Bioaccumulation and photosynthetic activity response of sweet sorghum seedling (*Sorghum bicolor* L. Moench) to cadmium stress

Z.C. XUE^{*}, J.H. LI^{*}, D.S. LI^{*}, S.Z. LI^{*,+}, C.D. JIANG^{**}, L.A. LIU^{**}, S.Y. WANG^{**}, and W.J. KANG^{**}

Institute New Energy Technology, Tsinghua University, Beijing 100084, China^{*} Institute of Botany, The Chinese Academy of Sciences, Beijing, 100093, China^{**}

Abstract

A hydroponic experiment was conducted to investigate bioaccumulation and photosynthetic activity response to Cd in sweet sorghum seedlings. The seedlings were treated with 0, 50, and 100 μ M Cd for 15 d. Our results showed that morphological characteristics of sweet sorghum were significantly affected by Cd treatments. The Cd concentrations in roots and shoots increased with increasing Cd concentrations in the nutrition solution; higher Cd accumulation was observed in the roots. Meanwhile, the photosynthetic activity decreased significantly and a shape of chlorophyll (Chl) *a* fluorescence transient in leaves was altered by Cd treatments. The Chl contents in the leaves decreased significantly, which was demonstrated by a change of spectral reflectance. Our data indicated that the higher Cd concentration reduced Chl contents and inhibited electron transport in the leaves, leading to the decrease of photosynthetic activity.

Additional key words: chlorophyll a fluorescence; energy plant; photosynthetic activity; soil contamination; spectral reflectance.

Introduction

Soil contamination with heavy metals has become a critical environmental issue throughout the world. Cadmium, which is one of the main toxic heavy metals in agricultural soils, is not needed by plants. It is mainly coming from livestock manures, atmospheric deposition, and fertilizers (Luo *et al.* 2009). Because of its high rate of soil-to-plant transfer, Cd can be easily acquired by plants, leading to inhibition of the growth and productivity of crops. Cd is efficiently retained by kidney and liver in the human body and it results in health issues after entering food chain (Satarug *et al.* 2010, Yuan *et al.* 2014, Zhong *et al.* 2015). Therefore, it is an urgent and necessary need to remediate the Cd contaminated agricultural soils for minimizing environmental impacts and ensure the safety

of food. Although various chemical (leaching and fixation) and physical (soil replacement, thermal desorption) methods have been used successfully for soil remediation, these methods are relatively expensive and are often unable to remove contamination completely. So phytoremediation has been adopted due to its cost-effective, nonintrusive, and ecofriendly nature (Gomes *et al.* 2016). Then, some researchers have suggested that using energy plants (Gomes 2012), such as maize (Vigliotta *et al.* 2016), rape seed (van Ginneken *et al.* 2007), and sweet sorghum (Tian *et al.* 2015, Zhuang *et al.* 2009) as phytoremediation plants, because of their rapid growth and high biomass production.

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⁺Corresponding author; phone: (86)010-62772123, e-mail: <u>szli@mail.tsinghua.edu.cn</u>

Abbreviations: ABS/RC – absorption flux per RC; DI_o/RC – dissipated energy flux per RC at t = 0; DM – dry mass; E – transpiration rate; ET_o/RC – electron transport flux per RC at t = 0; F₁ – fluorescence intensity at the I step (at 30 ms); F_J – fluorescence intensity; the J step (at 2 ms); F_m – maximal fluorescence intensity; F_o – fluorescence intensity at 20 µs; F_t – fluorescence emission from a darkadapted leaf at the time t; g_s – stomatal conductance; mND₇₀₅ – modified red-edge normalized difference vegetation index; M_o – slope of the curve at the origin of the relative variable fluorescence rise; mSR₇₀₅ – modified red-edge ratio; PI_{abs} – performance index; P_N – net photosynthetic rate; PRI – photochemical reflectance index; RC – reaction center; RC/CS_o – Q_A-reducing reaction centers per cross-section; TF – translocation factor; TR_o/RC – trapped energy flux per RC at t = 0; V_t – relative variable fluorescence at the time t; δ_{Ro} – probability that an electron is transported from the reduced intersystem electron acceptors to the final electron acceptors of PSI; ϕ_{Po} – maximum quantum yield for primary photochemistry; Ψ_{Eo} – probability that an electron moves further than Q_A⁻.

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Sweet sorghum [Sorghum bicolor (L.) Moench], which is considered as a great promising energy plant, shows a rapid growth, high yield, rich carbohydrate content in stalk, and wider adaptability (Almodares and Hadi 2009, Guo et al. 2018). The stalk yield of sweet sorghum can reach 60–90 t ha⁻¹ and sugar content of 10–15% (w/w). It is a good candidate for bioenergy production as s potential yield of sweet sorghum in producing ethanol is 3,000-5,220 L ha⁻¹ (Sathya et al. 2016). Previous studies have proved that sweet sorghum has the ability to absorb heavy metals (An 2004, Marchiol et al. 2007, Tian et al. 2015). Therefore, the phytoremediation technology based on sweet sorghum could effectively utilize the remedying plants to produce ethanol and power and prevent Cdcontaminated agro-products from entering the food chain. Sweet sorghum can extract more than 0.05 kg ha⁻¹ of Cd in a single crop, when the diethylene triamine pentaacetic acid-extractable Cd concentrations is 1.02 mg kg⁻¹(soil) (Zhuang et al. 2009). Tian et al. (2015) study showed that the morphological characteristics of sweet sorghum were not significantly changed with the Cd concentration $\leq 5 \text{ mg}$ kg⁻¹(soil). However, a plant height and dry mass of S. bicolor decreased 27.6-28.5% and 38.7-51.5%, respectively, when the Cd concentration in soil was 15 mg kg⁻¹ (Wang et al. 2017). When the Cd concentration increased

Materials and methods

Plant materials and treatments: Sweet sorghum [Sorghum bicolor (L.) Moench, cv. BL0602] plants were grown in pots (20 cm in diameter and 35 cm in height) containing quartz sand in a greenhouse. During the growth period, the average day/night temperatures were 32/20°C, the relative humidity were 70-95%, and midday PPFD was 800 μ mol m⁻² s⁻¹. The plants were supplied daily with half-strength Hoagland solution to avoid any potential nutrient and drought stresses. The plants were thinned to three plants per pot 5 d after sowing. Then, Cd treatments were applied to the plants after two weeks. The treatment solutions were prepared with CdNO₃·2.5 H₂O to give Cd concentration of 0, 50, and 100 µM. The treatments continued for 15 d. The sand was flushed with the sufficient treatment solution (about double amount of the water that the sand in the pot can hold) daily to maintain the concentration of nutrition minerals and Cd concentrations constant during the treatment period. The new fully expanded leaves were used for measurement of gas exchange, Chl a fluorescence, and spectral reflectance.

Morphological response: The leaf area of the new fully expanded leaves was measured using a *LI-3000C* portable area meter (*LI-COR Biosciences*, USA). The number of leaves, root and shoot length was measured at the end of the experiment. Then the dry masses of the roots and shoots were determined under 80° C to constant mass. Fifteen replicate measurements were made for each treatment and the results were averaged.

to 30 mg kg⁻¹, shoot, leaf, and seed biomass of Yajin No.1 decreased to 68.7, 75.1, and 70%, respectively (Tian et al. 2015). The inhibition of sweet sorghum growth under high Cd concentration occurs due to reduced photosynthetic activity in the leaves. Many studies have demonstrated that the decrease in photosynthetic rate under Cd stress might be a result of reduced Chl contents (Küpper et al. 1996, He et al. 2008), obstructed electron transport (Pagliano et al. 2006, Sigfridsson et al. 2004), as well as a perturbation of enzymes of CO₂ fixation (Parmar et al. 2013). Although the effects of Cd on the photosynthetic activity have been assessed in a variety of plants, such as rice (Wang et al. 2014, He et al. 2008), maize (Silva et al. 2017, Lysenko et al. 2015, Wang et al. 2009), and soybean (Xue et al. 2013), the effects of Cd on photosynthetic activity of sweet sorghum leaves have received only limited attention.

Therefore, in order to assess the photosynthetic activity response of sweet sorghum to the Cd stress, we used hydroponics experimental with a higher concentration of Cd treatment [50 and 100 μ m, approximately 5.62 and 11.24 mg kg⁻¹(soil)] to investigate gas exchange, Chl *a* fluorescence, and spectral reflectance in the leaves of sweet sorghum. Then, the effects can be observed in a short time.

Gas exchange: The net photosynthetic rate (P_N), stomatal conductance (g_s), and transpiration rate (E) were measured using a *CIRAS-2* portable photosynthetic system (*PP Systems*, USA) on a sunny day between 9:30 and 11:00 h. (Xue *et al.* 2014). The atmospheric conditions in the leaf chamber were controlled by the *CIRAS-2* during the measurement, the PPFD at 1,200 µmol m⁻² s⁻¹, temperature at 25°C, and CO₂ concentration at 360 mmol mol⁻¹. The light was provided by a red/blue LED source. Five replicate measurements were made for each treatment, and the results were averaged.

Chl *a* **fluorescence transient** was measured using a *Handy-PEA* fluorometer (*Hansatech*, UK). Following dark adaptation for 20 min, all leaves were immediately exposed to a saturating light pulse of 3,500 µmol(photon) $m^{-2} s^{-1}$ by red light (peak at 650 nm) for 2 s. Each transient obtained from the dark-adapted samples was analyzed according to the JIP-test (Appendix) (Strasser *et al.* 2004, Salvatori *et al.* 2015, Chen *et al.* 2016). Fifteen replicate measurements were made for each treatment, and the results were averaged.

Spectral reflectance measurements were measured using a Unispec SC field portable spectrometer (*PP Systems*, USA). Leaf reflectance was measured with a bifurcated fiber optic cable and a leaf clip (models UNI410, *PP* Systems, Haverhill, MA). Leaf illumination was provided by a tungsten halogen lamp in the spectrometer. Thirty measurements were made for each treatment, and the results were averaged. A linear interpolation routine was used to estimate values at 1-nm intervals prior to calculation of indexes. The following established vegetation indexes, which derived from spectral reflectance, were then calculated: (1) the photochemical reflectance index (PRI), PRI = $(R_{531} - R_{570})/(R_{531} + R_{570})$ (Gamon *et al.* 1992); (2) the modified red-edge ratio (mSR₇₀₅), mSR₇₀₅ = $(R_{750} - R_{445})/(R_{705} - R_{445})$ (Sims and Gamon 2002); (3) the modified red-edge normalized difference vegetation index (mND₇₀₅), mND₇₀₅ = $(R_{750} - R_{705})/(R_{750} + R_{705} - 2R_{445})$ (Sims and Gamon 2002).

Cd concentrations: The dried roots and shoots of the sweet sorghum plants were grounded to fine powder and digested in 6 mL of a mixture of nitric acid and hydrogen

Results and discussion

The Cd concentrations in both roots and shoots of sweet sorghum seedling increased with increasing Cd concentrations in the Hoagland solution (Fig. 1A). It is well known that Cd is easily acquired by root systems of plants through plasma membrane transporters (Kim et al. 2002, Song et al. 2017). Then Cd is transported to shoots driven by transpiration (Liu et al. 2016, Salt et al. 1995). There were found positive and linear relationships between the transpiration rate and the Cd accumulation in the shoots (Lai 2015, Liu et al. 2016). The more Cd available to be absorbed in the rooting medium, the more Cd can be acquired by the roots (Soudek et al. 2014), which is consistent with our observation. However, the Cd concentrations in the roots were significantly higher than those in the shoots, and the TF of the sweet sorghum was about 0.2under different Cd concentrations (Fig. 1A). It means that the sweet sorghum could mainly accumulate Cd in the roots, which could act as a major protective mechanism to reduce the Cd transport to shoots (Pinto et al. 2004). However, the excess Cd in the plants can profoundly interfere with a series of biochemical and physiological processes, such as inactivation enzymes activity, disturbance in nutrient uptake, and inhibition of photosynthesis (Jia et al. 2016, Parmar et al. 2013, Soudek et al. 2014, Siedlecka and Krupa 1999). This leads to a significant reduction of the biomass, or even to death of the plant under high Cd concentrations. In this study, the morphological characteristics of sweet sorghum were significantly changed by the Cd treatments. The root and shoot length, leaf area, root and shoot mass, and the number of leaves were significantly lowered with the increase of the Cd concentrations in Hoagland solution. Under 50 and 100 µM Cd treatments, the shoot length decreased by 20.4 and 44.5%, the shoot mass decreased by 21.8 and 48.2%, respectively (Table 1). Our results are consistent with those of Pinto et al. (2004) who reported that a significant decrease of biomass was observed for sorghum grown in nutrient solutions with 10 mg(Cd) L⁻¹. Due to the peroxide (2:1, v/v) at 160°C for 5 h. Then the Cd concentrations were determined, using an inductive coupled plasma mass spectrometer (*Agilent 7700X, Agilent Technologies*, USA). Three measurements were made for each treatment, and the results were averaged.

Statistical analysis: Translocation factor (TF) was used to present the ability of plants to translocate heavy metal from roots to shoots. It was calculated using the formula: TF = Cd concentrations in shoots [mg g⁻¹(DM)]/Cd concentrations in roots [mg g⁻¹(DM)]. Data were subjected to an analysis of variance (*ANOVA*), using *SPSS 22*. And significant differences between mean values were determined through least significant difference (LSD) test. Differences were considered statistically significant when *P*<0.05.



Fig. 1. The translocation factor (TF, A, right axis), Cd concentration in root and shoot (A, left axis) and accumulation (B) in the root and shoot of sweet sorghum plants after being treated with different concentrations of Cd for 15 d. The means \pm SE of three replicates are shown. The *different letters* indicate significant differences at P<0.05 between different treatments.

significantly declined dry mass, the Cd accumulation in the roots under 100 μ M Cd concentration was lower than that under 50 μ M (Fig. 1*B*).

Previous research has suggested that high Cd concentrations [100 mg(Cd) kg⁻¹(soil)] (Gill *et al.* 2012). As a C₄ crop, sweet sorghum has high photosynthetic efficiency. In the present study, the P_N , g_s , and E in the leaves of sweet sorghum decreased significantly by Cd treatment. The P_N decreased by 23.6 and 38% under 50 and 100 μ M Cd

Table 1. The root length, shoot length, leaf area, root mass, shoot mass, and number of leaves of sweet sorghum plants after being treated with different concentrations of Cd for 15 d. The means \pm SE of 15 replicates are shown. The *different letters* indicate significant differences at *P*<0.05 between different treatments.

Cd [µm]	Root length [cm]	Shoot length [cm]	Leaf area [cm ²]	Root mass [g]	Shoot mass [g]	Number of leaves
0 50 100	$\begin{array}{l} 17.63 \pm 1.69^a \\ 17.75 \pm 4.80^a \\ 12.57 \pm 2.43^b \end{array}$	$\begin{array}{c} 42.00 \pm 4.44^{a} \\ 33.42 \pm 3.11^{b} \\ 23.31 \pm 3.24^{c} \end{array}$	$\begin{array}{c} 19.92 \pm 3.63^{a} \\ 15.80 \pm 3.92^{b} \\ 12.48 \pm 2.45^{c} \end{array}$	$\begin{array}{c} 0.087 \pm 0.003^a \\ 0.070 \pm 0.002^b \\ 0.034 \pm 0.002^c \end{array}$	$\begin{array}{c} 0.166 \pm 0.003^a \\ 0.131 \pm 0.004^b \\ 0.086 \pm 0.003^c \end{array}$	$\begin{array}{c} 5.20 \pm 1.03^{a} \\ 3.00 \pm 0.52^{b} \\ 2.90 \pm 0.74^{c} \end{array}$

treatments (Fig. 2A), respectively, while E decreased by 35.3 and 47.5%, respectively. The reduction of g_s could result in the decrease of P_N and E. It was further supported by fact the g_s was well correlated with the P_N ($R^2 = 0.98$) and $E(R^2 = 0.98)$. Meanwhile, Chl contents were reduced by Cd. In this study, spectral reflectance techniques was adopted to gain insights of the change of Chl contents in the leaves. The spectral curves of the sweet sorghum leaves increased significantly in a visible range (500-700 nm), in dependence on Chl contents in the leaves, after treatments with different concentrations of Cd (Fig. 3A). From the difference in reflectance, we could observe the significant changes caused by the Cd treatment (Fig. 3B). The PRI can serve as a good indicator of the change in photosynthetic apparatus under environmental stress (Gamon et al. 1992, Zhang et al. 2017). In this study, the decrease of PRI in the leaves of sweet sorghum under Cd stress was similar to that of P_N (Fig. 4A), which is demonstrated by the significant correlation between the PRI and $P_{\rm N}$ in the leaves ($R^2 = 1.00$). The significantly decreased mND₇₀₅ (Fig. 4B) and mSR₇₀₅ (Fig. 4C), which have a high correlation with Chl contents across different leaf types (Sims and Gamon 2002), indicated that the Chl content in the leaves decreased significantly by the Cd treatment. Cd could induce the inhibition of Chl biosynthesis and the substitution of the central Mg²⁺ in Chl molecule (Küpper et al. 1996, Wang et al. 2014). The decrease of the Chl content can partly account for the decrease of $P_{\rm N}$, which is in agreement with previous reports (Wang et al. 2014).



Fig. 2. The net photosynthetic rate (P_N , A), stomatal conductance (g_s , B), and transpiration rate (E, C) in the leaves of sweet sorghum plants after being treated with different concentrations of Cd for 15 d. The means \pm SE of five replicates are shown. The *different letters* indicate significant differences at P<0.05 between different treatments.



Fig. 3. The reflectance (A) and difference in reflectance (B) in the leaves of sweet sorghum plants after being treated with different concentrations of Cd for 15 d. Each curve represents the average of thirty independent measurements.



Fig. 4. The photochemical reflectance index (PRI, A), modified red-edge ratio (mSR₇₀₅, B), and modified red-edge normalized difference vegetation index (mND₇₀₅, C) in the leaves of sweet sorghum plants after being treated with different concentrations of Cd for 15 d. The means \pm SE of thirty replicates are shown. The *different letters* indicate significant differences at *P*<0.05 between different treatments.



Fig. 5. The chlorophyll a fluorescence rise kinetics (OJIP) of sweet sorghum leaves after being treated with different concentrations of Cd for 15 d. Each curve represents the average of 15 independent measurements.

Cd in chloroplasts could interact with Chl-protein complexes and disrupt energy absorption and transfer in photosystems (Bashir *et al.* 2015). In this study, we estimated photosynthetic efficiency of Cd-treated plants by Chl *a* fluorescence technique, which is a very sensitive

Table 2. The φ_{Po} , Ψ_{Eo} , δ_{Ro} , PI_{abs} , RC/CS_o , DI_o/RC , ABS/RC, TR_o/RC , and ET_o/RC in the leaves of sweet sorghum after treated with different concentrations of Cd for 15 d. Fifteen replicate measurements were made for each treatment, and the results were averaged. The *different letters* indicate significant differences at P < 0.05 between different treatments.

Parameter	Cd concentratio 0	on [µM] 50	100	
$\begin{array}{c} \phi_{Po} \\ \Psi_{Eo} \\ \delta_{Ro} \\ RC/CS_o \\ ABS/RC \\ TR_o/RC \\ ET_o/RC \\ DI_o/RC \\ PI_{abs} \end{array}$	$\begin{array}{c} 0.75\pm 0.02^a\\ 0.62\pm 0.01^a\\ 0.32\pm 0.03^a\\ 173.41\pm 7.48^a\\ 2.67\pm 0.21^c\\ 1.99\pm 0.11^c\\ 1.24\pm 0.06^b\\ 0.67\pm 0.10^c\\ 1.89\pm 0.35^a\\ \end{array}$	$\begin{array}{c} 0.69 \pm 0.02^b \\ 0.55 \pm 0.02^b \\ 0.24 \pm 0.04^b \\ 153.33 \pm 9.06^b \\ 3.39 \pm 0.16^b \\ 2.35 \pm 0.09^b \\ 1.29 \pm 0.08^b \\ 1.04 \pm 0.09^b \\ 0.83 \pm 0.10^b \end{array}$	$\begin{array}{c} 0.67 \pm 0.02^{\circ} \\ 0.49 \pm 0.03^{\circ} \\ 0.20 \pm 0.05^{\circ} \\ 103.37 \pm 20.43^{\circ} \\ 4.30 \pm 0.53^{a} \\ 2.87 \pm 0.33^{a} \\ 1.41 \pm 0.19^{a} \\ 1.43 \pm 0.23^{a} \\ 0.47 \pm 0.12^{\circ} \end{array}$	

to stress and can gain insights of structure, conformation, and function of photosynthetic apparatus (Stirbet and Govindjee 2011, Salvatori et al. 2015, Kalaji et al. 2016). The change of the shape of Chl *a* fluorescence transients of sweet sorghum leaves by Cd treatment suggested that Cd significantly influenced the performance of photosynthetic machinery (Fig. 5). In order to reveal further a damage to PSII, the transients were analyzed according to the JIP-test. The parameters derived from Chl a fluorescence were analyzed and shown in Table 2. The results showed Cd-induced increase of DIo/RC, ABS/RC, TR_o/RC, and ET_o/RC, as well as a significant decrease of the $\phi_{Po},\,\Psi_{Eo},\,\delta_{Ro},\,PI_{abs},$ and RC/CSo. The decrease in ϕ_{Po} indicated that the photosynthetic efficiency of sweet sorghum was lowered, implying that the primary photochemical reactions were affected with the higher Cd concentration. Ψ_{E_0} represents the probability that an absorbed photon moves an electron further than Q_A^- , and δ_{R_0} represents the probability that an electron is transported from the reduced intersystem electron acceptors to the final electron acceptors of PSI (Strasser et al. 2004). The decrease in Ψ_{Eo} and δ_{Ro} of sweet sorghum leaves under Cd treatments suggested that the electron transport of PSII was blocked due to QA- accumulation. Whereas, the increase in DIo/RC, ABS/RC, TRo/RC, and ETo/RC suggests that a fraction of active reaction centers was inactivated, which was also confirmed by decreases in RC/CS_o. It was supported by the decreased Chl contents in leaves under Cd treatments (Fig. 4) and the decrease of Chl a content was more pronounced than that of Chl b under Cd treatments (He et al. 2008). The performance index, PIabs, which combines three main structural and functional characteristics of PSII (ABS/RC, ϕ_{Po} , Ψ_{Eo}), is closely related to the ability of energy conservation and the activity of photosynthetic apparatus. A significant decrease in the PI_{abs} of sweet sorghum leaves with the enhanced Cd concentrations indicated that the electron transfers were blocked. Previous research has suggested that Cd could exert multiple effects on both donor and acceptor sides of the PSII (Pagliano *et al.* 2006, Sigfridsson *et al.* 2004). Cd could exchange with Ca²⁺ in oxygen-evolving complex on the donor side and decrease the rate of electron transfer from Q_A to Q_B due to interaction with non-heme Fe and conformational modification of Q_B pocket (Parmar *et al.* 2013).

However, different plant species (Li *et al.* 1997), even cultivars (Franić *et al.* 2017), have different Cd tolerance. So further investigation is needed to elucidate the mechanism of the Cd distribution in the different sweet

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sorghum genotypes and choose cultivars according to the environmental conditions.

Conclusion: The results demonstrated that the Cd concentration in the sweet sorghum seedling increased with the increasing Cd concentration in the nutrition solution and more Cd accumulated in the roots. The higher Cd concentration decreased Chl contents and obstructed electron transport in the leaves, leading to the decrease of photosynthetic activity.

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Appendix

Formulae and explanation the technical data of the OJIP curves and the selected JIP-test parameters used in this study.

Fluorescence parameter	Description
Ft	Fluorescence emission from a dark-adapted leaf at the time t
Fo	Fluorescence intensity at 20 µs
FJ	Fluorescence intensity at the J step (at 2 ms)
FI	Fluorescence intensity at the I step (at 30 ms)
F _m	Maximal fluorescence intensity
$M_o = 4 (F_{300\mu s} - F_o)/(F_m - F_o)$	Slope of the curve at the origin of the relative variable fluorescence rise
$V_t = (F_t - F_o)/(F_m - F_o)$	Relative variable fluorescence at the time t
$\varphi_{\rm Po} = (F_{\rm m} - F_{\rm o})/F_{\rm m}$	Maximum quantum yield for primary photochemistry
$\Psi_{\rm Eo} = 1 - V_{\rm J}$	Probability that an electron moves further than Q_A^-
$\delta_{\rm Ro} = (1 - V_{\rm I})/(1 - V_{\rm J})$	Probability that an electron is transported from the reduced intersystem
	electron acceptors to the final electron acceptors of PSI
$RC/Cs_o = \varphi_{Po}(V_J/M_o) F_o$	Q _A -reducing RCs per CS
$ABS/RC = M_o (1/V_J) (1/\phi_{Po})$	Absorption flux per RC
$TR_o/RC = M_o (1/V_J)$	Trapped energy flux per RC at $t = 0$
$ET_o/RC = M_o (1/V_J) \Psi_{Eo}$	Electron transport flux per RC at $t = 0$
$DI_o/RC = (ABS/RC) - (TR_o/RC)$	Dissipated energy flux per RC at $t = 0$
$PI_{abs} = (RC/ABS) \left[\phi_{Po}/(1 - \phi_{Po}) \right] \left[\Psi_{Eo}/(1 - \Psi_{Eo}) \right]$	The performance index