Cytokinin-induced photosynthetic adaptability of Zea mays L. to drought stress associated with nitric oxide signal: Probed by ESR spectroscopy and fast OJIP fluorescence rise

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A R T I C L E   I N F O

Keywords:
Cytokinin
Drought stress
Fast OJIP fluorescence rise
Nitric oxide
ROS

A B S T R A C T

Nitric oxide (NO), as a diffusible molecule, performs important roles in diverse physiological processes. Interestingly, NO signaling is based on interactions with plant hormones. The aim of this study was, first, to test the effect of cytokinin (CTK) on the primary reaction of photosynthesis under drought stress, and then to examine whether NO is involved in CTK-induced photosynthetic resistance due to its role as a second messenger in stress response. Under drought stress, plants were treated with CTK, or CTK plus the NO scavenger (Hemoglobin [Hb]) for 6 h. The effects of CTK and Hb on fast OJIP fluorescence rise were then examined. At the same time, NO and reactive oxygen species (ROS) signals in all the treatments were detected by electron spin resonance (ESR) spectroscopy. The results showed that CTK-regulated fluorescence transient rise under drought stress and increased the electron donation capacity of photosynthesis system (PS) II. The plant photosynthetic performance index (PI) on an absorption basis and corresponding three PI components (RC/ABS, P N and P E ) also increased. High NO signal intensity alleviated drought-induced ROS damage to plants; thus, the signal probably played a direct role in eliciting CTK regulation to energy absorption (RC/ABS) and excitation energy trapped (P E ) in response to drought. Although CTK stimulated more excitation energy conversion to electron transfer (P ET ), because NO was probably bound to the plastoquinone pool (PQ) of the electron transport chain, CTK decreased electron transport to the acceptor side of PSII (see V t, Sm and N). Furthermore, CTK stimulated more NO signal formation, probably mainly via a nitrate reductase (NR) source under the conditions of the study, and Hb prevented stimulation from CTK. However, these results will require confirmation from future studies.

Introduction

Nitric oxide (NO) is a uniquely volatile molecule in plants (Velikova et al., 2008). The finding that NO acts as a multifunctional messenger has changed our understanding of the free radical as a toxic by-product in oxidative metabolism to a key regulator of cellular functions. The messenger, which is released from plant leaves subject to environmental stimuli (Arasimowicz and Floryszak-Wieczorek, 2007), is involved in the activation of antioxidant enzymes through defense gene up-regulation (Xie et al., 2008), so that plant resistance to environmental stresses such as drought, salt, and heat stress is enhanced (Qiao and Fan, 2008). In summary, it is widely accepted that NO plays a crucial role in plant tolerance to adverse environments.

Drought is one of the key environmental stresses that commonly interfere with plant water homeostasis. Under water stress, photosynthesis maintenance depends on photosynthetic apparatus adjustment (Shangguan et al., 1999). In addition,
photochemical quenching and photosynthetic quantum conversion decrease with increase in water stress (Shangguan et al., 2000). In recent years, photosynthetic sites were recognized as endogenous NO cellular sources (Jasid et al., 2006). Some studies reported the cyto-protective role of NO in plant photosynthesis (Velikova et al., 2008) via mediating stomatal closure, as well as by interaction with Ca2+ signals (Courtis et al., 2008). Other data showed that NO was a reversible inhibitor to photosynthetic adenosine triphosphate (ATP) synthesis (Takahashi and Yamazaki, 2002), and that its production occurred downstream of reactive oxygen species (ROS) in guard cells during stomatal closure (Srivistava et al., 2009). Furthermore, some reports showed that the binding sites of NO within the photosynthetic system (PS) II complexes were the nonheme iron between the primary quinone acceptor QA and the secondary quinone acceptor QB (Wodala et al., 2008). Therefore, the role of NO in photosynthetic function in intact but drought-injured leaves has been poorly addressed by interaction with Ca2+ signals (Courtois et al., 2008). Other data reported the cyto-protective role of NO in plant photosynthesis endogenous NO cellular sources (Jasid et al., 2006). Some studies showed that NO was a reversible inhibitor to photosynthetic activity are numerous and likely related to other still unidentified signals. Based on these results, we hypothesized that the NO signal is involved in regulating photosynthesis via CTK under environmental stresses.

Drought decreases the photosynthetic rate because of a proportional increase in inactivated reaction centers (RCs) (non-QA reducing or heat sink centers). Photosynthetic electron transfer is then inhibited, depending on the water deficit level and plant species. Among the techniques suitable for assessing water stress conditions in terms of photosynthesis, the measurement of direct chlorophyll (Chl) fluorescence in PSII and “JIP-test” analysis (i.e., the analysis of the OJIP polyphasic transient rise) (Strasser et al., 2000; Strasser et al., 2004) are relatively inexpensive. The kinetics of OJIP transient rise provides detailed information on the structure and function of photosynthetic apparatus of plants suffering from water deficit (Oukarroum et al., 2007), and the transient rise is thought to largely depend on the changes in the redox state of QA (Strasser et al., 2004). Furthermore, it reflects the reduction of the photosynthetic electron transport chain (Schansker et al., 2005). Fluorescence parameters derived from OJIP transient rise, such as the photosynthetic performance index (PI) on an absorption basis and its components derived by flux theory, have been suggested to describe changes of energy absorption, trapping, and conversion (Strasser et al., 2000). The analysis of these parameters is called a JIP-test.

The objectives of our study were, first, to investigate the effect of CTK on the regulation of photosynthetic capacity under drought stress as revealed by the JIP-test, and then to employ the electron spin resonance (ESR) technique to test the hypothesis that CTK-induced photosynthetic resistance to drought is associated with NO signals. In addition, CTK stimulation of NO formation via NR under the conditions of this study began to emerge and deserves further molecular and physiological investigation.

Material and methods

Plant material and growth conditions

Maize (Zea mays L. cvs Shandan 2552) seeds were obtained from Northwest Agriculture and Forestry University (Yangling, Shaanxi, China). The seeds were soaked in water containing 0.2% (w/v) tiuram and benomyl for 24 h to prevent fungal infection, then placed on moist gauze spread in disks, and finally kept in the dark to germinate at 28 °C for 4 d. Uniformly germinated seeds were planted in pots filled with clean quartz sand and cultured with whole Hoagland’s nutrient solution in a growth chamber (Hangzhou Qishi Artificial Environment Company, Ltd., Hangzhou, China) for 20 d. During the culture, the photoperiod was 13 h (day, 600 μmolCO2 m−2 s−1)/11 h (night), the temperature was 28 °C (day)/22 °C (night), and the midday relative humidity was 60% (day/night).

After the maize seedlings had grown for 20 d, 18% PEG (−0.5 MPa) was added to the Hoagland solutions and the seedlings were kept to grow for 6 d. Then the drought-stressed plants were then incubated at 25 °C at light for 6 h in distilled water with either: (1) −0.5 MPa osmotic solution, i.e. 18% PEG (D); (2) osmotic solution+0.01 mmol L−1 6-BA (D+BA); (3) osmotic solution+0.01 mmol L−1 6-BA+5% Hb (D+BA+Hb); and (4) osmotic solution+5% Hb (D+Hb). In addition, the control plants, which suffered no drought stress over the entire study period, were then incubated with distilled water. The treatments were randomly arranged and repeated four times. The uppermost fully expanded leaves were chosen for relevant measurements. The parameters concerned were measured at the 6th hour after each treatment was carried out.

Analysis of Chl fluorescence transient rise: “JIP-test”

Induced by a red saturating light, the OJIP rising transient was measured with leaves that were dark-adapted for 20 min. On a logarithmic time scale, the rising transient from F0 (F measured at 50 μs when all the RCs of PSII were open, i.e., when QA was fully oxidized) to Fv (where Fv=Fm under the saturating excitation light, of which the excitation intensity was high enough to ensure the closure of all the RCs of PSII, i.e., the full reduction of all the RCs) showed a polyphasic behavior. The analysis of the transient took into consideration fluorescence values at 50 μs (F0, step O), 100 μs (F100 μs), 300 μs (F300 μs, step K), 2 ms (F2 ms, step J), 30 ms (F30 ms, step I), and maximal level (Fm, step P).

The JIP-test represents a translation of original data into biophysical parameters that quantify the energy flow through PSII. The photosynthetic activity of an RC complex is regulated in three steps: absorption of light energy (ABS), trapping of excitation energy (TR), and conversion of energy to electron transport (ET). PI is introduced as a multi-parametric expression of the three independent steps contributing to photosynthesis. PI and its three components are calculated according to original fluorescence measurements (Strasser et al., 2000; Strasser et al., 2004).

The expression of RC/ABS=[(F2 ms–F0)/4(F300 μs–F0)]·(Fv/Fm) represents the active RC density on a Chl basis. Decrease in
Nitrate reductase activity

Nitrate reductase activity was measured as described by Luo et al. (2006). A fresh leaf sample (1 g) for enzyme extraction was ground in liquid N and 5 mL extraction buffer. For NR assays, the 5 mL extraction buffer contained 25 mmol L\(^{-1}\) potassium phosphate buffer (pH 8.8), 10 mmol L\(^{-1}\) cysteine, and 1 mmol L\(^{-1}\) ethylenediaminetetraacetic acid (EDTA). After the leaf sample was ground until it thawed and transformed into a solution, the solution was centrifuged at 20,000 \(\times\) g for 20 min. Then its extracted aliquots were an enzyme extracts reaction mixture that contained 1.2 mL 0.1 mmol L\(^{-1}\) potassium phosphate buffer (pH 7.5), 0.1 mmol L\(^{-1}\) KNO\(_3\), and 0.4 mL 0.25 mmol L\(^{-1}\) nicotinamide adenine dinucleotide (NADH) was added to the 0.4 mL enzyme extracts and mixed at 25 \(^\circ\)C for 30 min. The control plants were prepared by adding 0.1 mmol L\(^{-1}\) potassium phosphate buffer (pH 7.5) instead of NADH. After 30 min, the reaction was stopped by adding 10 g kg\(^{-1}\) sulphanilamide. Then, 2 mol L\(^{-1}\) HCl and 0.2 g kg\(^{-1}\) N-(1-naphthyl)-ethylenediammonium dichloride (NED) were also added to the reaction solution for chromogenic reaction and were centrifuged at 20,000g for 4 \(^\circ\)C for 15 min. The NO\(_2\) produced from the supernatant was calorimetrically measured at 540 nm. NR activity was expressed in \(\mu\)g NO\(_2\) g\(^{-1}\) FW h\(^{-1}\).

ESR detection of leaf NO and ROS

The validity of the DCFH (2,7-′-dichlorofluorescin) method in monitoring ROS generation in cells in the presence of oxidizing systems has been questioned by some researchers (Bonini et al., 2006). In order to determine leaf ROS or NO variations in our experiments, the ESR technique was adopted to specifically detect leaf ROS and NO in Z. mays L. seedlings.

Leaf ROS and NO variations of Z. mays L. seedlings were studied by a novel method that can simultaneously detect NO and ROS in a biological system by ESR in some modified forms (Cao et al., 2006). A sample of fresh leaf tissue (0.2 g) was ground on ice with 1,200 \(\mu\)L of 0.2 M phosphate buffer solution (pH 7.4) containing 0.32 M sucrose, 10 mM Hepes, 10 mM PBN, 2 mM DETAPAC, 0.5% Tween 80, and 5 mM thiourea. The ground solution was centrifuged at 13,201 \(\times\) g for 20 min, and 460 \(\mu\)L of its supernatant was mixed with 10 \(\mu\)L of 0.5 M Na\(_2\)S\(_2\)O\(_3\), 0.3 M FeSO\(_4\), 0.6 M DETC, and 10 mM L-arginine, respectively. Then the mixture solution was kept at 37 \(^\circ\)C for 2 h and placed on ice. 300 \(\mu\)L ethyl acetate was quickly added to the solution. Next, the solution was kept on a vortex agitator for 15 s. After having been centrifuged at 13,201 g and 4 \(^\circ\)C for 10 min, the upper organic solvent layer of the solution was separated and collected for ESR detection.

All the experiments were conducted at room temperature with a Bruker ER200D-SRC spectrometer. The conditions of the instrument were as follows: X-band, 100 kHz modulation with 1 G (singlet oxygen) or 3.2 G (NO and ROS) amplitude; 20 mW power, 3385 G central magnetic field with 200 G \(1\) G (singlet oxygen) or 400 G (NO and ROS) scan, and 200 s scan time.

Statistical analysis

Data from the independent experiments presented in the figures are in the form of means + standard deviation of three or four replicates. Significance of differences were (at \(P < 0.05\) or \(P < 0.01\)) tested using SAS for Windows V8 (SAS Institute, Cary, NC, USA), and the differences among the treatments were tested by Duncan’s multiple range test, which is a standard routine of SAS.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{Shape of polyphasic fluorescence rise O-J-I-P transients in the treatment with 6-BA under drought stress, plotted on a logarithmic time scale from 50 \(\mu\)s to 1 s. The transients were normalized on \(F_o\). The legend symbols stand for the time points set in the JIP-test for calculating the structural and functional parameters in the five treatments. The signals were as follows: the fluorescence intensity \(F_o\) (the measured \(+\) at 50 \(\mu\)s when all the RCs of the PSII are open, i.e., when the primary acceptor quinone QA is fully oxidized); the fluorescence intensity \(F_t\) (at 300 \(\mu\)s); the fluorescence intensities \(F_1\) (at 2 ms) and \(F_2\) (at 30 ms); the maximal fluorescence intensity, \(F_{\text{m}}\); the fluorescence intensity \(F_0\) (at time denoted as \(F_{\text{eq}}\), when the excitation intensity is high enough to ensure the closure of all the RCs of PSII, i.e., the full reduction of all RCs); the fluorescence induction has a typical sigmoid shape presenting three main phases: O-J-I-P. Phase J-I-P is the part of the transient that runs kinetically in parallel to the reduction of the PQ-pool, so that it is sensitive to the electron transport capacity of PSII RCs that share a PQ-pool; Phase J-I-P is related to PSI activity (Schansker et al., 2005).
\end{figure}

\begin{table}
\centering
\begin{tabular}{|c|c|c|c|c|c|c|}
\hline
Treatment & \(F_{\text{m}}\) & \(F_{\text{pm}}\) & \(F_{\text{Po}}\) & \(F_{\text{Po}/C_0}\) & \(F_{\text{Po}/C_0}\) & \(F_{\text{Po}}\) \\
\hline
Control & 365a & 422a & 624a & 937a & 128a & 1549a \\
D & 376a & 436a & 630a & 883b & 108b & 1326b \\
D+Hb & 372a & 418a & 567b & 756b & 973b & 1219c \\
D+6-BA & 372a & 424a & 619a & 913a & 1272a & 1520a \\
D+BA & 376a & 433a & 625a & 907b & 128a & 1512a \\
\hline
\end{tabular}
\caption{Accepted parameters directly from OJIP fluorescence transients for fluorescence yields at 50 \(\mu\)s, 100 \(\mu\)s, 300 \(\mu\)s, 2 ms, 30 ms and the maximum level.}
\end{table}

The fluorescence signals were as follows: \(F_{\text{pm}}\) (the minimal reliable recorded fluorescence at 50 \(\mu\)s, when all the PSII RCs are open); \(F_{\text{Po}}\) (the measured fluorescence at 100 \(\mu\)s); \(F_{\text{Po}/C_0}\) (the measured fluorescence at the K step [300 \(\mu\)s] of O-J-I-P); \(F_{\text{Po}/C_0}\) (the measured fluorescence at the J step [2 ms] of O-J-I-P); \(F_{\text{Po}}\) (the measured fluorescence at the I step [30 ms] of O-J-I-P); and \(F_{\text{m}}\) (the maximal fluorescence intensity, when all the PSII RCs are closed). Different letters in the same columns showed significant differences at \(P < 0.05\). The 20-day-old plants grew in the PEG solution for 6 d and then were transplanted to the solutions containing 0.5 MPa osmotic solution (D) or plus 0.01 mmol L\(^{-1}\) 6-BA (D+BA), osmotic solution plus 0.01 mmol L\(^{-1}\) 6-BA+5% Hb (D+BA+Hb) or plus 5% Hb (D+Hb). The only controls that were not drought-stressed the same time were then treated with H\(_2\)O.
Results

Effects of CTK on polyphasic fluorescence rise and PI components

A JIP-test was used to determine Chl fluorescence transient. The shape of OJIP transient is sensitive to drought stress (Strasser et al., 2000). In all the treatments, the fast fluorescence OJIP transients of maize were distinct (Fig. 1). The shapes of the OJIP transients (Fig. 1) and fluorescence yield after 300 μs (Table 1) in the drought treatments were different from those in the control. Drought markedly decreased step I (see the $V_i$ in Table 2) and IP-phase (also presented as Sm and N); however, analysis of the other parameters ($V_o$, $V_i$) confirmed dehydration damage to electron donation of PSII. The changes in $V_o$, $V_i$, $V_0$ and $N$ in the treatment with 6-BA under drought stress did not differ from those in the control. However, in the presence of Hb, these parameters also recovered to the level of D treatment. When only Hb was present in the PEG solution, an interesting fluorescence feature was observed; $V_o$, $V_i$, and $V_0$ were slightly reduced, while Sm and N increased above those in D treatment.

PI is one of the Chl fluorescence parameters that provide useful quantitative information about plant status and vitality under drought stress. According to its definition by Strasser et al. (2000; 2004), PI combines three values quantifying the three functional steps of photosynthetic activity by a PSII RC complex, from light energy absorption through excitation energy trapping, and excitation energy conversion to electron transport occurrence in PSII. Effects of CTK on PI and its components were further investigated by JIP testing. PI and its two PI components ($P_{TR}$ and $RC/ABS$) decreased in the treatments with only PEG, or PEG plus Hb. For example, the PI dropped 35% and 36% in treatments D and D+Hb compared with the control, respectively. The decreases in $P_{TR}$ (18% and 29%) in these two treatments contributed mostly to this decreased PI, whereas decreases in RC/ABS contributed 14% and 19%, respectively. However, the PI and its two PI components in the treatments with 6-BA under drought stress recovered to the control values (< 10%, $P > 0.05$). In dark reactions after $Q_A$ drought stress led to a by 9% decrease in $P_{ET}$. When Hb was present in the PEG solution, $P_{ET}$ values increased to the control level. However, $P_{ET}$ increased above the control level by 5% or 6% in the treatments with 6-BA or plus Hb, respectively. Among the PI components, the increase of $P_{ET}$ by CTK contributed most to the high PI (Fig. 2).

Effects of CTK on NO and ROS generations

The principle of the ESR method for simultaneous detection of NO and ROS signals was discussed in a previous study (Cao et al., 2005). The leaf ESR signal increased under drought stress (Fig. 3B), and particularly in the treatment with 6-BA, in which the NO

![Image](318x89 to 329x97)

Table 2

Derived parameters from accepted parameters at OJIP fluorescence transient and used in the analysis of the OJIP fluorescence transient.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$V_k$</th>
<th>$V_i$</th>
<th>$V_0$</th>
<th>Sm</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.219b</td>
<td>0.48b</td>
<td>0.78a</td>
<td>19.68c</td>
<td>35.65c</td>
</tr>
<tr>
<td>D</td>
<td>0.267a</td>
<td>0.53a</td>
<td>0.75b</td>
<td>20.47b</td>
<td>41.01b</td>
</tr>
<tr>
<td>D+Hb</td>
<td>0.235a</td>
<td>0.46ab</td>
<td>0.67b</td>
<td>24.81a</td>
<td>50.71a</td>
</tr>
<tr>
<td>D+BA</td>
<td>0.215b</td>
<td>0.47b</td>
<td>0.78a</td>
<td>17.85c</td>
<td>32.56c</td>
</tr>
<tr>
<td>D+BA+Hb</td>
<td>0.221ab</td>
<td>0.47b</td>
<td>0.79a</td>
<td>20.54b</td>
<td>38.63b</td>
</tr>
</tbody>
</table>

Values in the relative variable fluorescence at step $K$: $V_k=(F_k-F_o)/(F_m-F_o)$; values in the relative variable fluorescence at step $J$: $V_i=(F_i-F_o)/(F_m-F_o)$; $Sm=area/(F_k-F_o)$; total electron carriers per RC of PSII; $N=Sm \times Mo \times (1/V_i)$, number of turnovers of $Q_A$ indicates how many times $Q_A$ is reduced in the time-span from 0 to $T_{max}$. Sm and N reflected changes of PSII acceptor side. Different letters in the same columns showed significant differences at $P < 0.05$. The 20-day-old plants grew in the PEG solution for 6 d and then were transplanted to the solutions containing ~0.5 MPa osmotic solution (D) or plus 0.01 mmol L$^{-1}$ 6-BA (D+BA), osmotic solution plus 0.01 mmol L$^{-1}$ 6-BA+5% Hb (D+BA+Hb) or plus 5% Hb (D+Hb). The only controls that were not drought-stressed all the time were then treated with H$_2$O.

![Fig. 2](400x497)

Fig. 2. Relative differences of PI and PI components of maize suffering drought in the treatment with 6-BA from the control. The 20-day-old plants grew in the PEG solution for 6 d and then were transplanted to solutions containing ~0.5 MPa osmotic solution (D) or plus 0.01 mmol L$^{-1}$ 6-BA (D+BA), osmotic solution plus 0.01 mmol L$^{-1}$ 6-BA+5% Hb (D+BA+Hb) or plus 5% Hb (D+Hb). The control plants were not drought stressed all the time. The values were the relative differences of PI and its three PI components in the treatments from those in the control. And the PI components were the active RC densities on a Chl basis (RC/ABS), the performances due to trapping probability ($P_{TR}$), and the performances due to electron transport probability ($P_{ET}$). The horizontal bars stand for the responses of plants stressed by drought relative to those of the control plants, which were calculated by the formula of the percentages [%]=$(value_{stress}-value_{control}) \times 100/value_{control}$. The alphabetic symbols indicate difference significances between the PI and PI components of plants under drought stress and those of the control plants (ANOVA, $P < 0.05$).
Effects of CTK on NR activity

In plants, the source of NO is unclear, and an NO synthase (NOS)-like enzyme and NR are commonly claimed as the potential sources (Modolo et al., 2005). NO can be synthesized from nitrite via NR. Thus far, nothing has been known about any possible regulation to NR-induced NO production by environmental stress factors, and most importantly, by drought stress. Thus, the NR activity was analyzed to investigate the source of NO production from CTK in this study (Fig. 5A). In the control treatment, NR activity was low. However, drought enhanced nitrate reduction. In particular, the NR activity increased by 144% in the treatment with 6-BA compared to that in the D treatment. In the presence of Hb, 6-BA-induced nitrate reduction was quickly suppressed ($P < 0.05$). When Hb was added to the PEG solution, NR activity decreased compared with that in the D treatment ($P < 0.05$). The NR activity and NO signal were linearly and significantly correlated with $R^2$ at about 0.93 (Fig. 5B). These results show that NR was likely the main source of CTK stimulating NO generation, in accordance with the results of Tun et al. (2001) showing that the NR was activated by CTK, and that NO was produced via NR.

Discussion

CTK reverses drought-induced changes in OJIP fast fluorescence rise

CTK has been shown to be involved in many processes of plant growth and development. Studies have used exogenous 6-BA to test whether CTK is beneficial for photosynthesis, and these studies have revealed that it plays a regulative role in tolerance responses of plant chloroplasts to adverse environments (Chernyad’ev and Monakhova, 2003). We were the first to study the biological roles of CTK in the photosynthetic apparatus in terms of Chl fluorescence transients rise. (The importance of the transients rise for analyzing information regarding the photosynthetic apparatus is described above in the Introduction Section) Subsequently, the NO spectra signal was tested by ESR measurement.

The notion that shape of the OJIP transient is sensitive to drought stress (Strasser et al., 2000) is supported by our observations. The changes in steps K and J showed that the donor side of PSII and oxygen evolving complex (OEC) were destroyed due to drought, and that the electron donation capacity of the PSI donor side decreased as a consequence of increase in closed PSII RCs (Fig. 1, Table 1, and Table 2). However, the acceptor side of PSII did not suffer from damage (see the changes in $V_J$ or $\phi_0$ $V_J$). Step I was positive to drought (Table 1 and Table 2). Further significant increases in Sm and N implied that the heterogeneity of PQ increased electron donation capacity and $Q_A$ reduction of the PSI acceptor side when leaf water loss occurred. CTK played a key role in electron donation in the PSII donor side. However, acting as a signaling molecule, NO was involved in the regulation of electron flux in photosynthetic units via CTK. On the acceptor side of PSII, when CTK hindered the electron transport beyond $Q_A$, NO should also be the causal signal because the phenomenon was nonexistent in the presence of Hb. NO is also itself a reactive nitrogen species (Jasid et al., 2006). Although NO probably acted as a breaker to scavenge deleterious ROS under our conditions, we suggest that the binding sites of NO within the chloroplast were not $Q_A$ and $Q_A$ in the PSI unit, and were probably linked with PQ. Thus, NO did not alleviate ROS-induced damage to the PQ-pool. This is likely the cause of CTK decreasing the activity of the electron transport beyond $Q_A$. However, some of the results on its target sites are controversial (Wodala et al., 2008). Most importantly, the specific binding sites of NO in the PQ-pool deserve further research.

Effects of CTK on PI and its corresponding components

The PSII switches from the process of converting light energy into biochemical energy storage to the energy conversion process...
that transforms absorbed light energy into heat dissipation (Thach et al., 2007). One of parameters used to analyze the response of the plant’s PSII is PI. PI has been created out of three independent expressions: the concentration of RCs per Chl (RC/ABS), an expression related to primary photochemistry ($P_{TR}$), and an expression related to electron transport ($P_{ET}$). This means that PI is sensitive to changes in either antenna properties, trapping efficiency or electron transport beyond QA (Oukarroum et al., 2007).

Fig. 4. Relative intensities of the leaf NO and ROS signals at the 6th hour after the treatment with 6-BA in the presence or absence of the NO scavenger Hb. The mean height of the three peaks in each signal was taken as the relative intensity of the NO and ROS signals. The 20-day-old plants grew in the PEG solution for 6 d and then were transplanted to the solutions containing – 0.5 MPa osmotic solution (D) or plus 5% Hb (D+Hb), osmotic solution plus 0.01 mmol L$^{-1}$ 6-BA (D+BA), osmotic solution plus 0.01 mmol L$^{-1}$ 6-BA and 5% Hb (D+BA+Hb). The control plants were not drought stressed all the time. The values represent the means of four replications and the bars stand for SE. Different letters indicate statistical difference significances among the treatments at $P < 0.05$ by Duncan’s multiple range test. The treatments differed significantly.

Fig. 5. Leaf NR activities of plants at the 6th hour after the treatment with 6-BA under drought stress in the presence or absence of NO scavenger Hb (see Fig. 5A). And the linear correlation between NR activity and NO signal is analyzed $R^2=0.9277$ (see Fig. 5B). The 20-day-old plants grew in the PEG solution for 6 d and then were transplanted to the solutions containing – 0.5 MPa osmotic solution (D) or plus 5% Hb (D+Hb), osmotic solution plus 0.01 mmol L$^{-1}$ 6-BA (D+BA), osmotic solution plus 0.01 mmol L$^{-1}$ 6-BA and 5% Hb (D+BA+Hb). The control plants were not drought stressed all the time. The values represent the means of the three replications and the bars stand for SE. Different letters indicate statistical difference significances among the treatments at $P < 0.05$ by Duncan’s multiple range test. The treatments differed significantly.
the PSII complex merit further molecular research.

Conclusions

The role of CTK in photosynthesis is poorly understood. After examining the effects of CTK on Chl fluorescence transients rise in maize leaves, we conclude that, under our conditions, CTK action was indeed associated with the NO signal. For example, CTK treatment increased NO production, and the Hb-scavenged NO signal was induced by CTK and drought stress. These results justify the conclusion that NO is involved in CTK regulation of photosynthesis adaptability to drought stress. NR activity measurement showed that CTK-triggered NO production was likely associated with an NR-like enzyme. In summary, these findings confirmed our hypothesis that NO is a potential regulator probably involved in CTK action in regulating energy absorption, trapping, and conversion. However, the cellular targets of NO in the PSII complex merit further molecular research.

Acknowledgements

This work was supported by the Important Direction Project of Innovation of CAS (KSCX2-YW-N-003), the West-action Program of CAS (KZCX2-XB2-05), and the Program for Changjiang Scholars and Innovative Research Team in University (IRT0745).

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