Photosystem II of barley seedlings under cadmium and lead stress

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ABSTRACT

Chlorophyll *a* fluorescence measurements were carried out on two barley (*Hordeum vulgare* L.) cultivars Arabi Abiad and Arabi Aswad at 8 and 14 days after emergence to identify their early tolerance mechanism for heavy metals (25 and 50μM of cadmium and lead). Transient fluorescence curves (OJIP curves) and energy flux models showed different specific reactions of photosystem II (PSII) of each cultivar to each type of stress. After 7 days of lead stress application plants of cv. A. Aswad showed weaker I and P peaks on the OJIP curve than control plants, and the appearance of a new K step; parameters of phenomenological energy fluxes for cv. A. Abiad were similar to those for control plants and only some silent reaction centers appeared. Generally, parameters of energy fluxes within PSII were directly shifted shortly (24 h) after the application of both heavy metals, especially in the case of plants grown under cadmium treatment. This suggests that these parameters could be good indicators for monitoring of these two pollutants in the environment at early stages of plant development.

Keywords: OJIP-test; chlorophyll fluorescence; heavy metals; bioenergetics

High environmental concentrations of heavy metals may be accumulated by plants and in certain concentrations will inhibit plant growth and development. However, despite some well-documented negative effects of heavy metals on physiological and photochemical processes (Clijsters and Van Assche 1985, Sujak 2005), there is no clear review of the overall photosynthetic response to this kind of stress.

Cadmium is both a non-essential and toxic element for plant growth. The sources of cadmium in the environment are phosphate fertilizers as well as contamination from industry, mining and petrol vehicles exhaust emissions (Joshi and Mohanty 2004). Cadmium inhibits photosynthesis, interferes with chlorophyll biosynthesis and degradation and affects many other photosynthetic processes (Joshi and Mohanty 2004).

The source of lead in soil and plants mainly originates in emissions from coal-fired power plants and from different industrial processes (Joshi and Mohanty 2004). Lead negatively affects photosynthesis (Moustakas et al. 1994). According to Parys et al. (1998) this effect is mainly caused by an influence on higher ABA synthesis resulting in a decrease of stomatal conductance.

Photosynthesis is usually suppressed by high concentrations of heavy metals but the effect of individual heavy metal can be specific for a given plant species and even cultivar (Küpper et al. 1996, Wierzbicka 1999, Küpper et al. 2002, Antosiewicz 2005, Romanowska-Duda et al. 2005, Sharma and Dubey 2005).

Chlorophyll *a* fluorescence kinetics is an informative tool for studying the effects of different environmental stresses on photosynthesis. A number of examples illustrate the effects of short or long-term exposure to heavy metals on photosynthetic activity expressed as chlorophyll *a* fluorescence parameters (Clijsters and Van Assche 1985, Stiborova et al. 1986, Joshi and Mohanty 2004).

To better understand the chlorophyll fluorescence signals changes under different stress conditions some advanced researches applied JIP-test, which is based on the theory of energy flow in thylakoid membranes (Strasser et al. 2004, Romanowska-Duda et al. 2005). This theory expresses the equi-

librium between the inflow and outflow of the entire energy for the analyzed system of photosynthetic pigments and it allows obtaining relevant information about the probability of the fate of the absorbed energy. Thus, JIP-test serves to obtain detailed information about the structure and function of the photosynthetic apparatus (mostly related to PSII) (Strasser et al. 1995, 2004). It includes the analyses of several groups of measured and calculated parameters, so called specific and phenomenological parameters of flow of energy. Part of calculated parameters within JIP-test is related to energy fluxes for absorption (ABS), trapping (TR) and electron transport (ETR) per reaction center (RC) or measured area of sample, which is called cross section (CS).

Barley (*Hordeum vulgare* L.) originates in the Eastern Mediterranean region where plants experience many abiotic stresses in the field. Its production has become more intense and complex in recent years, and thus crop managers need to better understand factors effecting the yield of this plant. This will consist of trials aimed at estimating responses of barley to different unfavourable conditions.

This work presents a trial for early detection of cadmium and lead stress effects on PSII activity of two barley Syrian landraces which are still cultivated as major crop in semi-arid areas but whose tolerance mechanisms to heavy metals are not clearly understood.

MATERIAL AND METHODS

Barley seedlings (*Hordeum vulgare* L.) cvs. Arabi Abiad and Arabi Aswad were grown in computer-controlled greenhouse in 1 l dark glass pots filled with modified Hoagland nutrients solution. Temperature was continuously controlled and it was 26/18°C (±0.5°C) for day/night, the relative humidity was between 50–60%, the photoperiod for the day/night cycle was 16/8 h, and the maximum photosynthetically active radiation was about 1400 μmol (photon)/m^2/s which was supplied by sodium lamps (Philips High pressure sodium, 600W/230V, 90 000 lm, Gavita, Norway). After 7 days of growth $CdCl_2$ or $Pb(NO_3)_2$ were added to final concentration of 25 and 50μM, respectively.

Chlorophyll *a* fluorescence measurements were done 24 h (8 days after emergence) and 7 days after stress application (14 days after emergence) on the middle region of mature leaves using the Plant Efficiency Analyzer (HandyPEA fluorimeter, Hansatech Instruments Ltd., King's Lynn, Norfolk, England). Before measurements barley seedling were kept in darkness for 45–60 min at room temperature. For evaluating the fluorescence induction transients, the Biolyzer v. 3.0.6 software developed by the Laboratory of Bioenergetics, University of Geneva, Switzerland was used. The average values of 30 measurements done on 1st, 2nd and 3rd leaves for each treatment were shown. Data were tested statistically by ANOVA 2 software.

RESULTS AND DISCUSSION

Transient fluorescence curve

Transient fluorescence curves of both studied cultivars grown 24 h with cadmium were almost smooth and it was difficult to see any points characterizing standard OJIP curve, while other treatments clearly showed the characteristic OJIP phases. Transient fluorescence curves for plants treated with lead were very similar to those of control plants but their values were a bit lower (ca. 10%) than control (Figure 1).

Transient chlorophyll *a* fluorescence induction curves for plants of both cultivars grown for 7 days under cadmium treatment (Figure 2) were almost flat without O, J, I, and P peaks [similar values of F_0 (chlorophyll fluorescence intensity measured when all photosystem II reaction centers are open) and F_M (maximal chlorophyll fluorescence intensity measured when all photosystem II reaction centers are closed)]. The transient curve of cv. A. Abiad grown for 7 days under lead treatment was similar to the transient curve of control plants (Figure 2) but F_M value was ca. 15% lower compared with plants without heavy metals. Plants of cv. A. Aswad showed weaker I and P peaks compared with control plants and K step appeared (at 300–500 μs) (Figure 2). F_M value was ca. 50% lower while F_0 was ca. 20% higher.

Phenomenological energy flux (leaf model)

After 24 h of stress application, phenomenological energy flux parameters i.e. ABS/CS_0, TR_0/CS_0, ET_0/CS_0 and DI_0/CS_0 for both studied cultivars of barley plants grown without stresses shown on a leaf model were rather similar (Figure 3).

After cadmium supplement, phenomenological energy fluxes of both cultivars were similar to each other, but quite different from those grown without stress (Figure 3). There was almost no TR_0/CS_0 and ET_0/CS_0 for both cultivars and ABS/CS_0 and

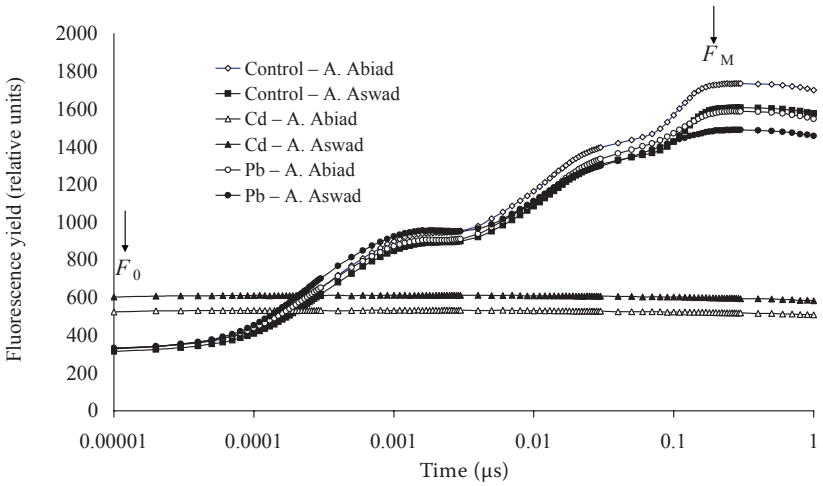

Figure 1. Transient chlorophyll *a* fluorescence induction curves of two Syrian landraces (cvs. Arabi Abiad and Arabi Aswad) grown under lead and cadmium treatment for 24 h

DI_0/CS_0 were much higher (ca. 28%) than those of control plants. Plants of both cultivars did not have reducing Q_A reaction centers, as almost all their reaction centers (ca. 98%) were silent.

Plants of both cultivars grown for 24 h with lead had similar ABS/CS_0, TR_0/CS_0, ET_0/CS_0 and DI_0/CS_0 to control plants (Figure 3). Only the number of active reducing Q_A reaction centers was slightly lower than that of control plants (8% and 16% for cvs. A. Abiad and A. Aswad, respectively).

Parameters describing phenomenological energy fluxes of both barley cultivars grown with cadmium after 7 days were quite different than these of control plants (Figure 4). For cv. A. Aswad it was impossible to calculate phenomenological energy fluxes because of very low values. Under lead treatment, parameters of phenomenological energy fluxes for cv. A. Abiad were similar to these for control plants and only silent reaction centers (20%) appeared (Figure 4). Plants of cv. A. Aswad showed lower ET_0/CS_0 (ca. 30%) and higher ABS/CS_0 (ca. 24%) and DI_0/CS_0 (ca. 245%) than control plants and they had more (ca. 40%) silent reaction centers (Figure 4).

Changes of studied fluorescence parameters of barley plants grown under cadmium in our experiment could be a complex result of harmful effects of this toxic element, as it causes inhibition of photosynthesis and influences chlorophyll biosynthesis (Krupa et al. 1993) and degradation, PSI and PSII activity, degradation of thylakoid lipids, damage of oxygen evolution complex (OEC) and LHC II antenna system (Joshi and Mohanty 2004). Strasser et al. (1995) showed that cadmium

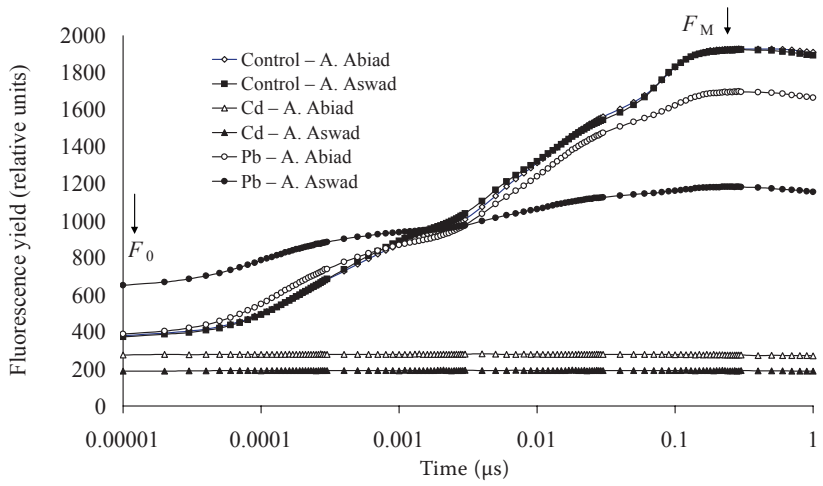

Figure 2. Transient chlorophyll *a* fluorescence induction curves of two Syrian landraces (cvs. Arabi Abiad and Arabi Aswad) grown under lead and cadmium treatment for 7 days

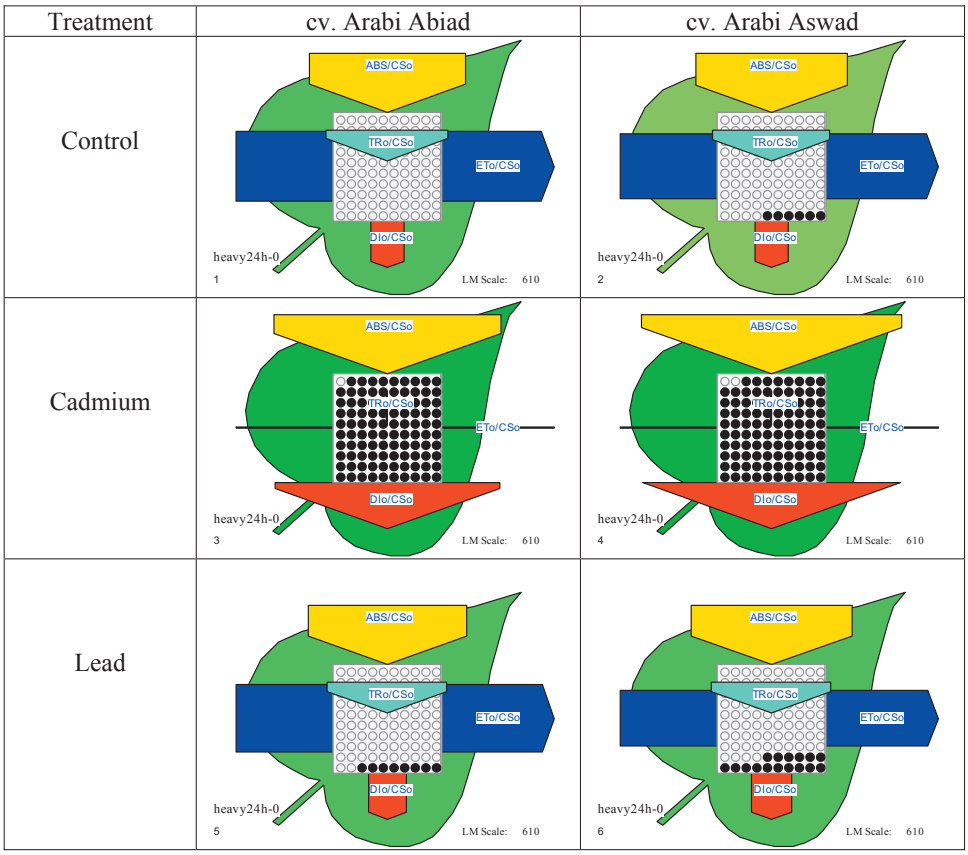

Figure 3. Leaf model showing phenomenological energy fluxes per excited cross section (CS) of barley cvs. Arabi Abiad and Arabi Aswad grown without stress (control) and after 24 h of lead and cadmium treatment

ABS/CS_0 – absorption flux per CS approximated by F_0; TR/CS_0 – trapped energy flux per CS; ET/CS_0 – electron transport flux per CS; DI/CS_0 – dissipated energy flux per CS. Each relative value is represented by the size of proper parameters (arrow), empty circles represent reducing Q_A reaction centers (active), full black circles represent non-reducing Q_A reaction centers (inactive or silent). Color intensity of leaves is proportional to its chlorophyll content calculated by Biolyzer software

slows both the reduction of Q_A and the oxidation of the reduced Q_A.

Phenomenological energy fluxes per excited cross section for plants of both cultivars grown 24 h and 7 days with cadmium were completely changed as compared with those of control plants (Figures 3 and 4). It could be a result of inhibition of many plant functions by cadmium, mainly by formation of covalent bonds with side groups of organic compounds such as proteins resulting in inhibition of their activities (McGrath et al. 2001); significant is especially the interaction of cadmium with the SH-groups of proteins (Franco et al. 1999) e.g. the inhibition of different enzymes including the protochlorophyllide reductase and plastocyanin or the Calvin cycle enzymes. Cadmium can also substitute the Mg^{2+} in the chlorophyll molecule.

Barley can grow even at concentrations above 10μM of cadmium under nutrient rich conditions, which is probably correlated with intracellular compartmentation and specific transport processes that allow the toxic effects of cadmium to decrease (Brune et al. 1995, Gonzalez et al. 1999); however, cadmium concentration used in our experiment (25μM) caused complete reduction of PSII efficiency (Figures 1 and 2). It is possible that the excess of cadmium in barley plants caused a down regulation of PSII to avoid over-reduction of primary electron acceptor Q_A and to reduce the load on the electron transport chain (Vassilev and Manolov 1999). PSII probably contains a common site(s) for heavy metal action in plants at the oxidative or reducing side of PSII (Küpper et al. 1996).

Under lead stress, photosynthesis is mainly inhibited by site of action of lead on Calvin cycle (Stiborova et al. 1986). It is believed that PSI is more tolerant to lead than PSII and the lead inhibition site is located at the donor side of PSII (Joshi and Mohanty 2004).

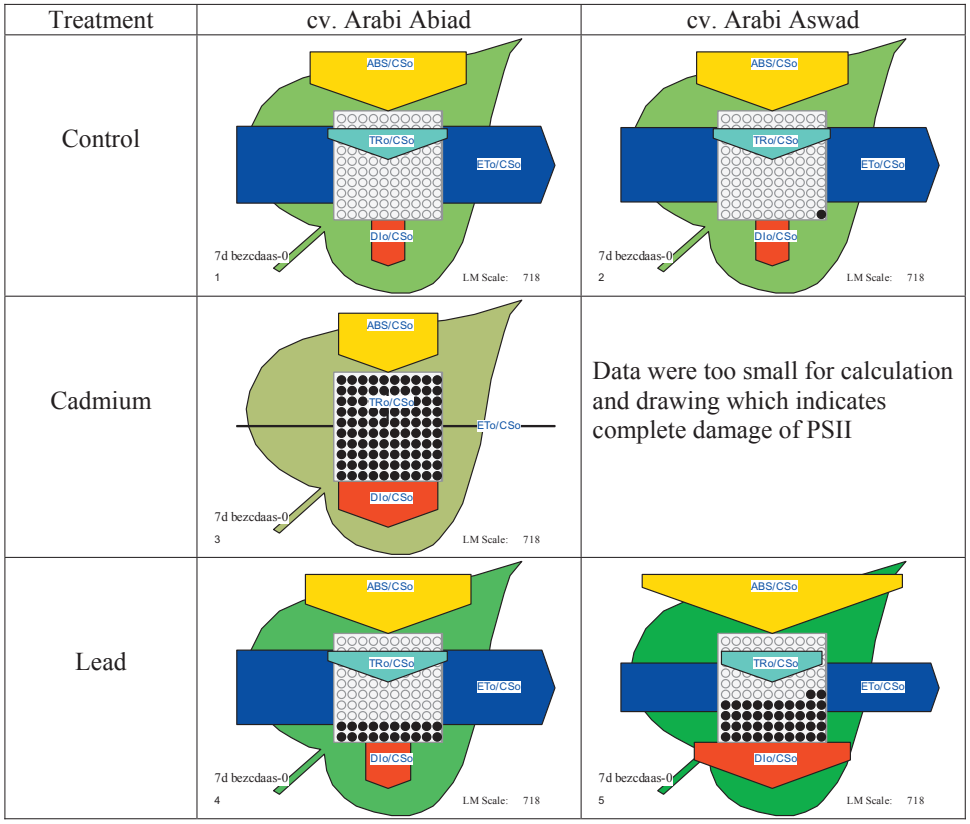

Figure 4. Leaf model showing phenomenological energy fluxes per excited cross section (CS) of barley cvs. Arabi Abiad and Arabi Aswad grown without stress (control) and after 7 days of lead and cadmium treatment

ABS/CS_0 – absorption flux per CS approximated by F_0; TR/CS_0 – trapped energy flux per CS; ET/CS_0 – electron transport flux per CS; DI/CS_0 – dissipated energy flux per CS. Each relative value is represented by the size of proper parameters (arrow), empty circles represent reducing Q_A reaction centers (active), full black circles represent non-reducing Q_A reaction centers (inactive or silent). Color intensity of leaves is proportional to its chlorophyll content calculated by Biolyzer software

Plants of cv. A. Aswad grown for 7 days with lead showed K step on OJIP transient fluorescence induction curve (Strasser et al. 2004), which can be related to inhibition of electron transport between evolving oxygen complex and reaction centre of PSII (Figure 2). Moreover, absorption and dissipation of energy fluxes within PSII were high while trapped energy and electron transport fluxes were low, but according to Parys et al. (1998) photosynthetic electron transport is weakly affected in lead-treated plants. We think that the above-observed changes in our experiment were caused by an increased number of silent reaction centres under lead treatment (Figure 4).

After 7 days of lead treatment reduction of the primary quinone acceptor of electrons Q_A in PSII (O-J phase) and quenching of the fluorescence controlled by the donor site of PSII and the characteristic activity of water splitting system (J-I phase) were slightly altered under lead treatment for cv. A. Abiad and more significantly altered for cv. A. Aswad.

Both studied heavy metals negatively influenced PSII activity of barley plants and this effect depended on the metal and time of stress; the stress mechanism was rather different in both studied heavy metals, as chlorophyll *a* fluorescence parameters characterizing PSII activity changed in different manner. Changes in PSII became more apparent with time.

Taking into account the reaction of both cultivars to studied heavy metals it appears that both cultivars are highly sensitive to cadmium stress while cv. A. Abiad seems to be more tolerant to lead stress than cv. A. Aswad; it is probably a result of its lower uptake or higher resistance to lead.

Phenomenological parameters (energy absorption, trapping and electron transport calculated on the basis of cross section) are directly shifted shortly after both heavy metals application (24 h),

especially for plants grown under cadmium treatment. This suggests that these parameters could be good indicators to monitor the negative influence of these two pollutants at early stages of their action.

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