Int. J. Environ. Sci. Technol. (2013) 10:125–132
DOI 10.1007/s13762-012-0072-4

ORIGINAL PAPER

Effects of inhibitors on ferricyanide uptake and assimilation by plants

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Received: 22 August 2011 / Revised: 25 November 2011 / Accepted: 31 January 2012 / Published online: 12 April 2012

Abstract Effects of inhibitors on uptake and assimilation of ferricyanide by different plants were investigated. Detached roots of plants were kept in a closed-dark hydroponic system with ferricyanide solution amended with various inhibitors. Dissociation of ferricyanide to free cyanide and iron in solution was negligible. The application of inhibitors affected both botanical assimilation and uptake of ferricyanide. Of the inhibitors tested, silver nitrate showed a significantly inhibitory effect on ferricyanide uptake by rice, soybean and maize ($P < 0.01$), while a negligible effect was found in willows spiked with the same inhibitor ($P > 0.05$). However, lanthanum chloride showed the most severe effect on botanical assimilation of ferricyanide by maize and rice ($P < 0.01$), whereas silver nitrate and tetraethylammonium chloride were the most sensitive inhibitors to soybean and willows, respectively ($P < 0.01$). Botanical assimilation of ferricyanide was observed positively in responses to temperatures, in which maize was more susceptible than other selected plants. In conclusion, application of inhibitors has a substantial influence on the uptake and assimilation of ferricyanide by plants, and the inhibitory efficiency is highly dependent on the species of inhibitors applied.

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Keywords Ferricyanide · Inhibitor · Metabolism · Removal · Root · Temperature

Introduction

Several cyanide-containing compounds have been detected in the environment, namely free cyanide, weak-acid dissociable cyanide, iron cyanides, and thiocyanate (Ebbs et al. 2008). Of the iron cyanides detected, ferricyanide $[Fe^{III}(CN)_6]^{-3}$ is one of the most commonly found species (Mansfeldt et al. 2004), which has a closer link to the presence of free cyanide (CN^- and HCN) derived from both anthropogenic and natural sources (Ebbs et al. 2010). There is abundant literature showing that a number of biotic and abiotic factors, including pH, redox potential, the availability and quality of solar radiation, biological activity and community, and the presence of organic are activated in the fate and speciation of ferricyanide in the environment (Ghosh et al. 2004; Rennert and Mansfeldt 2002; Yu et al. 2011a, b). Indeed, dissociation of ferricyanide due to the changes in the physicochemical conditions shows significant concerns, although it is less toxic than free cyanide (Ebbs et al. 2008; Zagury et al. 2004).

It has been proposed that there are two possible pathways involved in the botanical uptake of ferricyanide (Larsen and Trapp 2006; Yu and Gu 2010): (1) ferricyanide dissociates into iron (Fe^{3+}) and free cyanide (CN^-) first, and then both species are able to penetrate the root membrane through the defined transport pathways; (2) plants take Fe^{3+} and free cyanide (CN^-) as a complex without liberation. It is known that iron (Fe) is an essential element for plants because it plays critical role in biochemical process. However, phytoavailability of Fe often limits plant growth (Eide 1997; Harada et al. 2007). Plants have evolved different

mechanisms to acquire Fe from soils (Kim and Guerinot 2007). Grasses use the chelation-based strategy to acquire Fe in the presence of Fe-chelating substances phytosiderophores excreted by roots (Harada et al. 2007). However, a Fe-acquisition mechanism commonly found in higher plants is a reduction-based pathway, in which Fe becomes more phytoavailable by reducing Fe^{3+} to the more soluble Fe^{2+} in the presence of Fe^{3+} reductase at the root cell's plasma membrane (Kim and Guerinot 2007).

Although direct evidence of the plant-mediated uptake and assimilation of ferricyanide is still under investigation, several studies have been conducted to clarify the possible biological transport and fate involved (Ebbs et al. 2003, 2008; Larsen and Trapp 2006; Samiotakis and Ebbs 2004; Yu and Gu 2010). Unlike botanical uptake of small neutral chemical species, ferricyanide is unable to move through biological membranes by simple diffusion due to its physical–chemical properties (Ebbs et al. 2003; Federico and Giartosio 1983). It is known that diffusive and advective mass transfer are the driving mechanisms in the transport of free CN into root cells from the solution (Dzombak et al. 2005) and the uptake velocity is independent upon the presence of inhibitors (Yu et al. 2011a, b). If the dissociation of ferricyanide into free CN (CN^-) is negligible in the solution before uptake by roots, any effect of the inhibitors applied on the removal could be interpreted as an effect on ferricyanide transport. In this study, the uptake and assimilation of ferricyanide by two dicots (soybean and willow) and two monocots (maize and rice) were compared in the presence or absence of inhibitors, using a closed-dark hydroponic system that preserved cyanide speciation. A special study was also placed on estimation of the temperature coefficient (Q_{10}) to determine the sensitivity of different species of plants to changes in temperatures during the ferricyanide assimilation. This work was conducted at the Department of Environmental Sciences and Engineering, Hunan Agricultural University, P. R. China from March 2010 to May 2010.

Materials and methods

Plant materials

Seeds of rice (*Oryza sativa* L. cv. JY 98), soybean (*Glycine max* L. cv. WH), and maize (*Zea mays* L. var. HK) after cleaning were planted under laboratory condition at 25 °C until shoots appeared. Hoagland's nutrient solution at 25 % strength was used to support plant growth. Seedlings were harvested after 12–15 days of growth. Cuttings of weeping willows (*Salix babylonica* L.) were removed from a single mature tree and placed in buckets of tap water at 25 °C under until new roots appeared.

Plants with new shoots and roots were transferred to a pretreatment solution containing 1 mM $CaCl_2$ + 2 mM MES–TRIS buffer (pH 6.0) for 24 h to clear the cell-wall space of ions (Ebbs et al. 2008). Plant roots approximately 8–10 cm in length were excised from the root tip, and cut into small pieces to use in the subsequent experiments.

Potassium ferricyanide [$K_3Fe(CN)_6$] (Sinopharm Chemical Reagent Co. Ltd., Shanghai, P. R. China) of analytical grade with ≥95% purity was used. It should be noted that 1 mg $K_3Fe(CN)_6$ is equivalent to 0.474 mg CN.

Effect of inhibitors on ferricyanide uptake by detached roots

Excised roots were precisely weighted (1.0 g fresh weight) and placed in 50 mL flasks. Then 50 mL spiked aqueous solution (deionized oxygen-saturated water) containing potassium ferricyanide and different inhibitors (20 μM mercuric chloride ($HgCl_2$), 50 μM silver nitrate ($AgNO_3$), 200 μM lanthanum chloride ($LaCl_3$), 5.0 mM tetraethylammonium chloride (TEACl), or 20 μM sodium vanadate (Na_3VO_4) Sigma-Aldrich Inc., St. Louis, MO) were added. $LaCl_3$ is a Ca^{2+} channel blocker (Ebbs et al. 2008). Na_3VO_4, $HgCl_2$, and $AgNO_3$ are inhibitors of the ion transporting (Ebbs et al. 2008; Zhang et al. 2010). TEACl is an anion channel inhibitor (Zhang et al. 2010). The initial concentration of spiked solution was 10.50 (±0.225) mg CN/L. The initial pH of spiked solution was adjusted to 7.5 with 0.1 % NaOH. The flasks were closed with glass stopper. All flasks wrapped with aluminum foil were housed at an incubator with a constant temperature 25 ± 0.5 °C for 24 h.

Three replicates were prepared for each treatment. One set of control in three replicates was with plant materials and ferricyanide, but without addition of any inhibitor. A second control in three replicates (the flasks were also wrapped with aluminum foils) was only with the testing chemical to quantify the loss and dissociation of ferricyanide within the testing system during the exposure period.

Effect of temperature on ferricyanide uptake by detached roots

Precise 1.0 g plant roots (FW) were placed in 50 mL vessels, and then 50 mL spiked aqueous solution (deionized oxygen-saturated water) containing potassium ferricyanide was added. The remaining procedures were identical to those described above. The initial concentration of spiked solution was 10.51 (±0.421) mg CN/L. The flasks wrapped with aluminum foil were put in a chamber with constant temperatures of 15, 20, 25, and 30 ± 0.5°C for 24 h. Three replicates were prepared for each treatment temperature.

Chemical analysis

Analysis of free CN and total CN

The presence of total CN and free CN in solution was all analyzed just prior application and at the termination of exposure. Total CN in excised roots was also analyzed after 24 h of exposure.

The concentrations of free CN and total cyanide in the aqueous solution were determined spectrophotometrically by a standard method (State Environmental Protection Administration of China 1989, method number GB 7487-87) as described previously (Yu and Gu 2010).

The analysis of total CN in plant tissues was also analyzed by a standard distillation method (State Environmental Protection Administration of China 1989, method number GB 7486-87). After exposure of 24 h, the excised roots were collected and rinsed with water. The remaining procedures were identical to those described previously (Yu and Gu 2010).

Analysis of Fe^{3+}/Fe^{2+} in solution: Dissolved Fe^{2+} concentration was determined by the ferrozine method as described previously (Gibbs 1979). The total dissolved Fe including dissolved Fe^{2+} and Fe^{3+} was measured by reducing total dissolved Fe into Fe^{2+}, and then Fe^{2+} was determined by the ferrozine method without addition of sodium fluoride (NaF) solution (Greenberg et al. 1992). The content of dissolved Fe^{3+} in solution was obtained by computing the difference between total dissolved Fe and dissolved Fe^{2+}.

Determination of the assimilation rate

The assimilation rate of ferricyanide v (μg CN/g FW h) was calculated from

$$v = \frac{m_{(\mathrm{I})} - m_{(\mathrm{F})} - m_{(\mathrm{R})}}{\Delta t \times M}$$

where $m_{(\mathrm{I})}$ and $m_{(\mathrm{F})}$ are the initial and final total CN (μg) in hydroponic solutions, respectively. $m_{(\mathrm{R})}$ refers to the total CN (μg) in roots. M is the biomass of the roots (g), and Δt is the time period (h).

Determination of the temperature coefficient Q_{10}

The influence of temperature on the assimilation rate of ferricyanide was quantified by calculating the temperature coefficient Q_{10}, which is defined as the increase of the metabolic activity over a 10 °C increase in temperature. The temperature coefficient was derived using the equation of Atkin et al. (2002)

$$Q_{10} = 10^{\Delta T \times slope}$$

where ΔT is 10°C, and slope is the slope of the linear fit curve of log v_{P} versus temperature T (°C).

Statistical methods

Analysis of variance (ANOVA) and Tukey's multiple range test was used to determine the statistical significance at 0.01 or 0.05 between the treatments (Sachs 1992).

Results and discussions

Speciation of ferricyanide in hydroponic solution

In the control amended with ferricyanide without plant materials, change of total CN in the hydroponic solution was negligible after a 24-h period of incubation in the absence of light, while trace amounts of free CN were tracked in the solution (data not shown), probably due to dissociation during handling (Meeussen et al. 1992). In addition, the content of dissolved Fe^{3+} in the control spiked with ferricyanide was negligible over the 24-h period of incubation after analysis of the total dissolved Fe and Fe^{2+} in the hydroponic solution (data not shown). These results indicated that the dissociation of ferricyanide into free CN in the hydroponic solution in the absence of light is likely to occur, but the velocity is extremely slow, which is similar to previous studies (Ebbs et al. 2008; Larsen and Trapp 2006; Samiotakis and Ebbs 2004; Yu and Gu 2010; Yu et al. 2012). Since the majority of the solution CN remained as ferricyanide, the disappearance of the applied ferricyanide in hydroponic solution could then be attributed to the uptake of plant materials.

Effect of inhibitors on ferricyanide uptake by detached roots

The effects of TEACl, Na_3VO_4, $HgCl_2$, $AgNO_3$, and $LaCl_3$ on the uptake and assimilation of ferricyanide by detached roots were investigated. For the treatment with willows (Table 1), less amounts of the applied ferricyanide were removed from the hydroponic solution in the presence of different inhibitors ($P > 0.05$) compared to the control, judged by the total CN analyzed. However, significant difference in total CN detected in plant materials exposed to inhibitors was observed ($P < 0.01$) compared to the control, except the treatments amended with Na_3VO_4 and $HgCl_2$. Indeed, 33.2, 44, and 66.2 % of the ferricyanide lost from the aqueous solution were recovered in plant tissues in the presence of $LaCl_3$, $AgNO_3$, and TEACl, respectively, while approximately 12–19 % was detected in other treatments. Loss of the applied ferricyanide from control

Table 1 Measured total CN concentrations in hydroponic solution (mg CN/L) and in roots of weeping willows (μg CN/g FW) exposed to ferricyanide in the presence of different inhibitors

Treatment	Initial conc. (mg CN/L)	Final conc. (mg CN/L)	Mass reduction (%)	Accumulation by roots (μg CN/g FW)	Mass recovery by roots (%)	Loss rate (μg CN/g. h)
Control	10.93 (0.45)	6.18 (0.11)	43.5 (0.96)	28.78 (1.99)	12.1 (1.11)	8.70 (0.30)
T-TEACl	11.09 (0.45)	7.23 (0.50)	34.8 (4.54)	126.7* (6.48)	66.2* (5.17)	2.75* (0.80)
T-Na_3VO_4	9.74 (0.11)	6.63 (0.05)	32.0 (0.47)	29.57 (2.78)	19.0 (1.53)	5.25* (0.05)
T-$HgCl_2$	9.90 (0.11)	5.73 (0.05)	42.1 (0.46)	35.38 (1.99)	17.0 (0.79)	7.21 (0.04)
T-$AgNO_3$	10.46 (0.23)	7.02 (0.30)	32.9 (2.87)	75.51* (5.94)	44.0* (1.58)	4.01* (0.41)
T-$LaCl_3$	10.06 (0.11)	6.49 (0.21)	35.4 (2.08)	59.14* (5.09)	33.2* (1.21)	4.96* (0.25)

Exposure period 24 h, the values are the mean of three replicates; in brackets standard deviation

FW fresh weight

* Significantly different to the control at 95 % significance level

without plant materials was negligible. As a result, loss can be attributed botanical assimilation. The calculated loss rates are shown in Table 1. The rates significantly inhibited due to the application of TEACl, Na_3VO_4, $AgNO_3$, and $LaCl_3$ ($P < 0.01$). The loss rate of ferricyanide was 8.70 μg CN/g FW. h at the control, which was more than three-fold higher than that the treatment amended with TEACl.

For the treatments with the roots of maize (Table 2), all applied chemicals caused remarkable inhibition on ferricyanide uptake compared to the control without any inhibitor ($P < 0.01$). Only between 12.9 and 23.7 % of the applied ferricyanide were removed by plant materials from the hydroponic solution in the presence of different inhibitors, while 48.8 % of the ferricyanide was taken up by the roots of maize without addition of chemical inhibitors. Substantial difference in the contents of ferricyanide detected as total CN in plant materials between the treatments and the control was observed ($P < 0.01$). The concentrations of CN in roots detected as total CN were found in the range of 38.55–75.51 μg CN/g FW in the treatments, while a significantly higher concentration of 157.1 μg CN/g FW was observed in the roots from the control. The assimilation rates were also significantly inhibited by TEACl, Na_3VO_4, $AgNO_3$, and $LaCl_3$ ($P < 0.01$), except $HgCl_2$ ($P > 0.05$), in which inhibition of ferricyanide assimilation by TEACl, Na_3VO_4, $AgNO_3$, $LaCl_3$, and $HgCl_2$ was 82, 88, 91, 92, and 20 % in the treatment with maize roots, respectively.

For the control with rice roots exposed to ferricyanide solution without addition of any chemical inhibitor, more than 65 % of the applied CN was removed from the hydroponic solution (Table 3). The uptake rates of ferricyanide by plant materials varied with the applied chemical inhibitors, in which $HgCl_2$, $AgNO_3$, and $LaCl_3$ showed a significantly negative impact on the uptake of ferricyanide ($P < 0.01$), and TEACl and Na_3VO_4 had a measurable influence ($P > 0.05$) compared to the control. Remarkable

Table 2 Measured total CN concentrations in hydroponic solution (mg CN/L) and in roots of maize (μg CN/g FW) exposed to ferricyanide in the presence of different inhibitors

Treatment	Initial conc. (mg CN/L)	Final conc. (mg CN/L)	Mass reduction (%)	Accumulation by roots (μg CN/g FW)	Mass recovery by roots (%)	Loss rate (μg CN/g. h)
Control	10.93 (0.22)	5.60 (0.71)	48.8 (6.50)	157.1 (9.04)	58.7 (5.54)	4.56 (0.69)
T-TEACl	10.22 (0.34)	8.53 (0.12)	16.6* (1.18)	65.21* (4.06)	77.1 (0.73)	0.81* (0.09)
T-Na_3VO_4	10.46 (0.22)	9.11 (0.16)	12.9* (1.51)	54.65* (4.75)	81.1 (2.49)	0.54* (0.13)
T-$HgCl_2$	10.69 (0.11)	8.16 (0.29)	23.7* (2.67)	38.55* (3.30)	30.7 (4.61)	3.67 (0.61)
T-$AgNO_3$	10.14 (0.22)	8.79 (0.16)	13.3* (1.56)	58.08* (6.35)	86.2 (0.93)	0.39* (0.07)
T-$LaCl_3$	10.06 (0.34)	8.37 (0.05)	16.8* (0.45)	75.51* (4.06)	89.3 (2.58)	0.38* (0.08)

Exposure period 24 h, the values are the mean of three replicates; in brackets standard deviation

FW fresh weight

* Significantly different to the control at 95 % significance level

Table 3 Measured total CN concentrations in hydroponic solution (mg CN/L) and in roots of rice (μg CN/g FW) exposed to ferricyanide in the presence of different inhibitors

Treatment	Initial conc. (mg CN/L)	Final conc. (mg CN/L)	Mass reduction (%)	Accumulation by roots (μg CN/g FW)	Mass recovery by roots (%)	Loss rate (μg CN/g. h)
Control	11.09 (0.45)	3.79 (0.19)	65.8 (1.69)	146.5 (7.04)	40.1 (1.64)	9.11 (0.35)
T-TEACl	11.01 (0.34)	4.15 (0.20)	62.4 (1.81)	258.7* (3.91)	75.4 (1.15)	3.52* (0.26)
T-Na_3VO_4	10.06 (0.34)	4.30 (0.41)	57.2 (4.04)	104.3 (6.45)	36.3 (3.14)	7.65 (0.86)
T-$HgCl_2$	11.56 (0.22)	9.27 (0.21)	19.8* (1.81)	41.45* (3.32)	36.5 (5.85)	3.05* (0.54)
T-$AgNO_3$	11.33 (0.11)	9.74 (0.21)	14.0* (1.85)	39.99* (1.19)	50.9 (5.98)	1.64* (0.40)
T-$LaCl_3$	9.98 (0.22)	8.34 (0.28)	16.4* (2.79)	45.02* (5.01)	55.6 (6.27)	1.53* (0.46)

Exposure period 24 h, the values are the mean of three replicates; in brackets: standard deviation

FW fresh weight

* Significantly different to the control at 95 % significance level

difference in the concentration of ferricyanide recovered in the plant materials between the treatments was found, judged by the total CN analyzed ($P < 0.01$). Consequently, the loss rates of ferricyanide significantly inhibited by TEACl, $HgCl_2$, $AgNO_3$, and $LaCl_3$ ($P < 0.01$), except Na_3VO_4 ($P > 0.05$). The loss rate of ferricyanide was 9.11 μg CN/g FW. h at the control, which was almost six-fold higher than the treatment amended with $LaCl_3$.

The removal rates of ferricyanide by soybean roots also varied with the application of chemical inhibitors (Table 4). Between 7.2 and 24.3 % of the applied ferricyanide was removed from the hydroponic solution due to the application of different inhibitors. Na_3VO_4 and $AgNO_3$ showed significantly inhibitory effect on ferricyanide uptake ($P < 0.01$) compared to the control. The concentrations of ferricyanide detected as total CN in plant materials were also variable between the treatments. The rates were significantly inhibited by TEACl, Na_3VO_4, and $AgNO_3$ ($P < 0.01$), except $HgCl_2$ and $LaCl_3$ ($P > 0.05$).

Effect of temperature on ferricyanide uptake by detached roots

Table 5 gives the changes of concentrations of ferricyanide detected as total CN in the hydroponic solutions with roots at different treatment temperatures. It is obvious that uptake of ferricyanide by plant roots varied with treatment temperatures and plant species. Within the temperature range of 15–30 °C, between 31.4 and 84.2 % of the applied ferricyanide, judged by the total CN analyzed, was removed from the hydroponic solution by rice roots over a 24-h period of exposure, while only 9.6–28.9 % of the ferricyanide was taken up by soybean roots from the solution. The highest capacity to remove ferricyanide was by rice roots at all treatment temperatures, followed weeping willows. The lowest removal capacity had the roots of soybean.

Analysis of the total CN in roots at the termination of exposure showed that significant difference was observed

Table 4 Measured total CN concentrations in hydroponic solution (mg CN/L) and in roots of soybean (μg CN/g FW) exposed to ferricyanide in the presence of different inhibitors

Treatment	Initial conc. (mg CN/L)	Final conc. (mg CN/L)	Mass reduction (%)	Accumulation by roots (μg CN/g FW)	Mass recovery by roots (%)	Loss rate (μg CN/g. h)
Control	10.85 (0.11)	8.66 (0.20)	20.2 (1.84)	42.51 (3.20)	39.2 (6.29)	2.79 (0.50)
T-TEACl	10.22 (0.11)	7.74 (0.24)	24.3 (2.37)	87.92* (11.01)	70.7 (3.76)	1.51* (0.20)
T-Na_3VO_4	10.22 (0.11)	8.82 (0.20)	13.7* (1.95)	45.94 (3.63)	66.1 (6.26)	1.01* (0.32)
T-$HgCl_2$	10.30 (0.22)	8.67 (0.16)	15.6 (1.60)	31.15 (0.46)	38.9 (3.80)	2.06 (0.33)
T-$AgNO_3$	10.53 (0.34)	9.77 (0.05)	7.2* (0.43)	23.23* (1.21)	61.2 (4.39)	0.62* (0.09)
T-$LaCl_3$	10.22 (0.11)	8.03 (0.23)	21.5 (2.24)	58.61 (5.54)	53.5 (0.94)	2.13 (0.25)

Exposure period 24 h, the values are the mean of three replicates; in brackets standard deviation

FW fresh weight

* Significantly different to the control at 95 % significance level

Table 5 Measured total CN concentrations in hydroponic solution (mg CN/L) and in plant materials (μg CN/g FW) exposed to ferricyanide at different treatment temperatures

Plant species	Treatment temperature (°C)	Conc. (I) (mg CN/L)	Conc. (F) (mg CN/L)	Mass reduction (%)	Conc. (root) (μg CN/g FW)	Mass recovery by roots (%)
Weeping willows	15	10.46 (0.22)	7.79 (0.25)	25.5 (2.43)	17.43 (0.79)	13.1 (0.77)
	20	10.30 (0.22)	7.08 (0.16)	31.3 (1.60)	17.95 (1.21)	11.2 (0.99)
	25	10.93 (0.22)	6.39 (0.12)	41.5 (1.11)	21.91 (2.99)	9.6 (1.06)
	30	10.53 (0.11)	4.33 (0.25)	58.9 (2.42)	22.18 (2.86)	7.1 (0.66)
Maize	15	9.98 (0.45)	7.82 (0.33)	20.3 (1.59)	48.84 (5.39)	47.6 (5.90)
	20	9.82 (0.67)	7.15 (0.32)	27.1 (3.26)	56.50 (3.29)	42.7 (4.03)
	25	10.38 (0.11)	5.49 (0.18)	47.1 (1.76)	122.51 (8.01)	50.1 (1.44)
	30	9.43 (0.34)	2.85 (0.40)	69.7 (4.20)	184.55 (11.2)	56.2 (2.94)
Rice	15	11.01 (0.11)	7.55 (0.12)	31.4 (1.09)	54.92 (1.21)	31.8 (0.96)
	20	10.77 (0.22)	5.89 (0.24)	45.3 (2.24)	109.04 (7.14)	44.7 (0.79)
	25	10.69 (0.11)	3.85 (0.12)	63.9 (1.13)	138.61 (2.38)	40.6 (0.14)
	30	10.85 (0.11)	1.72 (0.20)	84.2 (1.84)	213.07 (12.2)	46.7 (3.18)
Soybean	15	10.69 (0.11)	9.66 (0.08)	9.6 (0.74)	15.31 (0.46)	29.9 (1.64)
	20	10.61 (0.22)	9.00 (0.11)	15.1 (1.14)	26.93 (2.38)	33.6 (2.41)
	25	11.01 (0.11)	8.74 (0.16)	21.1 (1.65)	40.12 (1.21)	38.1 (1.94)
	30	10.69 (0.34)	7.60 (0.32)	28.9 (2.96)	65.21 (2.99)	42.4 (2.59)

Exposure period 24 h, the values are the mean of three replicates; in brackets standard deviation

FW fresh weight

between the four species of plants at all treatment temperatures ($P < 0.01$). In the treatment with rice, total CN in roots increased sharply from 54.92 (±1.21) to 213.07 (±12.2) μg CN/g (FW), with a change in temperature from 15 to 30 °C, in which the CN detected from the plant materials accounted for 31.8–46.7 % of the ferricyanide lost from the hydroponic solution. However, total CN in roots of willows increased slightly from 17.43 (±0.79) to 22.18 (±2.86) μg CN/g (FW), while less 14 % of the ferricyanide lost from the solution was recovered from plant materials after a 24-h period of exposure at all treatment temperatures.

The total CN analysis performed on the hydroponic solution and plant roots allowed for a closure of the mass balance (Table 5). Loss from controls without plant materials was negligible; as a result, all loss from the closed testing system was likely to be botanical assimilation. This is comparable to other findings (Larsen and Trapp 2006; Yu and Gu 2010). The calculated botanical assimilated rates of ferricyanide are shown in Fig. 1. The rates significantly increased with temperatures at all treatments. From temperature 15 to 30 °C, ferricyanide assimilated rate increased from 4.84 (±0.50) to 11.99 (±0.43) μg CN/g FW. h at the treatment with willow roots and the linear trend was significant at $a = 0.01$ ($R^2 = 0.96$). The loss rates of ferricyanide were 1.50 (±0.15) and 3.71 (±0.55) μg CN/g FW. h at the treatment with soybean roots at 15 and 30 °C, respectively, in which the linear trend was also significant at $a = 0.01 (R^2 = 0.98)$.

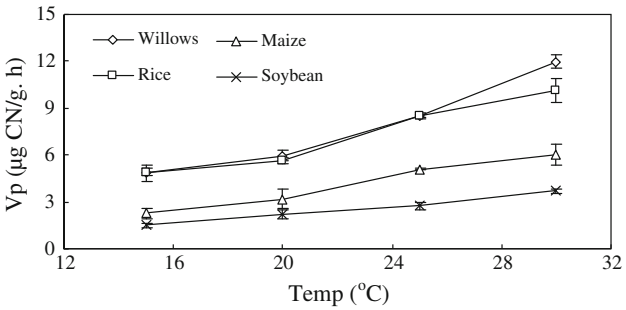

Fig. 1 Loss rates of ferricyanide by the roots of four plant species as affected by temperature (*vertical lines* represent standard deviation)

Calculation of the temperature coefficient Q_{10}

The response of enzymatic reactions to a temperature increase can be summarized by two functions, an increase of the forward reaction, and a decay due to enzyme denaturation as the temperature rises (Raison 1980). The trends from the treatments were all significant ($R^2 > 0.96$, figures not shown) at $a = 0.01$. The slope for the treatment with maize is 0.0295, and $Q_{10} = 1.97$ yields. With a slope of 0.0268 for willows, $Q_{10} = 1.85$. The lowest Q_{10} value of 1.67 was obtained for the treatment with rice. This suggested that the botanical assimilation of ferricyanide by

maize is more susceptible to the change of temperature than that by other species of plants.

It has been found that the speciation of ferricyanide in solution affects and/or alters the uptake mechanisms (Larsen and Trapp 2006). The presence of high Fe-affinity chelators and the change of solution pH probably cause the dissociation of iron CNs (Meeussen et al. 1992; Römheld and Marschner 1986). Indeed, the decomposition of iron CNs in soils in the absence of light was detected, but the rates appeared to be inversely related to the pH (Meeussen et al. 1992). In this study, the solution pH was quite stable during the entire period of incubation, with a range of 7.2–7.5. In addition, phytosiderophores excreted by the roots of graminaceous plants are metal chelators for Fe with high affinity (Römheld and Marschner 1986). It is known that the equilibrium constant (logK) is 52.63 for ferricyanide (Meeussen et al. 1992), while the log K value of Fe-phytosiderophores is only 18.1 (Mino et al. 1983). Therefore, the liberation of ferricyanide is highly unlikely to occur in the solution, due to the presence of phytosiderophores excreted. Indeed, only trace amounts of free CN and dissolved Fe^{3+} were detected in the solution without plant materials in this study, suggesting that the dissociation of ferricyanide in the solution is negligible. Hence, cyanide in the solution remained principally in the form of ferricyanide before uptake by plant roots.

After the enter of the complex into plant materials, the biological fate of ferricyanide is also highly dependent on the chemical species of cyanide present. There are two possible degradation pathways for phyto-assimilating ferricyanide by plants: (1) plants assimilate ferricyanide directly as a substrate through an undefined degradation pathway; (2) ferricyanide dissociates into Fe^{3+} and free CN (CN^-) first, and then free CN (CN^-) is metabolized by plants through the beta-cyanoalanine pathways (Larsen and Trapp 2006; Miller and Conn 1980). It has been observed that free CN did not accumulate in healthy plants (Larsen et al. 2005). In this study, significant amounts of CNs detected as total CN was detected in plant roots at the termination of exposure, implying that the dissociation of ferricyanide into free CN was unlikely to occur in plant materials, which was similar to previous work (Larsen and Trapp 2006). Therefore, there is a good reason to assume that ferricyanide was largely assimilated by plant materials directly without any in vivo phyto-decomposition.

In this study, the disappearance rates of ferricyanide from the aqueous solution varied with plant species. Rice showed the fastest removal potential for ferricyanide and the lowest capacity had soybean. From the inhibitors tested, $AgNO_3$ showed the significantly inhibitory effect on ferricyanide uptake by rice, soybean and maize ($P < 0.01$), while negligible effect of $AgNO_3$ was found in the treatment with willows ($P > 0.05$). Since trace amounts of free CN was detected in the solution, which is mainly in the form of HCN due to the neutral solution pH (Meeussen et al. 1992), the majority of cyanide in solution was ferricyanide. It is known that small neutral chemical species of HCN move through biological membranes principally by simple diffusion (Dzombak et al. 2005). Indeed, the membrane inhibitors are unable to influence the uptake of free CN (Yu et al. 2011a, b). Therefore, any effect of the inhibitors on the disappearance of ferricyanide in solution could be due to its effects on transport into plant materials from the water phase. In this study, the calculated botanical assimilation rates of ferricyanide were variable with species of inhibitors applied. Indeed, plants showed different responses to the application of inhibitors. $LaCl_3$ displayed the most severe effect on the loss rate of ferricyanide by maize and rice, while $AgNO_3$ and TEACl were the most sensitive inhibitors to soybean and willows, respectively.

Although Q_{10} values varied with species of plants (Fitter et al. 1998), Q_{10} values are frequently in the range of 1.1–2.9 (Azcón-Bieto 1992; Atkin et al. 2000). In this study, the Q_{10} values were determined to be 1.67-1.97, which are similar to the results obtained earlier. The Q_{10} values for Chinese elder (*Sambucus chinensis* L.) and weeping willows (*S. babylonica* L.) exposed to free CN were 1.84 and 2.09, respectively (Yu et al. 2005), while a relative lower Q_{10} value of 1.46 was found in the test with intact weeping willows during the removal of free CN (Yu et al. 2007). Compared to the Q_{10} value obtained in this study, a higher Q_{10} value of 2.75 was found in the treatment with maize seedlings (*Z. mays* L. var. ZN 304) exposed to ferricyanide, most likely due to different plant materials used. Even though the category of chemicals is very different, the Q_{10} values still fall into the range reported previously. Indeed, Q_{10} values of 2.41 and 1.42 were obtained for intact hybrid willows (*Salix matsudana* Koidz × alba L.) exposed to Cr(VI) and Cr(III), respectively (Yu et al. 2010).

Conclusion

The results presented here indicated that in vivo dissociation of ferricyanide to free cyanide was negligible during the phyto-removal of ferricyanide. A significant inhibitory effect on the removal rate were observed with rice, soybean, and maize in the presence of $AgNO_3$, while the assimilation rate of ferricyanide was variable to the application of inhibitors selected. Although variable with species of plants, the phyto-assimilation rate of ferricyanide was highly dependent on temperatures. According to the temperature coefficient Q_{10}, maize was more susceptible to the changes in temperature than other species of plants during the phyto-assimilation of ferricyanide.

Acknowledgments This work was financially supported by The National Nature Science Foundation of China (NSFC: 40971256).

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