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PROTECTION OF PHOTOSYSTEM II (PSII) BY NATURAL ANTIOXIDANTS FROM HEAVY METALS - INDUCED STRESS

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The comparative analysis of protective properties of biologically active composition (BAC) on the base of plumbagin (2-methyl, 5-hydroxi, 1.4-naphthoquinone) and Na-ascorbate (Na-asc) under toxic action of Zn^{2+} and Ni^{2+} on photosystem II (PSII) was carried out. The investigations were performed on wheat seedlings (Triticum aestivum L.) by means of introduction of metal ions and protective substances by two ways: simultaneously and consecutive incubations of wheat seedlings. The action of metal ions and defense effectiveness were evaluated by means of analysis of induction curves of delayed fluorescence of chlorophyll a in millisecond range (msec-DF of Chla) in wheat seedlings. The character of induction transitions changes of msec-DF of Chla under action of heavy metals was found to be indicated on suppression of radical pair primary charge separation and electron flow rate to primary quinone acceptor Q_A on donor side of PSII. BAC (at less degree) and Na-asc decreased toxic action of Zn^{2+} and Ni^{2+} that is likely to be due to neutralization of formed superoxide anion radicals, and thus restoration of oxidation-reduction equilibrium between photosystems.

***Keywords**: Ceratostigma plumbaginoides bunge, antioxidants, delayed chlorophyll fluorescence, Zn^{2+}, Ni^{2+}*

Introduction

In oxygenic photosynthesis various ROS (reactive oxygen species) such as superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($^{\bullet}OH$) are generated as a result of photosynthetic transport of electrons. Donor and acceptor sides PS II is known to be more vulnerable part of thylakoid membrane of chloroplast [4]. The generation of ROS is greatly accelerated under stress situation namely at action of heavy metals. Zn^{2+} is known to be main components of many enzymes and Ni^{2+} is in composition of limited plants enzymes. It is to be explained for the reason, that Zn^{2+} exchanged by 10^3 times more rapid than Ni^{2+}. The photosynthetic membrane of chloroplast thylakoids is considered to be main target under stress situation and more vulnerable are chlorophyll-protein complexes of PS II due to their structural peculiarities. Zn^{2+} is assumed to affect on oxidizing side of PS II and may to inhibit of manganese complex. Investigations of Baker *et al.* [3] proposed a second site of Zn^{2+} action situated between PS II and PS I in the electron transfer chain on the level of PQ. In a number of works concerning of Ni^{2+} toxicity on photosynthesis was shown that Ni^{2+} has reduced of Chl content in leaves due to inhibition of its synthesis and destroyed of electron transport from pheophytin through quinone Q_A and Fe on quinone Q_B on account of change of structure of carriers or proteins of reaction center of PSII.

To cope up with damages caused by ROS, cells possess by comprehensive and integrated endogenous antioxidant defense system, which is known to be composed by both enzymatic and as well as non-enzymatic compounds [2, 12]. The compounds belonging to several classes of phytochemical components such phenols, flavonoids, carotenoids, ascorbic acid, glutathione are known are able to scavenge free radicals and are classified as non-fermentative protective systems [15]. The function of antioxidants is to intercept and react with free radicals at rate faster than the substrate, prevent generation of ROS or to scavenge those formed. Low molecular compound Na-asc is known to be able strongly inactivate of free radicals. Na-asc is considered as the most powerful ROS-detoxifying compound because of it donate of electron to ascorbate-peroxidase thus activates this antioxidative enzyme [5].

Thus, Na-asc is considered to level of cell

from damages of plant growth and formation of Chl, induced by ROS [7]. Compound derived from plants still present a large source of natural antioxidants that have been shown to have an antioxidative and or/antiradical scavenging mechanisms [10, 11].

The aim of present paper in this connection was to conduct the comparative analysis of protective properties of natural antioxidant-biologically active composition (BAC) and Na-asc at oxidative stress, induced by heavy metal.

Materials and methods

Preparation and content of biologically active composition (BAC): BAC - bioactive composition on the base of plumbagin obtained by extraction from plant raw material of Ceratostigma Plumbaginoides Bunge. Plumbagin earlier was obtained by multi-stage extraction method with usage of organic solvents (hexane and sulfuric ether) and chemical substances NaOH and H_2SO_4. For our purposes plumbagin was obtained by modernized method of extraction that differs by technological simplicity. The fresh collected and grounded roots and rootlets of Ceratostigma plumbaginoides, Carawey (*Carum carvi*) and coriander (*Coriander sativum L.*) grounded seeds in optimal weight relation: 2 part ceratostigma, 1 part caraway, coriander mixed up and then after addition of water in 1:7 ratio is located indisstilation apparatus and is keeping during 24 hours under temperature of 60-65°. The mix then is distillated with water vapor. The biologically active composition contained 50 mg/l plumbagin (2-methyl, 5-hydroxi 1.4-naphthoquinone), 75ml/l ether oils of caraway and coriander.

The main compound of biologically active composition plumbagin is known to be a wide range natural antibiotic. The ether oils obtained from seeds of caraway and coriander equally with ensuring of dissolving of plumbagin with water high amount. The main components of caraway and coriander ether oils the carvacrol, carvone, limonene, linalool, n-simol amount 60-70% of composition [14].

Heavy metals treatmeant conditions:

Investigations were carried out on 7–9 days wheat seedlings (*Triticum aestivum* L.) grown on distillated water at room condition. To determine of heavy metal toxic action the 7–days seedling were incubated during 24 hours in solution containing $NiCl_2$ (10^{-3}M) and $ZnCl_2$ (10^{-3}M). To protect of thylakoid membranes from oxidative stress induced by action of heavy metal the 2 substances possessing by antioxidant properties: Bioactive composition ((BAC) 50mg/l) and Na-asc ($4•10^{-4}$M) that is considered as the most powerful ROS-detoxifying compound were used. The experiments were conducted into 2 variants: 1. 7-days seedlings of wheat (*Triticum aestivum L.*) were incubating during 24h in medium containing: BAC+Zn^{2+}, Na-asc+Zn^{2+}, BAC+Ni^{2+}, Na-asc+Ni^{2+}. 2. 7-days seedlings were incubated during 24h in solution, containing Na-asc and in solution with BAC and then were transferred to different solutions containing Zn^{2+} (BAC→Zn^{2+}, Na-asc→Zn^{2+}) and Ni^{2+} (BAC→ Ni^{2+}, Na-asc→Ni^{2+}) during 24h.

Measurement of PSII activity: To evaluate the functional activity of PSII in wheat seedlings and its stability to unfavorable factors of medium on the base of analysis of kinetic curves of induction transitions of chlorophyll *a* millisecond delayed fluorescence (msec-DF Chl*a*) were implemented. Analysis of induction curves of msec-DF Chl*a* characterized the state of reaction center of PSII and its nearest surroundings on donor (fast phase, (f.ph)) and acceptor (slow phase, (sl.ph)) sides. The measurements were conducted on fluoremeter including a phosphoscope in such way that 0.3 ms of excitation was following by 1.25 ms of dark and 0.3 ms of the delayed light emission registration. Investigations were carried out in vivo on cutting from leaves of seedlings, situated to special holder in front of photomultiplier.

Results and discussion

Influence of Zn and Ni ions to the activity of FSII: The state of PSII under toxic action of Zn^{2+} and Ni^{2+} salts on wheat seedlings were diagnosed by changes of induction of msec-DF Chl*a*, that in intact system is known to be in close connection with metabolitic processes and is regulated by cell in accordance with its energetic requirements. The character of induction transitions changes of msec-DF Chl*a* was dependent from action of heavy metal [6].

Table 1

Action of BAC and Na-asc on changes of steady state (s.s) level of induction curve of msec-DF of Chl*a*

	1st variant	2nd variant	1st variant	2nd variant
Zn^{2+} 1.4±0.06	BAC+ Zn^{2+} 1.7±0.1	BAC→ Zn^{2+} 1.7±0.13	Na-asc + Zn^{2+} 2.6±0.06	Na-asc→Zn^{2+} 1.1±0.1
Ni^{2+} 0.7±0.03	BAC+ Ni^{2+} 1.5±0.07	BAC→ Ni^{2+} 1.3±0.15	Na-asc + Ni^{2+} 0.7±0.03	Na-asc→Ni^{2+} 0.9±0.06

Note: Experiments were performed by 2 variants (*see*: Materials and methods). Intensity of steady state (s.s) level without of treatment by heavy metals (control) was taken as 1.0.

Let us to consider the results connected with steady state (s.s) level of induction curve of msec-DF of Chl*a*, reflecting of electrons outflow from Q_B to cytochrome b_6/f complex due to action of heavy metal (Table. 1).

Action of Zn ions lead to increasing of DFs.s up to 1.4 relatively to control. Simultaneously introduction of heavy metal with Na-asc is shown to increasing of DFs.s of msec-DF of Chl*a* (Table. 1).

Antioxidant activity of BAC during the influence of heavy metals: Under toxic action of Zn^{2+} and Ni^{2+} the antioxidant properties of BAC were determined. The Na-asc that is considered to play critical role in protection from oxidative stress was used [15]. Preliminary maintaining of seedlings in solution with Na-asc with subsequent action of Zn ions reduced of DFs.s almost up to control. BAC however is shown to not reveal of protective ability at presence of Zn^{2+} in both variants of its introduction to growing medium and leads to DFs.s increasing action of Ni ions in contrast to Zn ions is found to lead to decreasing of DFs.s level of msec-DF of Chl*a* by up to 30%, at persevere of Na-asc in the case of successive variant its action in respect to DFs.s character is weaken up to 20% and approaches to control. The effect of BAC as it is seen from Table.1 is absent at both variants and stable increases of DFs.s level. Action of Zn and Ni ions result in decreasing of DFf.ph/s.s and DFsl.ph/s.s (Fig.1 and 2). The effect of suppressing action of Zn^{2+} excised effect of Ni^{2+} action nearly by 2 times higher for DFsl.ph/s.s. The simultaneous introduction of Na-asc with heavy metal does not weaken of Zn^{2+} toxic action, whereas at the case of Na-asc+Ni^{2+} the value DFsl.ph/s.s is restored to control. Na-asc changed this correlation in the case of its successive introduction only in experiment with Zn^{2+}. The restoration of DFf.ph/s.s value nearly by 2 times higher is observed relatively to Zn^{2+} action and exceed control level the value of DFsl.ph/s.s is increased relatively action of Zn ions to 1.3 time (Fig.1 and 2).

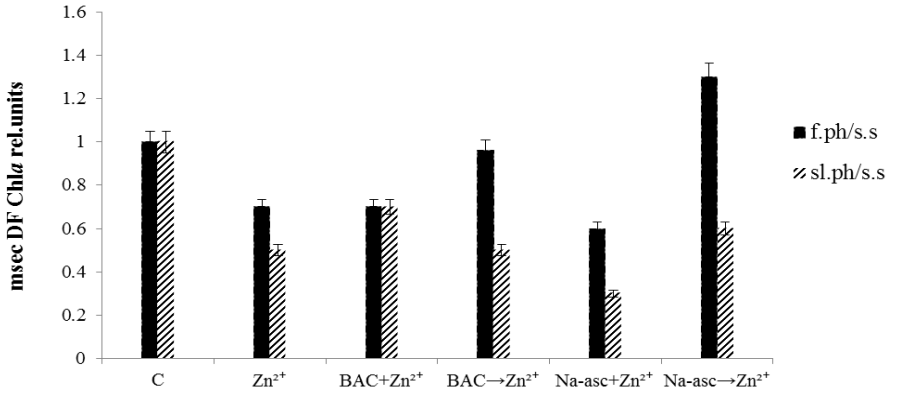

Fig.1. Action of BAC and Na-asc on changes of value of f.ph/s.s and sl.ph/s.s of msec-DF of Chl*a*, suppressed by Zn^{2+}. Experiments were performed by 2 variants: 1. 7–days seedlings of wheat (*Triticum aestivium L.*) were incubating during 24h in medium containing: Na-asc+Zn^{2+}, BAC+Zn^{2+} 2. 7–days seedlings were incubating during 24h in solution, containing Na-asc ($4 \cdot 10^{-4}$M) and in solution with BAC (50 mg/l) and then were transferred to solution containing Zn^{2+} (Na-asc →Zn^{2+}, BAC→ Zn^{2+}) during 24h

Effect of BAC at its simultaneous introduction with heavy metal was manifest only in presence of Zn^{2+}. The DFsl.ph/s.s value was increased by 1.4 times with respect to Zn^{2+}. The BAC effect was observed greater in the case of its simultaneously introduction with heavy metal. As it is seen on Figure (1 and 2) the value of DFf.ph/s.s with Zn^{2+} was restored to control level whereas in experiments with Ni^{2+} this value increased relatively to action of only Ni^{2+} by 1.2 times. The restoration of correlation value DFsl.ph/s.s has not observed. Observed change DFs.s fully reflect the state of oxidation-reduction reactions, connected with transfer of electrons. The change of correlation between charge separation at RC PSII with formation of the primary radical pair $P680^+$ Phe^- and electron transfer rate to primary quinone acceptor Q_A (DFsl.ph) to DFs.s characterized of donor and acceptor state of electron transfer chain.

Ascorbate is known to react and effectively neutralized superoxide anion radicals, formed under stress and detoxified of H_2O_2 as donor of electrons to ascorbate peroxidase or O_2^- and $^{\bullet}OH$ preventing by such way of enzymes inactivation and supports of oxidation-reduction equilibrium between photosystems [9]. An important role Na-asc plays in protecting of enzymes activity, containing of prosthetic ions of metals, supporting their in reduced forms. Many spicy plants are today considered as the important sources for the extraction of compounds with strong antioxidant activity [1]. Although there are some data about antioxidative effects of coriander and caraway essential oils, this activity is usually attributed to existing phenolic compounds. However, there are very few data that describe the potential antioxidant properties related to chemically well-characterized essential oils of coriander and caraway, which are crucial in phytotherapy [8]. The BAC main component-plumbagin is known to stabilized of light harvesting complex the most important component of photosynthetic membrane, keeping its stability due to ability of quinone antioxidants to dissolved in lipids and lowering of rate lipids peroxidation. BAC appears to be more accessible to site that is damaged by Ni^{2+} and is localized at the range of quinones functioning that is on acceptor side of PSII. It is explanation for effect only for state of DFf.ph/s.s (Fig. 2). Thus, protective effect of Na-asc under toxic action of Zn^{2+} relating to value of DFf.ph/s.s in second variant surpass of BAC effect by up to 36%,at that time in the case of Ni^{2+} protective effect of BAC is found to be greater by up to 20%.

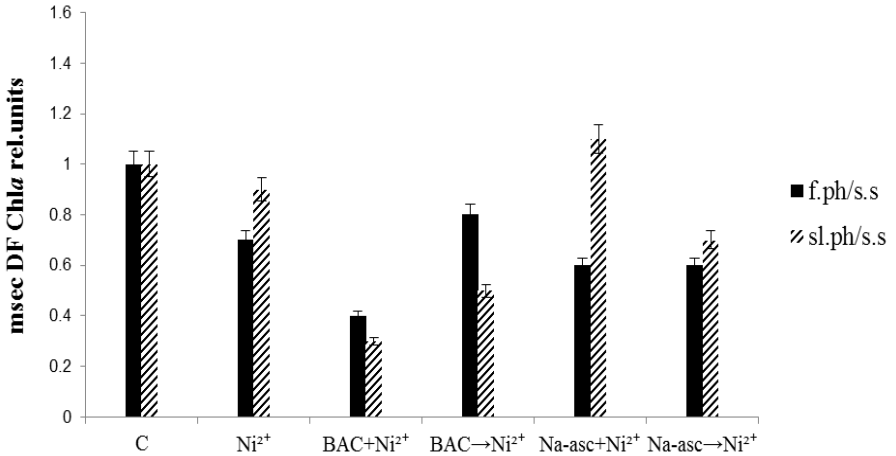

Fig.2. Action of BAC and Na-asc on changes of value of f.ph/s.s and sl.ph/s.s of msec-DF of Chl*a*, suppressed by Ni^{2+}. Experiments were performed by 2 variants: 1. 7-days seedlings of wheat (*Triticum aestivium L*) were incubating during 24h in medium containing: Na-asc+Ni^{2+}, BAC+Ni^{2+} 2. 7-days seedlings were incubating during 24h in solution, containing Na-asc ($4 \cdot 10^{-4}$M) and in solution with BAC (50mg/l) and then were transferred to solution containing Ni^{2+} (Na-asc →Ni^{2+}, BAC→ Ni^{2+}) during 24h

The toxic action of heavy metal leads to disturb the equilibrium between the photosystems and redox state of Q_A displacement, increasing of outflow of electrons to PSI [13]. This result in s.s increase and phases relation fall up to stationary level of fluorescence. It is not excluded that changes of fluorescent characteristics are connected with breakdown of chlorophyll in chlorophyll-protein complexes of PS II, which also brings its considerable changes to Chl*a* fluorescence and phase character of induction curve.

BAC, unlike from action of Na-asc, possibly is not always capable to fully neutralized of superoxide radicals (life time~2ms) that have time to migrate to PS II surrounding.

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FOTOSİSTEM II-NİN (FSII) AĞIR METALLARLA İNDUKSİYA OLUNMUŞ STRESSDƏN TƏBİİ ANTİOKSİDANTLARLA MÜDAFİƏSİ

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Zn^{2+} və Ni^{2}-in fotosistem II-yə (FSII) toksiki təsiri zamanı plumbagin (2-metil, 5-hidroksi, 1.4-naftoxinon) əsaslı bioloji aktiv kompozisiya (BAK) və Na-askorbatın (Na-ask) müdafiə qabiliyyətinin müqayisəli tədqiqi aparılmışdır. Buğda bitkilərində (*Triticum aestivum L.*) tədqiqatlar məhlulda cücərtilərin köklərinin inkubasiyası yolu ilə aparılmışdır. Buğda bitkisinə metal ionların təsiri və müdafiə qabiliyyəti xlorofil *a*-nın millisaniyə gecikmiş flüoressensiyasının (Xl*a* msan-GF) induksiya əyrilərinin analizi vasitəsilə qiymətləndirilmişdir. Ağır metalların təsiri nəticəsində Xl*a*-nın msan-GF induksiya keçidlərinin dəyişməsinin xarakteri sayəsində FSII-nin donor tərəfində Q_A ilkin xinon akseptoruna elektron axını və ilkin radikal cütlüklərin yüklərinin paylanmasının azalması müəyyən edilmişdir. BAK (az miqdarda) və Na-ask Zn^{2+} və Ni^{2+} təsirindən formalaşan superoksid anion radikallarını neytrallaşdıraraq onların toksiki təsirini azaltmış və fotosistemlər arasında oksidləşmə-reduksiya balansını bərpa etmişdir.

Açar sözlər: *Ceratostigma plumbaginoides bunge, antioksidantlar, xlorofilin millisaniyə gecikmiş flüoressensiyası, Zn^{2+}, Ni^{2+}*

ЗАЩИТА ФОТОСИСТЕМЫ II (ФСII) НАТУРАЛЬНЫМИ АНТИОКСИДАНТАМИ ОТ СТРЕССА, ИНДУЦИРОВАННОГО ТЯЖЕЛЫМИ МЕТАЛЛАМИ

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Проведен сравнительный анализ защитных свойств биологически активной композиции (БАК) на основе плюмбагина (5-гидрокси-2-метил-1,4-нафтохинон) и Na-аскорбата (Na-аск) при токсическом действии Zn^{2+} и Ni^{2+} на фотосистему II (ФСII). Исследования осуществлялись на проростках пшеницы (*Triticum aesivum* L.) путем введения ионов металлов и защитных соединений двумя путями: одновременной и последовательной инкубации проростков пшеницы. Действие ионов металла и эффективность защиты оценивались в результате анализа индукционных кривых замедленной флуоресценции хлорофилла *a* в миллисекундном диапазоне (мсек-ЗФ Хл*a*). Характер изменений индукционных переходов мсек-ЗФ Хл*a* при действии тяжелых металлов, как было найдено, отражается в подавлении разделения заряда первичной радикальной пары и потока электронов к первичному хиноновому акцептору Q_A на донорной стороне ФСII. БАК (в меньшей степени) и Na-аск уменьшали токсическое действие Zn^{2+} и Ni^{2+}, вероятно, путем нейтрализации образующихся супероксид анион радикалов, восстанавливая окислительно-восстановительное равновесие между фотосистемами.

Ключевые слова: *Ceratostigma plumbaginoides bunge, антиоксиданты, замедленная флуоресценция хлорофилла, Zn^{2+}, Ni^{2+}*