Journal of Environmental Biology **November 2009,** 30(6) 1055-1058 (2009)
©Triveni Enterprises, Lucknow (India) For personal use only
Free paper downloaded from: www.jeb.co.in Commercial distribution of this copy is illegal

Effect of seabuckthorn extract on delayed chlorophyll fluorescence on Cd and Co ions treated wheat seedlings

R.A. Ganiyeva[1], E.M. Novruzov[2], S.A. Bayramova[1], I.M. Kurbanova[1] and R.A. Hasanov*[1,3]

[1]Biophysics laboratory, [2]Plant Resources Department, Institute of Botany, National Acadamy of Science, Baku-AZ 1073, Azerbaijan
[3]Department of Biophysics and Molecular Biology, Baku State University, Baku-AZ1148, Azerbaijan

(Received: June 23, 2008; Revised received: November 25, 2008; Accepted: November 25, 2008)

Abstract: *The protecting effect of "Hypporamine PL" compound isolated from dry leaves of seabuckthorn (Hippophae rhamneides L.) on photosystem 2 (PS 2) activity suppression induced by $CdCl_2$ and $CoCl_2$ treatment in the 7 day old wheat seedlings (Triticum aestivum L.) under different pH of growth medium was investigated by measurement of millisecond delayed fluorescence (ms-DF) of chlorophyll intact leaves. The value o-i/p-s of ms-DF ratio was reduced under the Cd^{2+} and Co^{2+} treatments on 60 and 65% respectively at pH 6.7. Acidification of medium (pH 5.0) results in decreasing of ratio o-i/p-s only approximately on 30% in average. In the alkaline medium the lowering of o-i/p-s on 41% is observed in both ions treatments. This decreasing of o-i/p-s ratio occurred due to decreasing of fast phase o-i amplitude. At the same time the widening and increasing of slow phase p-s amplitude was observed. The compound "Hypporamine PL" limited the decrease of ms-DF components induced by heavy metals. It is suggested that the protective effect of "Hypporamine PL" on the photochemical reactions in the PS2 is due to catechins, epicatechins, quercetin and other polyphenols, containing in this compound, preventing the free radicals formation in the PS2 under treatment by heavy metal ions.*

Key words: *Heavy metal ions, Seabuckthorn extract, Delayed fluorescence, Photosystem 2*
PDF of full length paper is available online

Introduction

Increasing evidence suggests that membrane injury by various stress factors including metal toxicity is related to an increased production of highly toxic oxygen free radicals (Meriga *et al.*, 2004; Bertrand and Poirier, 2005; Umebese and Motajo, 2008; John *et al.,* 2007; Hasan *et al.,* 2009). Cadmium and Cobalt, a divalent cations, are most phytotoxic heavy metals, although has not been clearly established how plants respond to heavy metals. It is well known that cadmium exerts its phytotoxicity by interfering with several basic events of plant growth, development and physiology. A main effect of this metal, observed in most plants, studied to date is the inhibition of photosynthesis and chlorophyll being one of the targets (Somashekarich *et al.*, 1992). Studies investigating cadmium toxicity on chloroplast function and electron transport have suggested that this heavy metal can exert most of its effects on photosystem 2 (PS2). The effects of exposure of PS2 at different Cd concentrations is inactivation of the $CaMn_4$-cluster (Pagliano *et al.*, 2006). The donor side inhibition of PS2 by Cd^{2+} was described first by (Bazzaz and Govindjee, 1974). The toxicity of Cd^{2+} has been related with the increase of lipid peroxidation and alterations in antioxidant systems by increasing superoxide radical production and lipid peroxidation or by changes the enzymatic and nonenzymatic antioxidants (Gallego *et al.*, 1996; John *et al.*, 2007) in addition DNA damage (Min *et al.*, 2008). The effect of growing pea plants with $CdCl_2$ on different physiological parameters and enzymatic antioxidants was studied in order to know the possible involvement of cadmium in the generation of oxidative stress. Results obtained suggest that Cd^{2+} can induce a concentration-dependent oxidative stress situation in leaves (Sandalio *et al.*, 2001) as a result of inhibition of the antioxidative enzymes.

* **Corresponding author:** ra38hasan@yahoo.com

The high concentrations of Co^{2+} inhibit the electron transport at the acceptor side of PS2 (Mohanty *et al.*, 1989). These authors reported that the effect of Co^{2+} is possibly due to their role in modifying the function of Q_B, thereby impairing the PS2 activity. Co^{2+} can impair the Q_B function in three possible ways. Interruption of electron flow between Q_A and Q_B, direct modification of Q_B or alteration of components beyond Q_B.

It was shown that metals toxicity and subcellular distribution depend on pH (Burzynski, 2001; Rout and Das, 2003). Recent studies indicate that compensatory mechanisms exist to afford adequate protection to the photosynthetic apparatus as the whole set of antioxidant (flavonoids, isoprenoids and enzymatic antioxidants) defenses (Russo *et al.*, 2000; Hernander *et al.,* 2004). DF is known to be the recombination process between primary donor in PS2-P 680 with intermediate products containing as a rule a reactive oxygen species, generated as a result of incomplete reduction of oxygen during photosynthesis.

In the present work, the effect of exposure of wheat chloroplasts to cadmium and cobalt action at different pH of growth solution on suppression of PS2 activity by means delayed fluorescence (DF) of Chl *a* measurement *in vivo* were analyzed. The protecting effect of "Hypporamine PL" compound on wheat seedlings treated by heavy metal ions was also examined.

Online Copy

Materials and Methods

The leaves of wheat (*Triticum aestivum*) seedlings after 2 days of germinating were transferred to solution supplement with the same $CdCl_2$ and $CoCl_2$ (10^{-3}M) concentrations on various pH of medium (6.7; 5.0; 9.0) for 5 days. The kinetics of ms-DF was measured using a phosphoroscope as described previously (Rubin *et al.*, 1984; Gasanov *et al.*, 2007). A sample of leaves in special holder was irradiated with continuous white light (250 W m^{-2} s^{-1}) passing through 2 cm of $CuSO_4$ solution. The light was passing through holes on the rotating wheel of the phosphoroscope (three holes, 120° apart) in such way that 0.3 ms of excitation was following by 1.25 ms of dark and 0.3 ms of registration of the delayed light emission (three cycles of excitation/dark/registration per one full turnover of the phosphoroscope wheel. The delayed light was measured using red glass cut off filter (λ >680 nm). The analyses were run in 3-replications for each variants of measurement on 4-6 standard leaflets.

To obtain the compound named as "Hypporamine PL" the leaves of seabuckthorn (*Hippophae rhamnoides L.*), collected on the fruiting phase were used. The compound was obtained after inactivation of leaves at 105°C (10 min) and drying at 45-55°C to constant weight. The dry leaves were pounded and extracted with 70% methanol. An obtained extract was filtrated and drived off under vacuum up to water-base sediment. The sediment was treated by chloroform and condensated up to 1/10 of initial volume and mixed with equal volume of ethanol. The compound was found to be of polyphenols of seabuckthorn leaves was used for antioxidant activity determination and investigation of active substances – flavonoids and catechins. The quantitative composition of flavonoids and catechins were analyzed by means of two-dimension chromatography on paper in the system H-butanol – acetic acid – water 4:1:2 and 15% acetic acid (Novruzov *et al.*, 1983; Novruzov, 2001). The individual components of flavonoids were obtained by dividing of flavonoids sum from compound "Hypporamine PL" by means of column chromatography with polyamide as a sorbent. The elutions of substances were performed by chloroform – ethanol mixture with increase of latter. The catechins were dividing on column, filling with Silicagel L 40/100μ. An elution by chloroform – petroleum ether 9:1, saturated by water. The quantitative content of flavonoids was determined spectrophotometricaly (Petrechenko *et al.*, 2002), catechins photometricaly (Zaprometov, 1974). The identification of individual components on the base of investigation of acid hydrolysis products, UV-spectroscopy and comparison of physico-chemical constants with reliable samples were carried out.

Results and Discussion

The functional state of wheat seedlings treated by Cd^{2+} and Co^{2+} was evaluated by DF of Chl *a*, stipulated by recombination initial charges separation in the reaction center of PS 2. The induction curves of DF of Chl. *a*, reflecting the different phases of this processes in the leaves of seedlings is shown in the Fig. 1a. The fast phase *o-i* reflects the charge recombination, changes of proton gradient and reduction of primary electron acceptor on donor side of PS2 with its transfer to closed state. The phase *i-d* is conditioned by transitional processes in the electron transport chain resulting in the transfer of some PS2 centers to closed state. Phase *d-p* is connected with formation of chemical component of electrochemical proton gradient. The *p-s* phase is slow, indicating on the stability of electron flow to Q_A, phase *s-o* – stationary phase (Rubin *et al.*, 1984; Gasanov *et al.*, 2007).

To evaluate of PS2 state under stress induced by action of Cd^{2+} and Co^{2+} the ratio of fast phase *o-i* to slow phase *p-s* (Fig.1) reflecting an interaction between PS2 and electron transfer chain and proton gradient was estimated. As it is seen from Table 1 the ratio of *o-i/p-s* at different pH of medium in the control was relatively constant. In the case of pH 6.7 of growing medium in seedlings

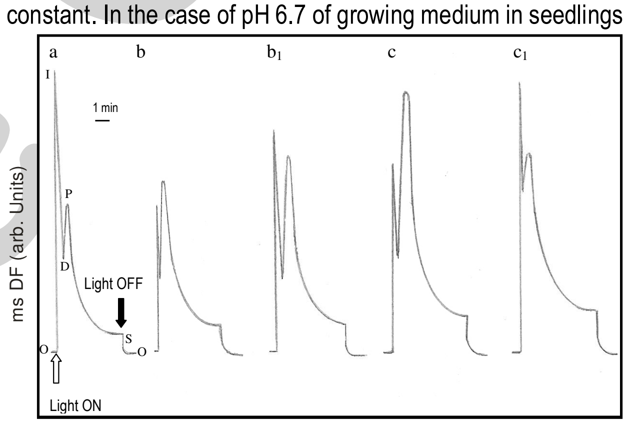

Fig. 1: Effect of "Hypporamine PL" on the induction curve of delayed fluorescence in the leaves of wheat seedlings, treated by Cd and Co ions at pH 6.7. (a) Control, (b) Co^{2+}; (b_1) Co^{2+} + PL; (c) Cd^{2+}; (c_1) Cd^{2+} + PL o-i – fast phase; i-d – transfer of centers of PS2 in closed state; d-p – chemical component of electrochemical proton gradient; p-s – slow phase; s-o – stationary level

Table - 1: Effect of "Hypporamine PL" on DF components ratio *(o-i/p-s)* in the wheat seedlings leaves treated by Cd^{2+} and Co^{2+} at the different pH of growth medium

pH of growth medium	Control (no $CdCl_2$ no $CoCl_2$ no PL)	$CdCl_2$ (10^{-3}M)	$CdCl_2$(10^{-3}M) + PL (2%)	$CoCl_2$ (10^{-3}M)	$CoCl_2$(10^{-3}M) + PL (2%)
		ms-DF components ratio (o-i / p-s), relative units			
6.7	2.01±0,28	0.8 ± 0.11	1.27 ± 0.18	0.7 ± 0.10	1.27 ± 0.18
5.0	2.07±0,30	1.26 ± 0.18	1.50 ± 0.21	1.46 ± 0.20	1.59 ± 0.21
9.0	2.00±0,28	1.18 ± 0.17	1.72 ± 0.24	1.18 ± 0.15	1.60 ± 0.22

Online Copy

Table - 2: The content of polyphenols (mg%) in the leaves of *seabuckthorn*

Total content of polyphenols	Catechins	Flavonoids	Leucoanthocyanids
6230	210	2690	127

treated by Cd^{2+} and Co^{2+} the induction curve of DF was changed (Fig. 1b, c). The value *o-i/p-s* ratio was reduced on 60 and 65% as compared with control (Table 1). Acidification of medium (pH 5.0) results in decreasing of ratio *o-i/p-s* on 37 and 27% accordingly in comparison with control. In the alkaline medium the lowering of *o-i/p-s* on 41% is observed in both cases as compared with control. This decreasing of *o-i/p-s* ratio relatively to control as at the effect of Cd^{2+} and as well as at treatment by Co^{2+} occurred due to decreasing of fast phase *o-i* amplitude (Fig.1) in the result of block up by heavy metal ions of primary electron acceptors reduction. At the same time the widening and increasing of slow phase *p-s* amplitude due to disruption of electron flow stability to Q_A was observed.

The compound "Hypporamine PL" was added to the growth medium of seedlings treated by Cd^{2+} and Co^{2+}. The content of polyphenols in the compound "Hypporamine Pl" from leaves of seabuckthorn is presented in the Table 2. The isolated flavonoids were identified as isorhamnetin-3-0-β-D glucopyranoside, izoramnethin 3-0-β-D glucopyranosil-7-0-α-L-rhamnoside, rutine- and quercetin. Catechins were identified as (2R, 35) (e-)epicatechin, (2R, 35)(+)gallocatechin, (2R, 3R) (e-) epigallocatechin, 3-galloil (2R, 3R)(-)epicatechin.

At the addition of "Hypporamine PL" to the medium, containing Cd^{2+} and Co^{2+} the protecting effect was observed. The kinetic curve, changed by heavy metals action was approached to the control (Fig. $1b_1$, c_1). The protecting effect of "Hypporamine PL" was revealed in the change of the ratio *(o-i/p-s)* value, depending on pH (Table 1) especially at pH 6.7 and 9.0.

It is well known that polyphenols (including flavonoids) have the capacity to quench oxidative damage, and scavenging active oxygen radicals (Pietta, 2000; Russo *et al*., 2000; Andrade *et al*., 2005). As was shown the toxicity of Cd^{2+} has been related with the alterations in antioxidant systems by increasing superoxide radical production or by changes the nonenzymatic antioxidants (Gallego *et al*., 1996; John *et al*., 2007). Moreover, Cd^{2+} can induce a concentration-dependent oxidative stress situation in leaves (Sandalio *et al*., 2001). It is suggested that the protecting effect of "Hypporamine PL" is due to the defense by catechin, epicatechin and other polyphenols perhaps preventing the development an oxygen free radicals state in the PS2, arising as an intermediate products due to action of heavy metals ions. If it is suggested that during 5 days wheat growth at different pH of medium the certain pH shift occur also in the cell, then the "Hypporamine PL" protecting effect strengthening at the alkaline pH may indicate on the action point of polyphenols, especially flavonoids, containing in the compound in the great number of catechins and quercetin which was reported to be among the most effective (Table 2) in the site of recombination $CaMn_4$-cluster with $P680Q_A$- (Gasanov *et al*., 2007). In all the considered mechanisms of "Hypporamine PL" action it should be taken into account that its protecting effect on the photosynthetic apparatus under action of heavy metal ions probably occur on the site close to water-oxidizing complex.

Acknowledgments

The authors thanks Dr. F. Mamedov (Uppsala University, Sweden) and Dr. R. Agalarov (Institute of Botany) for their helpful discussion and Dr. S. Aliyeva for technical assistance.

References

Andrade, R.G., L.T. Dalvi, J.M.C. Silva, G.K.B. Lopes, An. Alonso and M. Hermes-Lima: The antioxidant effect of tannic acid on the *in vitro* copper-mediated-formation of free radicals. *Arch. Biochem. Biophys*., **437**, 1-9 (2005).

Bazzaz, M.B. and Govindjee: Effects of cadmium nitrate on spectral characteristics and light reactions of chloroplasts. *Environ. Lett.,* **6**, 1-12 (1974).

Bertrand, M. and I. Poirier: Photosynthetic organisms and excess of metals. *Photosynthetica*, **43**, 345-353 (2005).

Burzynski, M.: Influence of pH and Cd and Cu uptake, distribution and their effect on nitrate reductase activity in cucumber (*Cucumis sativus* L.) seedlings roots. *Acta Physiol. Plant.*, **23**, 201-206 (2001).

Gallego, S.M., M.P. Benavides and M.L. Tomaro: Effect of heavy metal ions excess on sunflower leaves: Evidence for involvement of oxidative stress. *Plant Sci.*, **121**, 151-159 (1996).

Gasanov, R.A., S. Aliyeva, S. Arao, A. Ismailova, N. Katsuta, H. Kitade, Sh. Yamada, A. Kawamori and F. Mamedov: Comparative study of the water oxidizing reactions and the millisecond delayed chlorophyll fluorescence in photosystem II at different pH. *Photochem. Photobiol.,* **86**, 160-164 (2007).

Hasan, S. Aiman, Q. Fariduddin, B. Ali, S. Hayat and A. Ahmad: Cadmium: Toxicity and tolerance in plants. *J. Environ. Biol.,* **30**, 165-174 (2009).

Hernander, I., L. Allegre and S. Munne-Bosch: Drought-induced changes in flavonoids and other low-molecular-weight antioxidants in Cistusclusii plant grown under Mediterranean field conditions. *Tree Physiol.*, **24**, 1303-1311 (2004).

John, R., P. Ahmad, K. Qadjie and S. Sharma: Antioxidative response of *Lemna polyrriza L.* to cadmium stress. *J. Environ. Biol.*, **28**, 583-589 (2007).

Meriga, B., B.K. Reddy, K.R. Rao, L.A. Reddy and P.B. Kishor: Aliminium-induced production of oxygen radicals, lipid peroxidation and DNA damage in seedlings of rice (*Oryza sativa*). *Plant Physiol*., **161**, 63-68 (2004).

Min, Y.K., T.Y. Rhim, H.W. Shin and M.Y. Lee: Isolation cadmium-induced DNA-sequence in microalgal *Nannochloropsis occulata*. *J. Environ. Biol.*, **29**, 457-460 (2008).

Mohanty, N., I.Vass and S.Demeter: Impairment of photosystem 2 activity at the level of secondary quinone electron acceptor in chloroplast treated with cobalt, nickel and zinc ions. *Physiol. Plant*, **76**, 386-390 (1989).

Novruzov, E.N., N.M.Ismailov and C.Ch.Mamedov: The phenolic compounds of *Hippophae rhamnoides* L. leaves (Azerbaijan). *Plant Resourses* (*Russia*) **19**, 354-356 (1983).

Novruzov, E.N.: Flavonoids of different forms of seabucthorn *(Hippophae rhamnoides L.)* growing in Azerbaijan. *In*: Proc. of Internnational workshop on Seabuckthorn (*Eds.*: V. Singh and P.K. Khosla). Delhi, India pp. 140-146 (2001).

Pagliano, C., M.Raviolo, F.D.Vecchia, R.Gabbrielli, C.Gonneli, N.Rascio, R.Barbato and N.Rocca: Evidence for PSII donor-side damage and photoinhibition induced by cadmium treatment on rice *(Oryza sativa L.). Photochem. Photobiol.*, **84**, 70-78 (2006).

Online Copy

Petrechenko, V.M., T.V. Suchikina and N.C. Furca: The spectroscopic method of flavonoids analysis in *Euphoria brevilipa. Plant Resourses* (*Russia*), **38**, 104-109 (2002).

Pietta, P.G.: Flavonoids as antioxidants. *J. Nat. Prod.*, **63**, 1035-1042 (2000).

Rout, G.R. and P.Das: Effect of metal toxicity on plant and metabolism: I. Zinc. *Agronomie.*, **23**, 3-11 (2003).

Rubin, A.B., T.E. Krendeleva, P.C. Venediktov and D.N. Matorin: The initial processes of photosynthesis and photosynthetic productivity. *Agric. Biol.,* **6**, 81-92 (1984).

Russo, A., R. Acquaviva and A. Campisi: Bioflavonoids as antiradicals, antioxidants and DNA cleavage protectors. *Cell Biol. Toxicol.*, **16**, 91-98 (2000).

Sandalio, L.M., H.C. Dalurzo, M. Gomez, M.C. Romero-Puertous and L.A. del Rio: Cadmium-induced changes in the growth and oxidative metabolism of *pea* plants. *J. Exp. Bot.*, **52**, 364, 2115-2126 (2001).

Somashekarich, B.V., K. Padmija and ARK Prasad: Phytotoxity of cadmium ions on germinating seedlings of *mung bean (Phaseolus vulgaris)*: Involvement of lipid peroxides in chlorophyll degradation. *Physiol. Plant,* **85**, 85-89 (1992).

Umebese, C.E. and A.J. Motajo: Accumulation, tolerance and impact of alluminium, copper and zinc on growth and nitrate reductase activity of *Ceratophyllum demersum* (Hornwort). *J. Environ. Biol*., **29**, 197-200 (2008).

Zaprometov, M.N.: Biochemistry of phenolic compounds (*Ed.*: O.V. Makarova) Moscow. High School Publ. p. 212 (1974).

Online Copy