on pigment absorption in extracts from *Zea mays* leaves

Control		SA		Cadmium		Cadmium + SA	
λ_{max}	Absorbance	λ_{max}	Absorbance	λ_{max}	Absorbance	λ_{max}	Absorbance
14-days-old plants							
665	2.780±0.144	*664*	2.763±0.134	*665*	2.385±0.091	*664*	2,628±0,068
619	0.864±0.019	*619*	0.947±0.035	*619*	0.637±0.024	*618*	0.794±0.039
586	0.590±0.024	*587*	0.639±0.017	*586*	0.422±0.018	*587*	0.542±0.029
539	0.350±0.020	--	--	*538*	0.250±0.007	*539*	0.298±0.009
457	2.917±0.084	*457*	3.162±0.085	*456*	2.248±0.109	*458*	2.856±0.079
429	4.068±0.113	*430*	3.633±0.131	*428*	3.810±0.145	*428*	3.980±0.155
21-days-old plants							
663	1.726±0.047	*664*	1.753±0.065	*664*	1.343±0.058	*644*	1.874±0.054
618	0.393±0.015	*618*	0.511±0.027	*619*	0.327±0.012	*618*	0.487±0.014
584	0.243±0.013	*586*	0.371±0.014	*586*	0.214±0.009	*586*	0.331±0.017
539	0.119±0.003	*541*	0.246±0.007	*540*	0.110±0.045	*540*	0.191±0.010
433	2.693±0.097	*432*	2.671±0.035	*434*	2.126±0.108	*432*	2.985±0.116
28-days-old plants							
664	2.133±0.086	*664*	2.552±0.133	*664*	1.468±0.082	*664*	2.141±0.083
619	0.548±0.021	*618*	0.688±0.026	*619*	0.334±0.016	*618*	0.510±0.027
587	0.364±0.018	*586*	0.444±0.021	*587*	0.208±0.007	*586*	0.318±0.011
541	0.200±0.007	*540*	0.231±0.009	*537*	0.106±0.005	*540*	0.146±0.008
433	3.339±0.131	*433*	3.463±0.149	*431*	2.618±0.102	*432*	3.701±0.096

Table 3

Chlorophyll absorption peaks in extracts from wheat and maize leaves influenced by cadmium and salicylic acid, nm

Treatment	14-days-old plants	21-days-old plants	28-days-old plants
	T. aestivum L.		
Control	663.1±0.2	663.0±0.2	663.0±0.1
SA	663.0±0.1	663.0±0.1	663.0±0.1
Cadmium	**664.0±0.1**	**663.9±0.2**	663.2±0.2
Cd + SA	663.2±0.2	663.1±0.2	663.1±0.1
	Z. mays L.		
Control	663.1±0.1	663.0±0.1	663.0±0.1
SA	663.0±0.1	663.0±0.1	663.1±0.1
Cadmium	**664.6±0.2**	663.3±0.2	663.2±0.2
Cd + SA	**663.9±0.1**	663.2±0.1	663.0±0.1

Thus, our data supplement investigations of the protective effect of exogenous short-term application SA on photosynthetic pigments in Cd-stressed plants. Detected changes of pigment absorption maxima indicate appearance of the negligible amount of pigment derivatives in stressful conditions. Such changes due to degradation of pigment-protein complexes and activation of enzymatic activities in thylakoid envelope, observed earlier [1, 2]. Absence of [Cd]-chlorophyll complex in leaf extracts support proposition of other researchers [5] about indirect effect of Cd on chlorophyll, in contrast to other HMs.

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Стаття: надійшла до редакції 27.02.13

прийнята до друку 22.04.13

СПЕКТРАЛЬНИЙ АНАЛІЗ ФОТОСИНТЕТИЧНИХ ПІГМЕНТІВ РОСЛИН ЗА ДІЇ ІОНІВ КАДМІЮ ТА САЛІЦИЛОВОЇ КИСЛОТИ

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Встановлено зміни спектрів фотосинтетичних пігментів рослин пшениці та кукурудзи під впливом іонів кадмію і саліцилової кислоти. Вплив кадмію ініціював зсуви максимумів поглинання пігментів, що вказує на появу незначної кількості похідних пігментів. Зменшення кількості зсувів піків абсорбції пігментів під впливом саліцилату підтвердило його захисну роль в умовах стресу. [Cd]-хлорофіл не був виявлений у екстрактах досліджуваних рослин, що свідчить про непрямий вплив іонів кадмію на хлорофіл.

Ключові слова: спектральні характеристики, абсорбція, максимуми поглинання, фотосинтетичні пігменти, хлорофіл, кадмій, саліцилова кислота.

СПЕКТРАЛЬНЫЙ АНАЛИЗ ФОТОСИНТЕТИЧЕСКИХ ПИГМЕНТОВ РАСТЕНИЙ ПОД ВОЗДЕЙСТВИЕМ ИОНОВ КАДМИЯ И САЛИЦИЛОВОЙ КИСЛОТЫ

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Установлены изменения спектров фотосинтетических пигментов растений пшеницы и кукурузы в условиях воздействия ионов кадмия и салициловой кислоты. Воздействие кадмия инициировало сдвиги максимумов поглощения пигментов, что указывает на появление незначительного количества производных пигментов. Сокращение количества сдвигов пиков абсорбции пигментов под влиянием салицилата подтвердило его защитную роль в стрессовых условиях. [Cd]-хлорофилл не был обнаружен в экстрактах исследованных растений, что свидетельствует о косвенном влиянии ионов кадмия на хлорофилл.

Ключевые слова: спектральные характеристики, абсорбция, максимумы поглощения, фотосинтетические пигменты, хлорофилл, кадмий, салициловая кислота.