

Lead-induced toxicity and interference in chlorophyll fluorescence in *Talinum triangulare* grown hydroponically

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Abstract

We investigated the lead (Pb) effect on chlorophyll (Chl) fluorescence and photosynthetic electron transport system in *Talinum triangulare* (Jacq.) Willd. Plants were exposed to different concentrations of $\text{Pb}(\text{NO}_3)_2$ (i.e. 0, 0.25, 0.5, 0.75, 1.0, and 1.25 mM) for seven days in hydroponic experiments. Pb-treated leaves exhibited a relative decrease in Chl fluorescence induction curve, which resulted in the decrease of maximal fluorescence yield, maximal quantum yield, and effective quantum yield of PSII, while nonphotochemical quenching, quantum yield of regulated and nonregulated energy dissipation of PSII significantly increased during Pb stress. Furthermore, Pb concentrations also caused a decrease in maximal P700 change, photochemical quantum yield, nonphotochemical quantum yield, and ETR of PSI. We suggested that the changes in these parameters were a manifestation of Pb interference in the electron transport chain in both PSII and PSI. The sensitivity of PSII was greater than that of PSI in *T. triangulare* leaves.

Additional key words: ceylon spinach; chlorophyll fluorescence; electron transport rate; lead; photosynthesis; photosystems.

Introduction

Lead is a known environmental toxicant, widely present in the various ecosystems as a result of natural and technogenic activities. It is introduced into the environment through the smelting of Pb ores, automobile exhaust, sewage sludge, armory, paints, microelectronics, batteries, burning of fossil fuel in thermal and glass manufacturing industries, etc. (Sengar *et al.* 2008, Caboche *et al.* 2010, Chenery *et al.* 2012). Due to good commercial applications, Pb is also associated with insecticides, rubber, plastics, safety matches, food, alcoholic beverages, plasters, and medicine industries (Caboche *et al.* 2010, Chenery *et al.* 2012). As one of nonredox, active heavy metals (HMs), Pb can be easily absorbed in the plant cell. It causes molecular damage, either directly or indirectly through the formation of reactive oxygen species (ROS) (Kumar *et al.* 2012). The ROS attack on the polyunsaturated fatty acid of membrane lipids causes lipid peroxidation, which further leads towards oxidative stress and alters various biochemical and metabolic activities of the cells (Pourraut *et al.* 2011, Sytar *et al.* 2013).

Chl molecules are highly sensitive to HMs as well as play an important role in the ROS formation. During stress, the electron flow is inhibited due to instability of

Chl molecules or damage of the electron transfer system. In response, the excited Chl ultimately ends up in the production of ROS, which results in a functional loss of carbon fixation rather than in quenching of harvested light energy (Sytar *et al.* 2013). HMs also cause the inhibition of photosynthetic pigment biosynthesis, damage of oxygen-evolving complex (OEC), decline of PSII and PSI activities, and inhibition in the energy transfer to PSII-reaction center (RC) as well as electron transport between OEC and PSII-RC (Kalaji and Loboda 2007, Subrahmanyam 2008, Cenkci *et al.* 2010, Dążkiewicz and Baszyński 2010, Sytar *et al.* 2013). Pb has been reported to affect the LHC of both PSII and PSI, and the chloroplast coupling factor (Miles *et al.* 1972).

Talinum triangulare (Jacq.) Willd. (Ceylon spinach, Talinaceae) is an ornamental, leafy vegetable, and medicinal plant. This plant is the important source of flavonoids, minerals, tocopherol, vitamin C, proteins, and other antioxidants for the diet of a major human population (Kumar and Prasad 2010, Panyaphu *et al.* 2011). The use of polluted water and metal-containing pesticides, knowingly or unknowingly, for irrigation and cultivation is a common practice for local farmers in Nigeria, India,

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Abbreviations: Chl – chlorophyll; ETR_{PSI} – electron transport rate of PSI; ETR_{PSII} – electron transport rate of PSII; F_s – steady-state fluorescence yield; F_m – maximal fluorescence yield; F/F_m – maximal PSII quantum yield; HMs – heavy metals; OEC – oxygen evolving complex; P_m – maximal P700 change; Q_A – primary electron acceptor of PSII; q_N – coefficient of nonphotochemical quenching; q_P – coefficient of photochemical quenching; RC – reaction center; ROS – reactive oxygen species; SP – saturation pulse; Φ_{PSII} – effective PSII quantum yield; Y_{PSI} – photochemical quantum yield of PSI; Y_{ND} – nonphotochemical quantum yield of PSI; Y_{NO} – quantum yields of nonregulated energy dissipation of PSII; Y_{NPQ} – quantum yields of regulated energy dissipation of PSII.

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and other parts of the world (Sekhar *et al.* 2007, Uwah *et al.* 2009). These practices directly or indirectly affect growth and overall plant performance. It has been reported that *T. triangulare* can accumulate higher amounts of HMs in its tissues from contaminated media (Sekhar *et al.* 2007, Kumar *et al.* 2012). Analysis of toxic HMs effects on the edible part of the plant is always desirable (Uwah *et al.* 2009, Pourraut *et al.* 2011).

Our previous studies confirmed that Pb treatment

Materials and methods

Plant material and treatment: *T. triangulare* was collected from a field bank maintained under uniform growth conditions for long durations in the greenhouse at the University of Hyderabad, India. The PPFD used for plant growth and development ranged from 900 to 1,200 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Plants of the same age group were propagated through stem cuttings (5.0–7.0 mm in diameter and 17–22 cm high) in conical flasks containing 100 ml of modified Hoagland's media in the plant growth chamber at 16/8 h (day/night) regime and at temperature of $28 \pm 2^\circ\text{C}$ (Kumar and Prasad 2010). Stem cuttings were propagated in Hoagland's media for 3 weeks, with changing of media every 3–4 d, which resulted in extensive adventitious roots, leaves, and vegetative propagules. After 3 weeks of acclimatization, uniform stem cuttings with adventitious roots and leaves were treated for 7 d under above conditions with $\text{Pb}(\text{NO}_3)_2$ at different concentrations: 0 (control), 0.25, 0.5, 0.75, 1.0, and 1.25 mM. After 7 d of treatment, leaves of the same insertion level were used for Chl fluorescence and P700 analysis.

Chl fluorescence and P700: Prior to simultaneous assessment of both the Chl fluorescence and the P700 measurements, *T. triangulare* leaves were dark-adapted for 45 min at room temperature (25°C) to obtain oxidation-reduction equilibrium of PSII-PSI electron transport carriers. The Dual-Pulse Amplitude Modulated fluorometer (Dual-PAM-100, Heinz Walz GmbH, Germany) was used to measure the rapid polyphasic Chl fluorescence and P700 kinetic (Strasser and Strasser 1995, Anonymous 2006, Klughammer and Schreiber 2008a, b).

After the dark adaptation, the Chl fluorescence parameters, such as steady-state fluorescence yield (F_s) and maximal fluorescence yield (F_m), were determined by the application of a saturation pulse (SP) of 6,000 $\mu\text{mol}(\text{photon})$

causes oxidative stress in *T. triangulare*, which was scavenged through the activation of various enzymatic and nonenzymatic antioxidative and metabolic mechanisms (Kumar *et al.* 2012, 2013). In order to clarify the mechanism of Pb action, we aimed to analyze Chl fluorescence under different Pb concentrations in *T. triangulare*. The study provides information regarding Pb-induced toxicity and inhibition of photosynthetic mechanisms in higher plants.

$\text{m}^{-2} \text{s}^{-1}$ for 800 ms. The maximal PSII quantum yield (F_v/F_m) was calculated according to Handbook of operation with Dual-PAM (2006). Further, the application of an actinic photon flux density of 339 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 10 SP were triggered to measure the maximal Chl fluorescence in the light-adapted state. The effective PSII quantum yield (Φ_{PSII}), the coefficient of nonphotochemical quenching (q_N), the coefficient of photochemical quenching (q_P), the quantum yield of regulated energy dissipation (Y_{NPQ}), the quantum yield of nonregulated energy dissipation (Y_{NO}), and electron transport rate (ETR) were calculated using the formulas described by Klughammer and Schreiber (2008a) and Schreiber and Klughammer (2008). The intensity of measuring light for Chl fluorescence and P700 was $\sim 10 \mu\text{mol m}^{-2} \text{s}^{-1}$.

P700 was measured in the dual-wavelength mode (difference of intensities of 875 nm and 830 nm pulse-modulated measuring light reaching photodetector (DUAL-Detector Blue/Red fluorescence measuring light, Heinz Walz GmbH, Germany). The P700 was determined by the SP application of 6,000 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ for 800 ms and actinic light of 339 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The maximal P700 change (P_m), the analogy to F_m , was determined and used for calculating both the quantum yields of PSI (Y_{PSI}), and nonphotochemical quantum yield of PSI (Y_{ND}) (Klughammer and Schreiber 2008b).

Statistical analysis: The data presented corresponded to the mean values \pm standard deviation (SD) of three replicates. One-way analysis of variance (ANOVA) and Duncan's multiple range test by using SIGMASTAT software (version 11.0) were performed to confirm the variability of results and for the determination of significant ($P \leq 0.05$) difference between treatment groups, respectively.

Results

Pb-induced changes in Chl fluorescence and P700: Our results proved that the F_s decreased in the leaves of Pb-treated plants (Fig. 1A), which showed a good correlation ($R = 0.877$) with the increasing concentrations of Pb. The F_s decreased significantly after all Pb treatments, which accounted for 18.4–40.1% at 0.25–1.25 mM Pb in comparison with control, respectively. Furthermore, the treatment reduced significantly F_m with increasing Pb concentrations (*i.e.*, 19–45% in comparison with control,

Fig. 1B). The response of F_m showed a good correlation with the response of F_s ($R = 0.946$).

The F_v/F_m (Fig. 1C) and Φ_{PSII} (Fig. 1D) showed a linear decrease during Pb treatment. The maximum significant decrease in F_v/F_m was observed at 0.75 (8.8%), 1.0 (16.2%), and 1.25 mM Pb (19%). Similarly, in Φ_{PSII} , a significant decrease of 23–53% was observed at 0.25–1.25 mM Pb, respectively.

The trend of q_P (Fig. 2A) was almost similar to that of

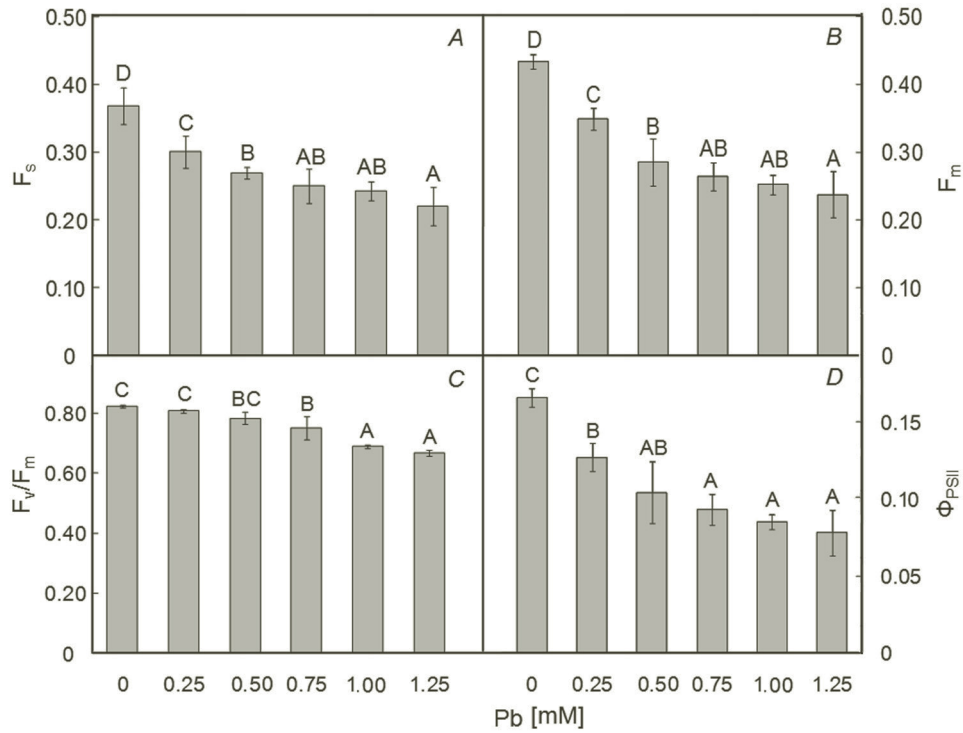


Fig. 1. Changes in (A) steady-state fluorescence yield (F_s), (B) maximal fluorescence yield (F_m), (C) maximal PSII quantum yield (F_v/F_m), and (D) effective PSII quantum yield (Φ_{PSII}) in leaves of *Talinum triangulare* after seven days of Pb treatments. Bars represent mean values (\pm SD). Bars denoted by different letters are significantly ($P \leq 0.05$) different with each other.

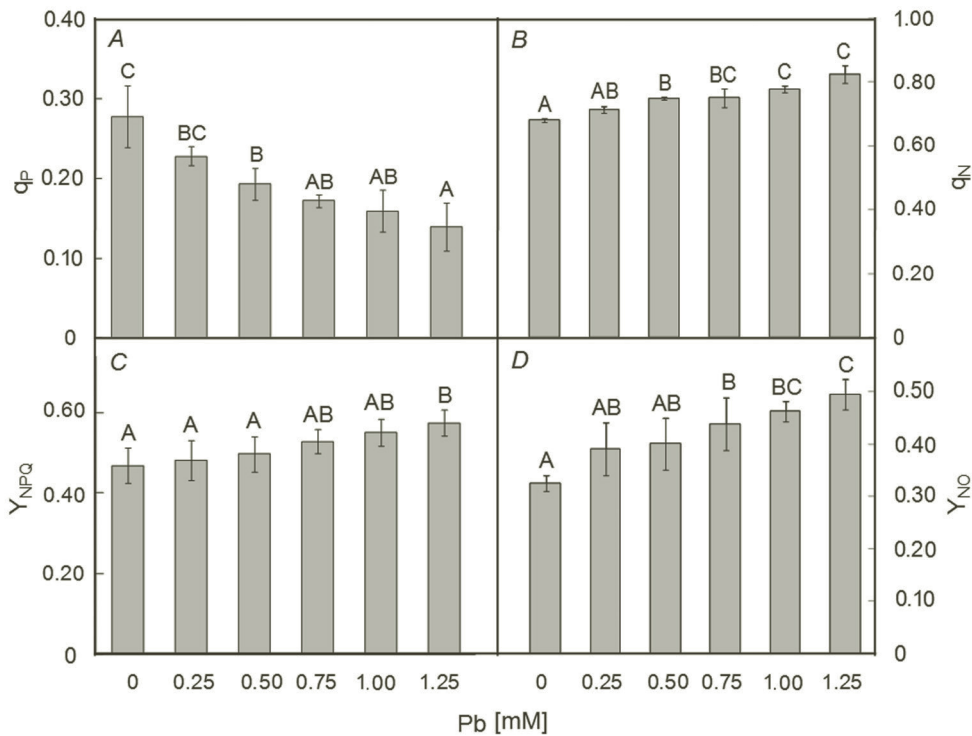


Fig. 2. Changes in photochemical quenching coefficient (q_p) (A), nonphotochemical quenching coefficient (q_N) (B), and quantum yields of regulated (Y_{NPQ}) (C) and nonregulated (Y_{NO}) (D) energy dissipation of PSII in leaves of *Talinum triangulare* after seven days of Pb treatments. Bars represent means values (\pm SD). Bars denoted by different letters are significantly ($P \leq 0.05$) different with each other.

the F_v/F_m . The q_p decreased significantly at 0.50–1.25 mM Pb, which was 30.5–50% as compared with control. On the contrary, q_N increased significantly by 4.8–21.1% at 0.25–1.25 mM Pb (Fig. 2B). Similarly, a gradual increase in the Y_{NPQ} (Fig. 2C) and Y_{NO} (Fig. 2D) was observed in

Pb-treated leaves. In comparison to control, Y_{NPQ} increased significantly to 22.5% only at 1.25 mM Pb. On the other hand, Y_{NO} increased significantly by ca. 35, 42, and 52% at 0.75, 1.0, and 1.25 mM Pb, respectively.

PAM analysis revealed that the photosynthetic electron

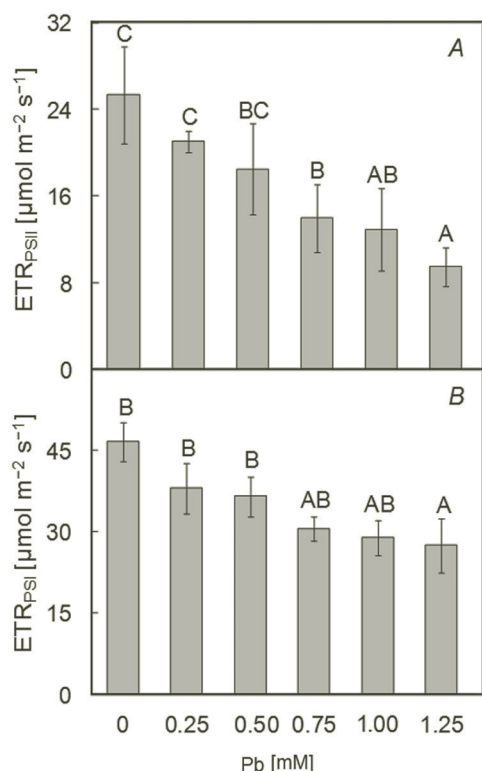


Fig. 3. Inhibition of electron transport rate of PSII (ETR_{PSII}) (A) and PSI (ETR_{PSI}) (B) in leaves of *Talinum triangulare* after seven days of Pb treatments. Bars represent mean values (\pm SD). Bars denoted by different letters are indicating significantly ($P \leq 0.05$) different with each other.

transport rate of both PSII (ETR_{PSII}) and PSI (ETR_{PSI}) decreased after 7 d of Pb treatment, respectively (Fig. 3). A significant decrease in ETR_{PSII} was observed at 0.75 (45%), 1.0 (49%), and 1.25 mM Pb (62.5%) in comparison with control (Fig. 3A). Similarly, ETR_{PSI} also significantly decreased by 37.8% and 41% at 1.0 and 1.25 mM Pb, respectively (Fig. 3B). ETR_{PSII} showed a good correlation ($R = 0.916$) with ETR_{PSI} .

Results of P700 or PSI showed that P_m , Y_{PSI} , and Y_{ND} decreased in *T. triangulare* leaves after 7 d of Pb treatment (Fig. 4). A significant decrease in the P_m was observed at 0.50–1.25 mM Pb after 7 d of treatment, which ranged from 21.2% to 51.2% in comparison with control, respectively (Fig. 4A). Furthermore, a maximum significant decrease of 37% and 46% in the Y_{PSI} was observed at 1.0 and 1.25 mM Pb compared with control, respectively (Fig. 4B).

Discussion

The results of our study suggested that Pb showed an adverse effect on photosynthetic processes. Our previous study revealed that Pb can accumulate in *T. triangulare* tissues and cause phytotoxicity and oxidative stress; phytotoxicity manifestation includes a significant decrease in Chl content and transport of nutrient elements (Kumar *et al.* 2012). Measurement of Chl fluorescence provided simple and reliable information

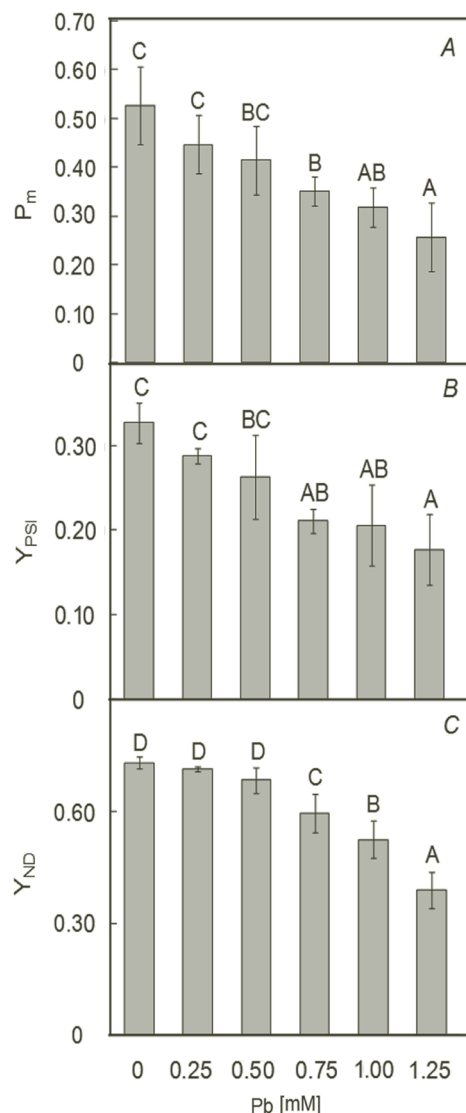


Fig. 4. Pb-induced changes in P700 activities. (A) maximal P700 change (P_m), (B) photochemical quantum yield of PSI (Y_{PSI}), and (C) nonphotochemical quantum yield of PSI (Y_{ND}) in leaves of *Talinum triangulare* after seven days of Pb treatments. Bars represent mean values (\pm SD). Bars denoted by different letters are significantly ($P \leq 0.05$) different with each other.

Simultaneously, Y_{ND} also significantly decreased by 28.2% and 46.9% at 1.0 and 1.25 mM Pb, respectively (Fig. 4C).

about the malfunction of both PSII and PSI under different Pb concentrations (Maxwell and Johnson 2000) (Figs. 1–4).

We proved that Pb had a negative impact on the Chl fluorescence induction curve as well as the activity of both PSII and PSI (Fig. 3). Pb treatment caused a decrease in the F_s intensity in comparison with control, which was directly related to the changes in Φ_{PSII} and q_p . The

decrease in F_m reflected the inhibition on the donor side of PSII when all the molecules of the primary electron acceptor (Q_A) are in reduced state or PSII-RC are closed. This inhibition is the manifestation of the decrease in the electron transport rate (Govindjee 1995, Mallick and Mohn 2003). A similar trend was observed in our study during Pb treatment. It has been reported that a decrease in F_m in Cr-treated *Datura innoxia* was associated with the structural alteration in the PSII pigment-protein complexes as well as changes in the ultrastructure of the thylakoid membranes (Vernay *et al.* 2008). However, decrease in the F_m in Pb-treated *T. triangulare* resulted from decrease in the Chl fluorescence intensity when all PSII reaction centers were closed. Similar response reported in Pb-, Hg-, and Cu-treated spinach (Boucher and Carpentier 1999), Cr-treated duckweed (Oláh 2010), and Ni-treated maize (Drażkiewicz and Baszyński 2010).

Decreased F_m caused a decrease in F_v/F_m , the ratio known as the most sensitive indicator for photoinhibition under various stress conditions, which further corroborated with a decrease in Φ_{PSII} (Ekmekçi *et al.* 2008). Further, Pb-induced decrease in F_v/F_m in *T. triangulare* can be explained by the negative effect of Pb on the efficiency of PSII photochemical reaction and electron transport chain. The decreased F_v/F_m can also be associated with the limited reoxidation of Q_A , which further resulted in a decrease in electron transport from PSII to PSI (Mallick and Mohn 2003). Similarly, a gradual decrease in F_v/F_m and Φ_{PSII} were observed in *D. innoxia* under Cr treatment (Vernay *et al.* 2008).

Along with F_v/F_m and Φ_{PSII} , q_p also decreased, while q_N increased in Pb-treated *T. triangulare* leaves. A similar trend was observed in maize treated with different Pb concentrations (Ekmekçi *et al.* 2008). The decrease in q_p represents an increased number of reduced (closed/inactive) PSII-RC in the leaves of Pb-treated plants. Higher number of closed RC signified that the light absorption exceeds the capacity of ETR and CO_2 fixation, which was significantly responsible for an increase in the q_N (Vassilev and Manolov 1999). It has been reported that Cr treatment caused a simultaneous decrease in the q_p and an increase in the q_N in *Triticum aestivum* (Subrahmanyam 2008) and in *D. innoxia* (Vernay *et al.* 2008), indicating a decreased utilization of NADPH due to Cr stress (Vernay *et al.* 2008). The increased q_N clearly signifies an enhanced rate constant of thermal dissipation of excitation energy, which downregulates the photosynthetic electron transport (Subrahmanyam 2008, Vernay *et al.* 2008). Pb caused a significant increase in Y_{NO} and Y_{NPQ} , which was in accordance with the decrease in F_m that was used to calculate these parameters. The Y_{NO} reflects the part of

energy dissipated as fluorescence and heat, due to closed PSII-RC. Increased Y_{NO} and Y_{NPQ} values can compensate for a decrease in Φ_{PSII} , under different Pb treatments that can be considered as the Pb-induced damage of the photosynthetic apparatus (Klughammer and Schreiber 2008a).

Our experimental results showed that Pb concentrations caused a significant decline in ETR in both PSII and PSI reaction centers. The Pb treatment of 1.25 mM affected the ETR_{PSII} (62.5%) much more than the ETR_{PSI} (41%). It was suggested that the downregulation of ETR_{PSII} is due to the prevention of over-reduction of Q_A , to decrease the load on electron transport chain. Several studies have reported the altered rate of electron transfer in PSII and PSI under the influence of Pb and other HMs (Wong and Govindjee 1976, Vassilev and Manolov 1999, Subrahmanyam 2008, Ekmekçi *et al.* 2008).

The PSI pigment-protein complex converts light energy into transmembrane charge separation, which finally leads to CO_2 reduction. As aforesaid, PSI activity has been observed to be less sensitive than PSII, during Pb stress condition (Sárvári 2005, Mobin and Khan 2007). The P_m represents the maximal change of the P700 signal upon quantitative transformation of P700 from the fully reduced to the fully oxidized state, which significantly decreased during 0.50–1.25 mM Pb treatment. Different concentrations of Pb caused a decrease in the Y_{PSI} and Y_{ND} in *T. triangulare* leaves. The Y_{PSI} is defined by the fraction of overall P700 that is reduced in a given state and not limited by acceptor side. Y_{PSI} is calculated from the complementary Y_{ND} , which represents the fraction of overall P700 that is oxidized in a given state and it is a determinant of the donor side limitation of PSI. It can be suggested that during Pb stress, no charge separation is possible due to acceptor side limitation, which resulted in decreased Y_{ND} followed by the failure of adaptation to the P700 donor-side limitation (Schreiber and Klughammer 2008). The decreased P_m , Y_{PSI} , and Y_{ND} directly point towards the inhibition of the PSI reaction center by Pb.

It can be concluded that *in vivo* assay of Pb toxicity using the Chl *a* fluorescence may provide a quick and sensitive method for assessing phytotoxic effects of agents such as Pb, and their sites of action. Thus Chl fluorescence parameters F_s , F_m , F_v/F_m , Φ_{PSII} , ETR, q_p and q_N are equally sensitive parameters to evaluate the impact of Pb stress in *T. triangulare*. A change in ETR_{PSII} and quantum yields might be directly related to the change in q_N . Moreover, the PSI photochemical reactions, Y_{PSI} , and ETR_{PSI} were also affected during Pb treatment conditions. Pb treatment affected or inhibited PSII function to a much greater extent than that of PSI.

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