

Original Research

Sb Uptake and Photosynthesis of *Zea mays* Growing in Soil Watered with Sb Mine Drainage: an OJIP Chlorophyll Fluorescence Study

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Received: 11 August 2009

Accepted: 19 February 2010

Abstract

Maize (*Zea mays*) growing in soil watered with Sb mine drainage (SMD) could accumulate Sb and transferred Sb from roots to shoots. The photosynthetic processes in the leaves were clearly inhibited by SMD. Chlorophyll synthesis was inhibited. Photosystem II (PSII) was sensitive to SMD. PSII activity (F_v/F_m and PI_{ABS}) was reduced due to SMD. Irrigation with SMD resulted in an increase in the proportion of the closed PSII reaction centers (RCs) and a decrease in the proportion of Q_B binding centers. The electron transport on both the donor side and the acceptor side was inhibited by SMD. The total electron transport flux in PSII was reduced and the electron transport beyond Q_A^- was the primary target site for toxicity of SMD. PSII RCs were transformed into dissipative sinks for excitation energy under stress of SMD. Sb accumulation in crops due to SMD irrigation may not only reduce crop growth and yield, but also pose a threat to food security and human health.

Keywords: antimony, *Zea mays*, uptake, photosynthesis, SMD, photosystem II, JIP-test, Chlorophyll fluorescence

Introduction

Antimony (Sb), with a molecular weight of 121.76 and an atomic number of 51, is widely used in making alloys, brake linings, flame-proof retardants, paint pigments, ceramics, glass, and catalysts [1, 2]. The huge demand for antimony from various industries has led to unprecedented exploitation of Sb deposits. Substantive amounts of antimony-containing compounds have been released into the environment as a result of industrial use of Sb and its exploitation. Sb pollution is a serious problem in mining areas.

The Sb level in soil in five British former mining and smelting sites were up to 700 mg kg⁻¹ [3]. Soil Sb levels in an abandoned open-sky antimony mine in Tuscany (Italy) ranged from 27.7 to 16,388.8 mg kg⁻¹ of dried soil [4].

Sb is not necessary for biological metabolism and is potentially toxic at very low concentrations. Sb has been listed as a priority pollutant by the U.S. Environmental Protection Agency [5] and Council of the European Communities due to its potential carcinogenicity [6]. Limited studies show that Sb is also toxic to plants [7, 8] and microorganisms [9]. Sb has been reported to inhibit rice growth (root growth and sprouts), and reduce rice yield [7]. Recently, it was shown that Sb at 5 mg L⁻¹ and 20 mg L⁻¹

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significantly decreased biomass of three species of fern [8]. Sb also has an inhibitory effect on the growth of some bacteria species and microbial enzymatic activities in soil [9]. Increasing attentions have been paid to this emergent pollutant in recent years due to its high level in potential environmental toxicity [2].

More than 80% of the total antimony (Sb) reserve of the world is located in southwestern China. There are several large-scale antimony mines in this area, including Xikuangshan Antimony Mine in Hunan province, and the Dushan and Qinglong Sb mines in Guizhou province. Huge amounts of antimony have been released into the surface water and farmland soil via drainage discharge containing high levels of Sb. Unfortunately, the Sb mine drainage (SMD) or the surface water mixed with drainage have been used to irrigate the farmland. Sb in top soil around the Sb mine area was as high as 5,045 mg·kg⁻¹ [10]. However, unlike other heavy metals such as Pb, Zn, Cd, Hg, and Cu, the effects of irrigation with SMD containing high levels of Sb on growth and physiology of crops is still unknown. Chlorophyll fluorescence is a sensitive, rapid, non-invasive, and reliable method to assess photosynthetic performance under various environmental stresses, including pollution [11-13]. In the present study, the effect of Dushan Sb Mine drainage on maize photosynthesis (*Zea mays*) was investigated.

Materials and Methods

Sb Mine Drainage

SMD was collected from the Dushan Sb Mine. Its pH is 8.21 and EC 1.63 ms cm⁻¹. Its composition (in mg L⁻¹) was: Na⁺ 58.14, K⁺ 22.64, Mg²⁺ 26.91, Ca²⁺ 438.23, NO₃⁻ 5.19, Cl⁻ 7.61, F⁻ 5.18, SO₄²⁻ 881.26, and PO₄³⁻ 0.38. The heavy metals in the drainage are Hg²⁺ 0.15 µg L⁻¹, As 1.81 µg L⁻¹, and Sb 28.75 mg L⁻¹. SMD was filtrated through a 0.45 µm membrane.

Plant Materials

Maize plants (*Zea mays*) were germinated from seeds in sterilized quartz sand in a thermostat-controlled, darkened chamber at 95% relative humidity and 25°C for 3 days. Two weeks later the seedlings were transplanted into soil. Soil was collected from Qianling Park. The levels of Sb, As, Hg, and other heavy meals in the soil was very low. Every three days, the plants were watered with aliquots of half-strength modified Hoagland solution containing various percentages of SMD. The plants watered with half-strength modified Hoagland solution containing no SMD were used as the control. The modified Hoagland solution contained the following nutrients: 28.7 mg·L⁻¹, NH₄H₂PO₄, 0.71 mg·L⁻¹ H₃BO₃, 164.1 mg·L⁻¹ Ca(NO₃)₂, 0.02 mg·L⁻¹ CuSO₄, 2.66 mg·L⁻¹ ferric tartrate, 60.19 mg·L⁻¹ MgSO₄, 0.45 mg·L⁻¹ MnCl₂, 0.004 mg·L⁻¹ MoO₃, 151.65 mg·L⁻¹ KNO₃, and 0.055 mg·L⁻¹ ZnSO₄. The plants were cultivated at 25/20°C

(day/night) with 85% relative humidity and with a 10 h/14 h light/dark period of illumination (250 µM m⁻² s⁻¹). The plants were grown in the soil for 21 days at PPFD 100 mmol under a day/night regime of 14/10 h and 25/18.

Chlorophyll Estimation

Chlorophyll content of the seedling leaves was determined at the end of each trial. About 1 g of fresh leaves was homogenized using a pestle and mortar in 3.0 mL of 95% ethanol with a small amount of quartz sand. The homogenate was filtered and diluted to 25 mL with 95% ethanol. The diluted solution was used for chlorophyll estimation. The absorbance of pigment extract was measured at 663 and 645 nm by a UV-Vis spectrophotometer (Unico 2000, Shanghai, China). The chlorophyll content was calculated using equation (1) as follows [14]:

$$\text{Chlorophyll content} = 20.2A_{645} + 8.02A_{663} \quad (1)$$

Measurement of Chlorophyll Fluorescence and JIP-Test

Chlorophyll fluorescence was measured using a hand-held fluorometer (Fluopen, PSI, CZ). The third leaf was analyzed with chlorophyll fluorescence tests. All samples were dark-adapted for five minutes before measurement. Each leaf was tested five times and the average value was used.

The chlorophyll fluorescence transients were recorded up to 1s on a logarithmic time scale, with data acquisition every 10 µs for the first 2 ms and every 1ms thereafter. The polyphasic fluorescence induction kinetics were analyzed according to the JIP-test [15]. The polyphasic fast-phase fluorescence induction curve provides valuable information on the magnitude of stress effects on the photosystem II (PSII) function. In the present study, the following data were directly obtained from the fast-rise kinetic curves: F₀, the initial fluorescence, was measured at 50 µs, when reaction centers (RCs) are open; F_J and F_I are the fluorescence intensity at J step (at 2 ms) and I step (at 30 ms); F_m, maximal fluorescence, was the peak fluorescence at P step when all RCs were closed after illumination; F₃₀₀ was the fluorescence at 300 µs. Selected parameters quantifying PSII behavior were calculated from the above original data as the formulae in Table 1 [15].

Q_B binding centers were calculated according to double hit experiments [16]. Dark adapted leaves were exposed twice for 1s with saturating light at an interval of 10 s dark. During the first exposure, all the RCs of PSII get closed (Q_A⁻ total). However, during 10 sec of darkness, the Q_A⁻ is reoxidized. When the plants are re-exposed to saturating light after 10 s of dark adaptation F₀ of the second illumination can be higher due to remaining reduced Q_A⁻. The difference in the relative variable fluorescence at time zero between the second and the first illumination ΔV₀ corresponds to the fraction of the RC that did not reopen during

Table 1. Formulae and terms used in the JIP-test [15, 20].

Formulae and terms	Illustrations
$V_J = (F_{2ms} - F_O)/(F_M - F_O)$	Relative variable fluorescence intensity at the J-step
$M_O = 4(F_{300\mu s} - F_O)/(F_M - F_O)$	Approximated initial slope of the fluorescence transient
$S_m = \text{Area}/(F_M - F_O)$ where: $\text{Area} = \int_0^{t_{F_{\max}}} (F_M - F_t) dt$	The working integral of the energy needed to close all reaction centers
$N = S_m \cdot M_O / V_J$	Turn-over number indicating how many times Q_A has been reduced to Q_A^- in the time span from t_0 to $t_{F_{\max}}$
$\varphi_{P_o} = \text{TR}_O/\text{ABS} = [1 - (F_O/F_M)] = F_V/F_M$	Maximum quantum yield for primary photochemistry (at $t=0$)
$\varphi_{E_o} = \text{ET}_O/\text{ABS} = [1 - (F_O/F_M)] \cdot \Psi_O$	Quantum yield for electron transport (at $t=0$)
$\Psi_O = \text{ET}_O/\text{TR}_O = (1 - V_J)$	Probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A (at $t=0$)
$\text{ABS}/\text{RC} = M_O \cdot (1/V_J) \cdot (1/\varphi_{P_o})$	Absorption flux per RC
$\text{TR}_O/\text{RC} = M_O \cdot (1/V_J)$	Trapped energy flux per RC (at $t=0$)
$\text{ET}_O/\text{RC} = M_O \cdot (1/V_J) \cdot \Psi_O$	Electron transport flux per RC (at $t=0$)
$\text{DI}_O/\text{RC} = (\text{ABS}/\text{RC}) - (\text{TR}_O/\text{RC})$	Dissipated energy flux per RC (at $t=0$)
$\text{PI}_{\text{ABS}} = (\text{RC}/\text{ABS}) \cdot [\varphi_{P_o}/(1 - \varphi_{P_o})] \cdot [\Psi_O/(1 - \Psi_O)]$	Performance index on absorption basis

the 10 s dark period. This fraction is called very slowly reopening RCs or non- Q_B binding centers. Therefore, the fraction of Q_B -binding centers can be calculated as:

$$1 - \Delta V_0 = (1 - F_0/F_M)_{(\text{second hit or second exposure})} / (1 - F_0/F_M)_{(\text{first hit or first exposure})}$$

Detection of Sb, Hg, and As Content in Plant Tissue

For Sb analysis, the dry root or shoot biomass was cut into small pieces and digested in a mixture of 5 ml of concentrated HNO_3 and 3 ml of concentrated HCl for 2 h at 160°C under pressure in a sealed heater. Sb, Hg, and As content in the digested solution was determined using an atomic fluorescence spectrometer (AFS-800, Jitian Instrument Inc., China).

Statistical Analysis

Each treatment was at least quintuplicated and the mean values were used. Student's t-test was employed for statistical analysis of experimental data. Significance was declared at $P < 0.05$.

Results and Discussion

Sb Accumulation in *Zea mays*

No As and Hg were detected in the plant tissues. Sb content in roots and shoots of *Zea mays* increased with the percentage of SMD in the culture solution (Fig. 1).

The shoots accumulated a higher level of Sb than the roots, suggesting the high mobility of Sb from the roots to shoots. The translocation coefficients of Sb from the roots to the shoots were in the range of 1.24-1.93. Sb accumulation and its translocation from the underground tissue to the above-ground tissue were dependent on the plant species. Some aquatic plants (*Typha latifolia*, *Scirpus sylvaticus* and *Phragmites australis*) were reported to accumulate Sb to a high level (100-1,300 mg kg^{-1} dry biomass) in roots but low level (15-19 mg kg^{-1} dry biomass) in aboveground tissue [17], with very low Sb translocation coefficients of Sb from the roots to the shoots. Pratas et al. [18] reported that Sb accumulation in sixteen plant species at abandoned mines

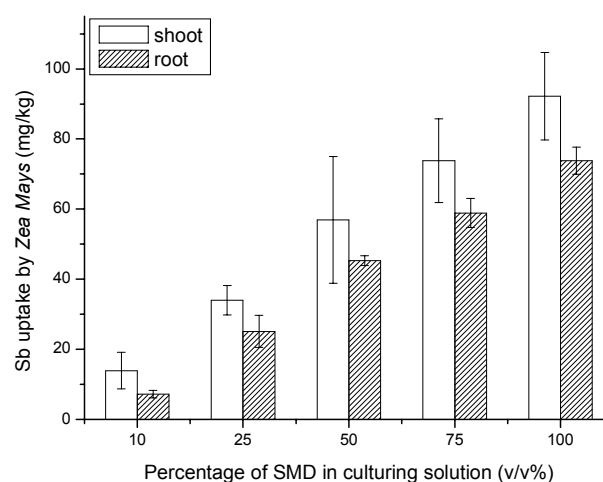


Fig. 1. Sb accumulation in roots and shoots of *Zea mays* growing in soils watered with culturing solution containing different percentages of SMD. Each data point represents the mean value \pm SE of five seedlings.

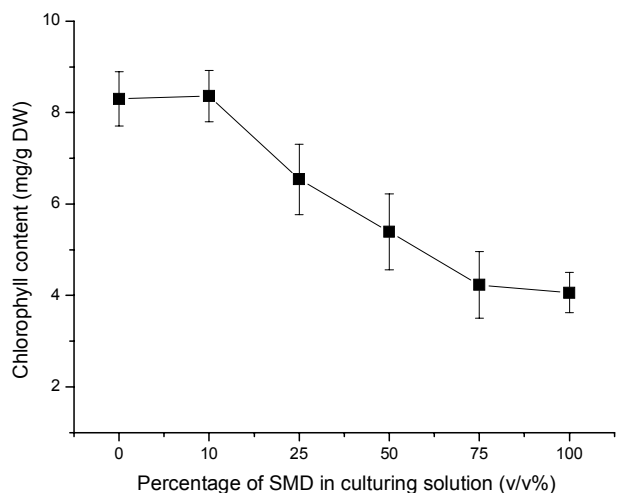


Fig. 2. Chlorophyll content in leaves of *Zea mays* growing in soils watered with culturing solution containing different percentages of SMD. Each data point represents the mean value±SE of five seedling leaves.

was largely at $\mu\text{g}\cdot\text{kg}^{-1}$ dry biomass, suggesting that Sb was not highly available to plants. Sb translocation coefficients from roots to the aboveground tissue for the same plant species may also vary significantly. Baroni et al. [19] showed that Sb translocation coefficients from roots to leaves of *Plantago lanceolata* varied from 0.21 to 2.76.

Effect on Chlorophyll Content

Effect of SMD treatment on chlorophyll content in leaves of *Zea mays* was shown in Fig. 2. It was found that a low percentage of SMD slightly altered chlorophyll content, indicating that SMD destroyed chlorophyll synthesis. Treatment with more than 10% SMD clearly decreased chlorophyll fluorescence. When the plants were watered with 100% SMD, the chlorophyll content was reduced by 39%.

Effect on PSII Function

The O-J-I-P fluorescence transient provides information on the status of Q_A , Q_B , and PQ pools [20]. Fig. 3a showed

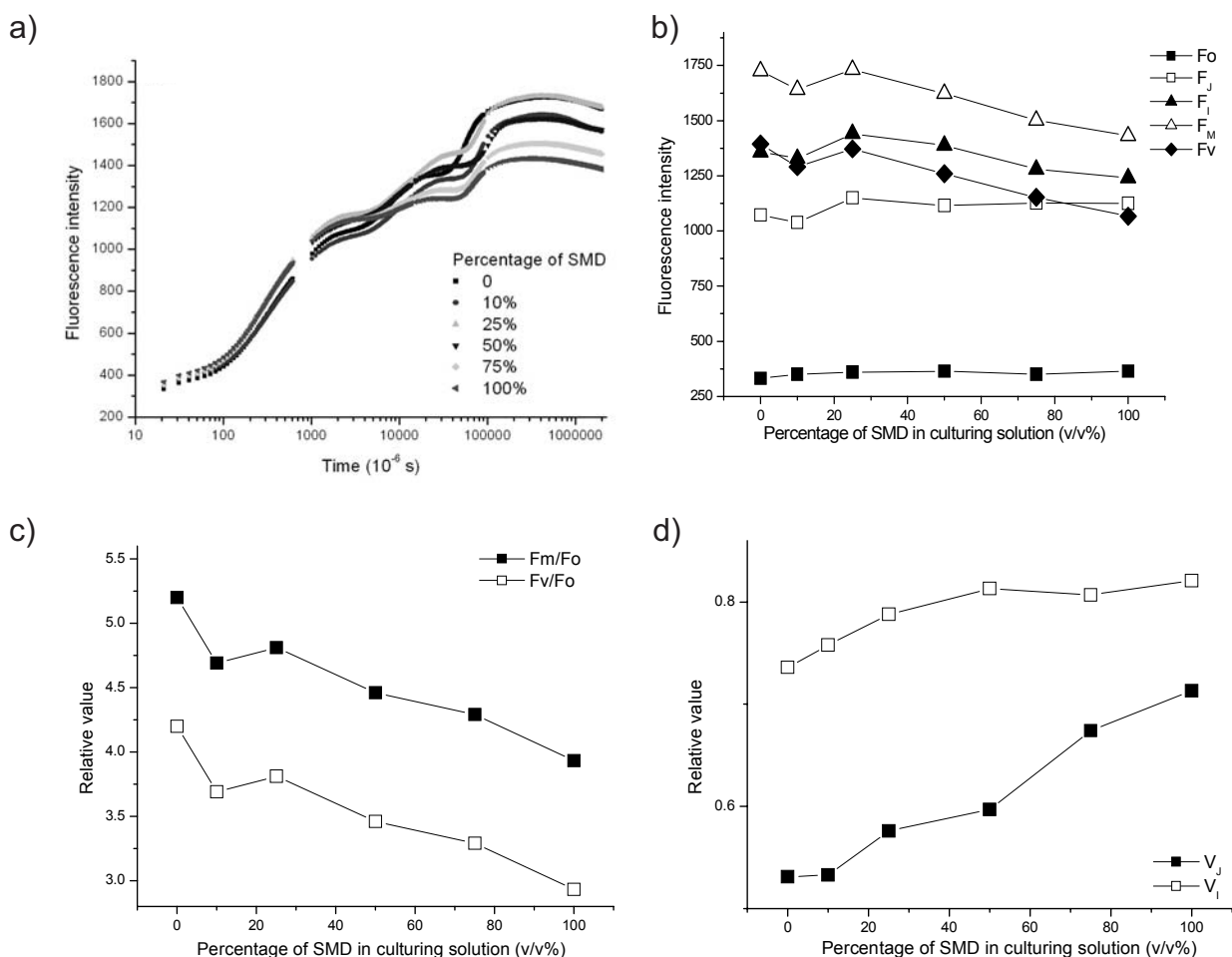


Fig. 3. OJIP transient curves and basic fluorescence parameters for seedlings watered with a culturing solution containing different percentages of drainage in culturing solution (v/v %).

- a) OJIP curves,
- b) F_0 , F_J , F_I , F_M , and F_V ,
- c) V_J and V_I , and
- d) F_M/F_0 and F_V/F_0 .

Each data point represents the mean value of five measurements.

the representative fast kinetic induction curves of plants growing in soils watered with culture solution containing different proportions of SMD. Compared with the control, fluorescence intensity at O (F_0) and J step (F_J) generally increased for plants watered with culture solution contain-

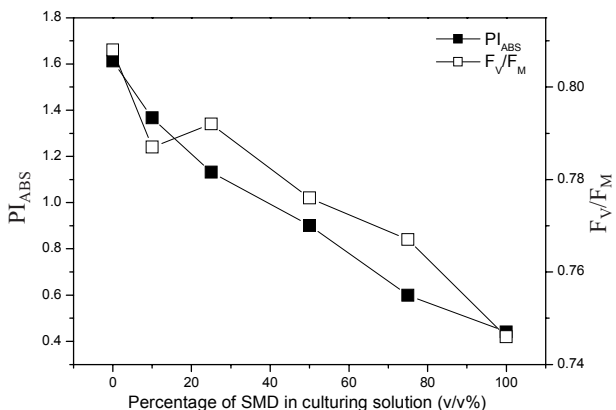


Fig. 4. PI_{ABS} and F_V/F_M for seedlings watered with culturing solution containing different percentages of SMD (v/v%). Each data point represents the mean value of five measurements.

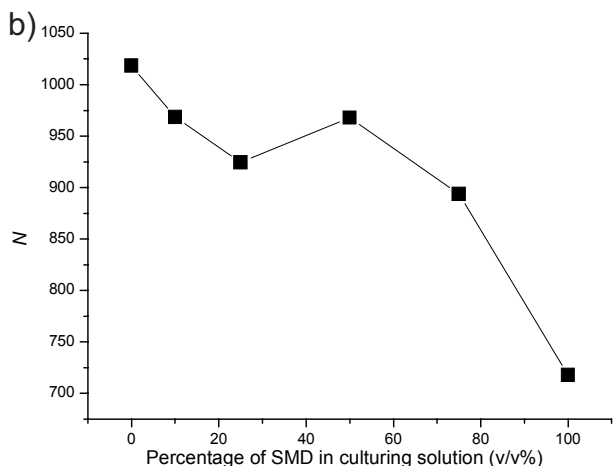
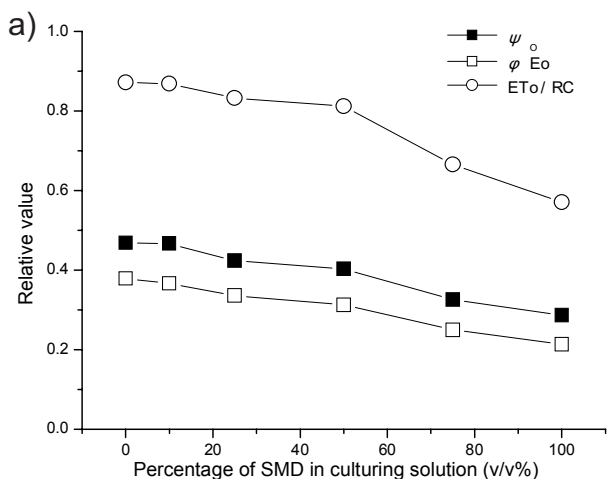


Fig. 5. Electron transport parameters a) and turnover number (N) b) for seedlings watered with culturing solution containing different percentages of SMD (v/v%). Each data point represents the mean value of five measurements.

ing low proportions of SMD and changed little when increasing the percentage of drainage in the culture solution (Fig. 3b). The increase in F_0 means photoinhibition, correlating with the occurrence of RCs with damage at the acceptor side of PSII [21]. Fluorescence intensity at I (F_I) and P step (F_M) generally showed a decreasing trend with increasing of the percentage of drainage (Fig. 3b). This resulted in a decrease in F_V , F_M/F_0 , and F_V/F_0 with increasing percentage of drainage (Figs. 3b and c). The lowering of F_M might have resulted from an increase in the proportion of the closed PSII RCs that did not participate in electron transport. The decrease in F_V indicates the lowered PSII capacity to reduce plastoquinone due to the disturbance of the PSII donor side [22] or the damage of the oxygen-evolving system [23]. F_V/F_0 indicates the number of active photosynthetic centers in the chloroplast and the decreasing of F_V/F_0 suggests that the photosynthetic process on the donor side of PSII was inhibited [24] and the water-splitting site was severely impaired [25].

SMD treatment caused a steady increases in V_J and V_I (Fig. 3d). The rise in V_J suggests an increase of the proportion of closed PSII RCs and the proportion of reduced Q_A at J step. The increasing of V_I indicates that the reduced Q_A and plastoquinone which cannot transfer electrons to the dark reactions accumulated.

The maximum photochemical yield of PSII (F_V/F_M) generally decreased with increasing percentage of drainage in the culturing solution (Fig. 4). The photosynthetic performance of PSII (PI_{ABS}) also decreased progressively with an increasing percentage of drainage.

The electron transport of PSII was inhibited by the SMD. The probability of electron transfer beyond Q_A (ψ_o) and the yield of electron transport beyond Q_A (ϕE_0) decreased with increasing percentage of drainage (Fig. 5a), suggesting that electron transport on the acceptor side was inhibited by drainage. Electron transport per RC (ET_o/RC), related to the reoxidation of reduced Q_A via electron trans-

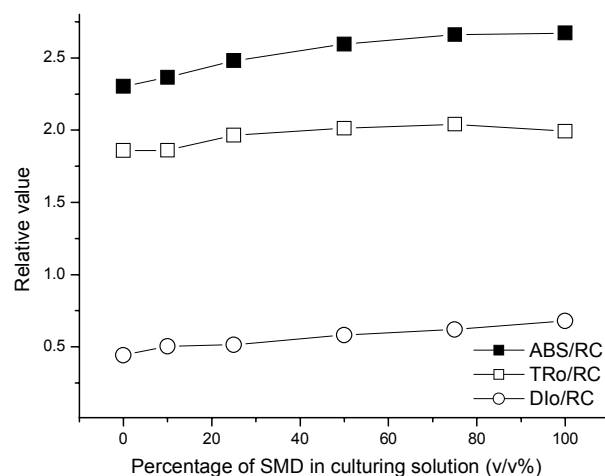


Fig. 6. Energy flux through PSII for seedlings watered with culturing solution containing different percentages of SMD (v/v%). Each data point represents the mean value of five measurements.

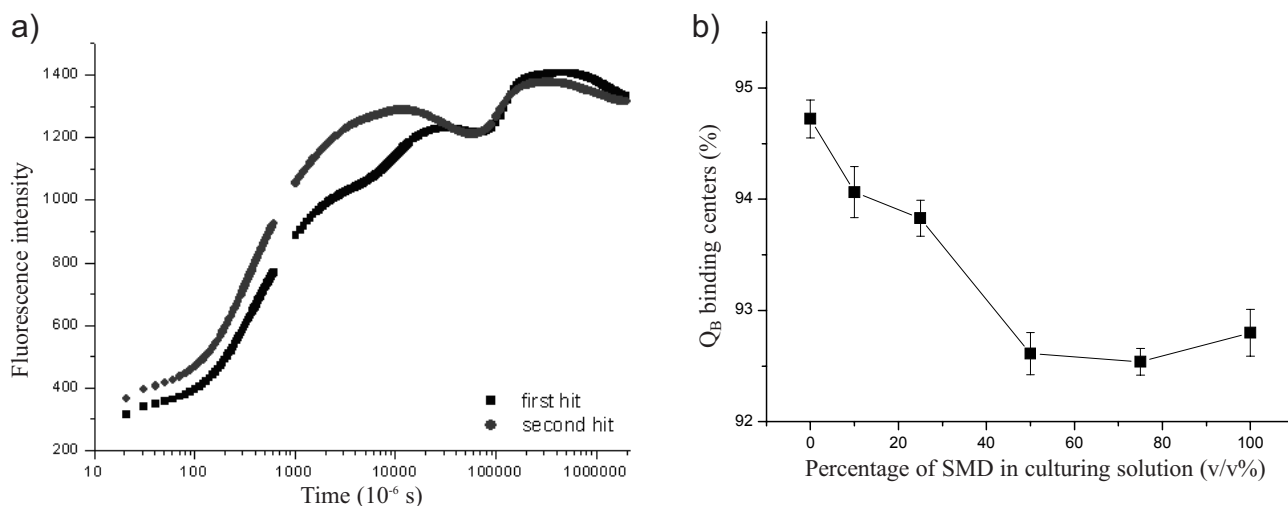


Fig. 7 a) representative curves in double-hit experiments and b) proportions of Q_B binding centers in PSII of seedlings watered with culturing solution containing different percentages of SMD. Each data point in b) represents the mean value \pm SE of five measurements.

port in an active RC, also dropped as the drainage percentage as the culturing solution increased. Effect of drainage on electron transport beyond the oxidation of Q_A^- was also evaluated by using the turn-over number (N). N indicates how many times Q_A has been reduced to Q_A^- in the time span from t_0 to tF_{max} . N showed a decreasing trend with increasing percentage of drainage (Fig. 5b), indicating that electron transport beyond the oxidation of Q_A^- was hindered by drainage.

Fig. 6 showed the effects of drainage on the specific energy fluxes through PSII at the RCs. The increase of drainage percentage resulted in the increase of the functional antenna size (ABS/RC), indicating that maize is unable to regulate the light-harvesting capacity in order to adapt to drainage stress. The trapping rate of the RC (TRo/RC) also increased with increasing percentage of drainage. The increase of the dissipation energy (DIo/RC=ABS/RC-TRo/RC) as the percentage of drainage increased, suggesting that PSII RCs are transformed into dissipative sinks for excitation energy under stress of drainage [26]. This was in accordance with the increase in F_0 after drainage treatment, which suggested the decreased efficiency of energy transfer from the antenna chlorophyll a to the RCs and/or the inactivation of PSII RCs [27].

The proportions of Q_B binding centers in PSII of seedlings irrigated with a culturing solution containing different percentages of SMD were evaluated. The typical curves in a double-hit experiment were shown in Fig. 7a. The fraction of Q_B -binding centers was clearly reduced after treatment with drainage (Fig. 7b). The proportion of Q_B -binding centers decreased drastically as a percentage of drainage increased up to 50% and then changed slightly with increasing percentage of drainage further. In the control plant, non- Q_B -binding centers accounted for about 5.3%. About 2.1% of Q_B -binding centers were inactivated after treatment with 50% drainage. Appenroth et al. [16]

reported similar findings. In their study, more than 6% binding centers were inactivated after 10 days of 50 μ M Cr treatment.

Conclusions

Maize growing in soil irrigated with SMD could accumulate Sb and translocate Sb from roots to shoots. The chlorophyll synthesis in maize seedling leaves was inhibited by Sb accumulation. Sb clearly reduced PSII activity (F_v/F_M and PI_{ABS}) in maize seedling leaves. Irrigation with SMD increased the proportion of the closed PSII RCs and decreased Q_B binding centers. Electron transport on both the donor side and the acceptor side was inhibited by drainage, with the steps beyond Q_A^- being the primary target site. PSII RCs were transformed into dissipative sinks for excitation energy under the stress of drainage.

Acknowledgements

This work was supported by the Knowledge Innovation Program of the Chinese Academy of Sciences (KZCX2-YW-335 and KZCX2-YW-135), the 100 Distinguished Young Scientists Program of the Chinese Academy of Sciences, the National Natural Science Foundation of China (40673070, 40872169) and 863 program projects 2006AA06Z339 from MOST of China. We are grateful to the anonymous reviewers for their valuable comments and suggestions.

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