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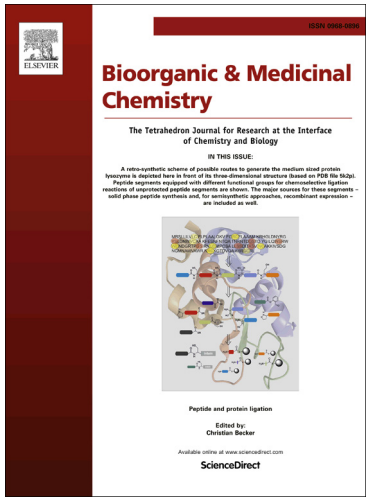

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A Rhodamine B-based fluorescent probe for imaging Cu^{2+} in maize roots

Ting Lv[a], Yongqian Xu[a], Hongjuan Li[a] and Fengyu Liu[b, *], Shiguo Sun[a, *]

[a] *Shaanxi Key Laboratory of Natural Products & Chemical Biology, College of Chemistry & Pharmacy, Northwest A&F University, Xinong Road 22, Yangling, Shaanxi 712100, P.R. China.*
[b] *State Key Laboratory of Fine Chemicals, School of Chemistry, Dalian University of Technology, No. 2 linggong Road, Ganjingzi District, Dalian 116023, China.*

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ABSTRACT

A new Rhodamine B-based fluorescent probe (RBO) is successfully designed and synthesized, which is a higher selective and sensitive chemosensor for Cu^{2+} than other ions. Under physiological conditions (pH = 7.0), the non emission RBO displays a rapid fluorescence increase together with a color change after addition of Cu^{2+} and the detection limit is down to 28 nM, which can clearly illustrate the distribution of Cu^{2+} with the help of laser scanning confocal microscope in plant tissues. Eventually, it confirmed that the Cu^{2+} accumulates mostly in the vascular cylinder and very less in the epidermal cells of maize roots, which is important to understand how the plants take up, transport and store in the Cu^{2+}.

1. Introduction

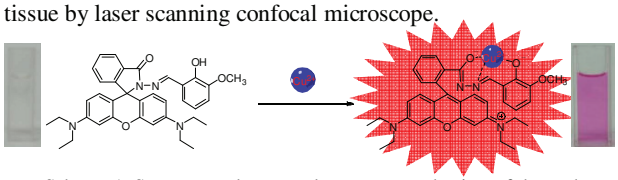
As an essential micronutrient, Cu^{2+} plays a pivotal role in all plants normal growth and development[1-2]. Because of its redox properties on physiological conditions, the Cu^{2+} is regarded as a catalytic cofactor for many enzymes such as superoxide dismutase in the cytosol and chloroplasts, and cytochrome oxidase within the mitochondria etc[3-4]. These enzymes are very important in plant multiple metabolic pathways. When excessive level of Cu^{2+} fills with the substrate where plants grow, it can accumulate in organs or any other tissue via the absorption[5-6]. Therefore, the uncontrolled high level of Cu^{2+} triggers the formation of reactive oxygen radicals that make ion leakage of cell membrane, destroy of pigment even further reduce the protective enzyme activity, photosynthesis, growth inhibition and the death of plant[7-13]. Based on these, it is significant to develop analytical methods for detecting and monitoring Cu^{2+} in plants.

Up to now, there are lots of methods to detect metal ions in tradition, such as atomic absorption spectrometry[14-16], electrochemical analysis[17], laser ablation inductively coupled plasma mass spectrometry[18-21], voltammetry[22] and atomic emission spectrometry[21] etc. Compared with these detecting technologies, fluorescent probes possess excellent properties like low cost, non-destructive, and easy to handle optical bio-imaging etc, which has attracted great attention on many kinds of metal ions monitored in plants. For example, Prof. Ishijima[23] utilized a commercial probe to detect Mg^{2+} in spinach chloroplasts. Prof. Bag[24] firstly developed a rhodamine-based probes for the selective detection of Pb^{2+} in A. lanata root in 2013. After three years, this professor[25] utilized a pyridine and pyrrole coupled rhodamine derivative for Co^{2+} detection in root and shoot tissues of Hybanthus enneaspermus plant species, again. Prof. Jung[26] utilized a naphthalene-based fluorescent probe to detect Zn^{2+} in Arabidopsis in 2015. Next year, Prof. Zhou[27] developed a highly selective probe for rapidly detecting Zn^{2+} in Arabidopsis and Prof. Bai[28] and coworkers developed a sensitivity small probe to detect Zn^{2+} in wheat leaves and roots etc. Unfortunately, not any reports on the fluorescent detection of Cu^{2+} in plants can be observed till now, which is a worthwhile goal.

In our previous studies, three luminescent probes have been designed for detection of Cu^{2+}. For example, based on oxidative cyclization mechanism, we designed two ruthenium(II) complex luminescent sensors to detect Cu^{2+} in pea aphids[29-30], meanwhile, a highly sensitive and selective turn-on fluorescent probe was developed for imaging of Cu^{2+} in living MCF-7 cells based on Rhodamine B[31]. In continuation of our research on this track, herein, a Rhodamine B-based fluorescent probe (**RBO**, Scheme 1) is designed for in-situ detecting and imaging of Cu^{2+} in the complex plant tissues. The proposed probe can be divided into two parts. One is rhodamine moiety which is non-fluorescent from pH 6 to 9 because of its spirocyclic structure. The other is aldehyde moiety which is working as a receptor for recognition of Cu^{2+}.

Based on the chelation mechanism[32] of controlling turn-on spiro conjugated system, **RBO** can provide a useful tool to visualize the bioaccumulation of Cu^{2+} in maize roots. The probe **RBO** is higher selective and sensitive for imaging Cu^{2+} than others[33] in plant tissue with the formation of chelating complex, accompanied strong fluorescence emerging and simultaneous color change due to the ring-opening form. Especially, this probe can be easily produced (yield 80%), monitor and quantify Cu^{2+}

both in solution by the naked eye, and visualize Cu^{2+} in plant tissue by laser scanning confocal microscope.



Scheme 1. Structure and proposed response mechanism of the probe **RBO** towards Cu^{2+}.

2. Experimental section

2.1 Reagents and apparatus

All solvents and chemical reagents purchased from Aladdin (China) are analytical grade. Stock solution of the metal ions (10 mM) was prepared in deionized water. The maize seeds got from the college of life science in Northwest A&F University. ^{1}H NMR and ^{13}C NMR spectra were obtained with a Bruker AVANCE III 500 MHz instrument (Germany) with chemical shifts reported in ppm at room temperature. Mass spectra were recorded on a Thermo Fisher LCQ Fleet mass spectrometer (USA) and a LC/Q-Tof MS spectrometry (**USA**). Absorption and emission spectra were collected by using a Shimadzu 1750 UV-visible spectrometer and a RF-5301 fluorescence spectrometer (Japan). The pH of the testing systems was determined by a PHS-3C pH Meter (China). The maize grew in growth cabinet using RX-2800 incubator with the temperature at 25 ± 2 ℃ and 70% - 80% relative humidity. The plant imaging was conducted by an vivo imaging system FX Pro (Leica TCS SP8, confocal microscope, Germany).

*2.2 Synthesis of the probe **RBO***

Rhodamine B hydrazide (**RBH**) is synthesized following the literature method[33]. In a drying 100 mL duplex round-bottom flask, **RBH** (0.92 g, 2 mmol) was dissolved in 25 mL anhydrous ethanol, and then 3-methoxysalicylaldehyde (0.28 g, 4 mmol) was added to the flask under the protection of nitrogen. The mixture was stirred and refluxed for 12 h. Then the reaction was cooled and evaporated in vacuo to give khaki oil. This oil was recrystallized from anhydrous ethanol, yield 0.71g (60%) of light orange solid. (Scheme 1) ^{1}H NMR (500 MHz, DMSO-d_6) δ (ppm): 10.18 (s, 1H), 9.12 (s, 1H), 7.90 (d, J = 7.0 Hz, 1H), 7.58 (m, 2H), 7.09 (d, J = 7.5 Hz, 1H), 6.92 (dd, J = 8.0, 1.0 Hz, 1H), 6.87 (dd, J = 8.0, 1.5 Hz, 1H), 6.73 (t, J = 8.0 Hz, 1H), 6.40 (dd, J = 9.4, 3.8 Hz, 4H), 6.32 (dd, J = 9.0, 2.5 Hz, 2H), 3.70 (s, 3H), 3.28 (q, J = 7.0 Hz, 8H), 1.05 (t, J = 7.0 Hz, 12H). ^{13}C NMR (125 MHz, DMSO-d_6) δ (ppm) 164.04, 153.23, 151.50, 150.51, 149.05, 148.34, 147.47, 134.47, 129.39, 129.20, 124.38, 123.53, 121.20, 119.52, 114.42, 108.66, 105.31, 97.82, 66.04, 56.23, 44.14, 12.90. HR-MS (ESI) m/z calc. for $C_{36}H_{39}N_4O_4^+$ $(M+H)^+$ 591.29658, found 591.29657.

2.3 Plant culture and imaging

The maize seeds were disinfected with a 70% ethanol and 2% sodium hypochlorite solution, washed thoroughly with distilled water several times, then cultured on Petri dishes under condition of 25℃, 70% relative humidity in the dark. When the seeds germinated, the control group was supplied only with 1/4 Hogland nutrient solution[14], but the experimental groups were treated by using Hoagland solution with different levels of Cu^{2+} (0, 100, 500, and 1000 μM) in a growth cabinet at 25℃, 75% humidity. (light/dark regime of 16 h/8 h, white light, 100 μmol photons/m^{2}s). Several days later, the roots were collected from the different groups and washed with deionized water. Then, these roots were prepared by simple hand section with 10-15 μm thicknesses putting in a dish[25, 34] and divided into three groups. The first group was the control. The second groups treated with different levels of Cu^{2+} (0, 100, 500 and 1000 μM) were dipped into a solution of **RBO** (1 mM) for 4 h, washing three times with deionized water. The third group was added 10 mM ethylenediaminetetraacetic acid disodium salt, a chelator of copper ions, for 6 h, washing three times with deionized water and then added **RBO** (1 mM) for 4 h, washing three times with deionized water again. All groups were observed under a laser scanning confocal microscope.

3. Results and discussion

*3.1 UV-Vis and Fluorescence Spectral responses of **RBO***

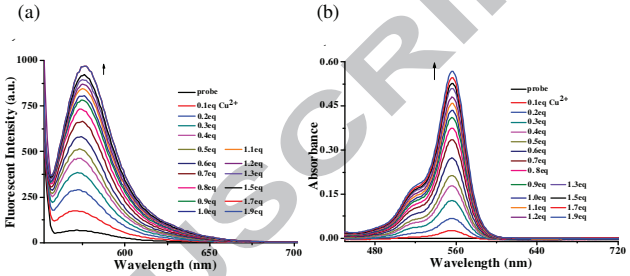


Figure 1. Fluorescent spectra (a) and UV-Vis absorption spectra (b) of **RBO** (10 μM) upon addition of Cu^{2+} in CH_3CN/H_2O (1 : 1, v/v) buffered with PBS, pH = 7.0, λ_{ex} = 540 nm.

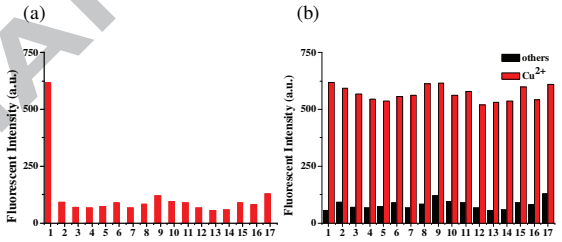


Figure 2. (a) Fluorescence intensity of **RBO** (10 μM) upon addition of Cu^{2+} (10 μM) and various metal ions (20 μM) in CH_3CN/H_2O (1 : 1, v/v) buffered with PBS, pH = 7.0, λ_{ex} = 540 nm. (b) Fluorescence intensity of **RBO** (10 μM) after addition of Cu^{2+} (10 μM) in the presence of various metal ions (20 μM) in CH_3CN/H_2O (1 : 1, v/v) buffered with PBS, pH = 7.0, λ_{ex} = 540 nm. Dark bars represent the fluorescent responses toward metal ions (1. Blank, 2. Al^{3+}, 3. Ca^{2+}, 4. Cd^{2+}, 5. Ce^{3+}, 6. Co^{2+}, 7. Cr^{3+}, 8. Fe^{2+}, 9. Fe^{3+}, 10. K^+, 11. Li^+, 12. Mg^{2+}, 13. Mn^{2+}, 14. Ni^{2+}, 15. Pb^{2+}, 16. Zn^{2+}, 17. Ba^{2+}); Red bars represent the subsequent addition of 10 μM Cu^{2+} to the aforementioned solutions.

The rhodamine derivative fluorescent probe is sensitive to acid environment where the ring opening makes the non-fluorescent probe emit red fluorescence[32, 35]. To find a suitable pH span where **RBO** could selectively and sensitively detect Cu^{2+}, the fluorescence property of **RBO** firstly tested in solution with different pH value (3.8 to 11.6, Figure S1). The fluorescence intensity was stable in a broad pH range of 5.7-9.3, which increased as the pH value lower than 5.8 because the H^+ can induce spirocyclic opening. Therefore, pH 7.0 was chosen to mimic physiological condition for avoiding any influence of **RBO** in an acidic environment. Then the dynamics of the probe to Cu^{2+} was investigated in CH_3CN/H_2O (1 : 1, v/v) buffered with PBS at room temperature. After the addition of 0.5 equiv Cu^{2+} to the solution of **RBO** (10 μM), the fluorescence intensity increased rapidly and reached a maximum in 5 min (Figure S2), then no further significant change occurred, which suggested that the optimal reaction time for Cu^{2+} detection for this probe was around 5 min. Hence, all the subsequent spectral measurements

were carried out after an equilibration time of 5 min. Then, the spectral properties of **RBO** with Cu^{2+} were investigated systematically. The **RBO** (10 μM) showed a moderate emission intensity at 572 nm in CH_3CN/H_2O (1 : 1, v/v) buffered with PBS solution (pH = 7.0). Nonetheless, upon gradually addition of Cu^{2+} over a concentration range of 0-19 μM, the fluorescent intensity of the probe increased to over 13 folds upon excitation at 540 nm with a little red shift (Figure 1a). These results confirmed our working hypothesis that the Cu^{2+} could bind with **RBO** forming **RBO**-Cu^{2+} complex. Moreover, the ratio of F/F_0 (F and F_0 represent the fluorescence intensity of **RBO** (at 575 nm) with or without adding the Cu^{2+}) followed a good linear relationship with the concentration of **RBO** over the range of 0-15 μM (Figure S3). According to the definition[36], the corresponding limit of detection (LOD) was down to 28 nM from 20 blank solutions, which is greatly lower than the maximal permitting level (1.30 ppm) of drinking water set by the U.S. EPA[19]. More importantly, the UV-Vis absorption spectra (Figure 1b) of **RBO** alone (10 μM) displayed no absorbance at 555 nm in CH_3CN/H_2O (1 : 1, v/v) buffered with PBS (pH = 7.0). With continuous added of $CuSO_4 \cdot 5H_2O$, the absorbance value increased from 0 to 0.56, corresponding to a significant change in solution from colorless to pink. This result demonstrated that **RBO** was effective in sensing Cu^{2+} even in minor level.

*3.2 Interference of **RBO** to Cu^{2+} over other metal ions*

In order to better evaluate the sensitivity and selectivity of **RBO** (10 μM) to Cu^{2+} than other metal ions like Al^{3+}, Ca^{2+}, Cd^{2+}, Ce^{3+}, Co^{2+}, Cr^{3+}, Fe^{3+}, K^+, Li^+, Mg^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+}, and Ba^{2+}. A 10 μM solution of other metal ions, as well as a 10 μM solution of Cu^{2+} was added respectively to a solution of 10 μM **RBO**. As is shown in Figure 2a, the change of fluorescent intensity of **RBO** with other ions was negligible. Whereas the **RBO** treated with Cu^{2+} exhibited a significant fluorescence enhancement. To further evaluate the interference by these metal ions, the competitive assays were carried out by recording the corresponding fluorescence spectra. As is shown in Figure 2b, the fluorescence change of **RBO** (10 μM) didn't cause any interference for the detection of Cu^{2+} in the presence of other interfering metal ions (20 μM). It was proved that **RBO** could be used as a specific fluorescent probe for Cu^{2+} in CH_3CN/H_2O (1 : 1, v/v) buffered with PBS, pH = 7.0, λ_{ex} = 540 nm.

*3.3 Reversibility experiment of **RBO***

To investigate the chelation ability, a cycle experiment was carried out. Notably, the solution exhibited no fluorescence before adding 2 equiv Cu^{2+}, then the fluorescent intensity increased rapidly and reached 4 times in 5 min. Then 2 equiv EDTA-Na_2 (10 μM) was added, the fluorescence decreased immediately (Figure 3). The cycle experiment determinations namely, eight times supplement of Cu^{2+} and subsequent EDTA-Na_2 were carried out with negligible intensity attenuation, which indicated that our probe could combine with Cu^{2+} strongly, proving potential applicability for in-situ fluorescent imaging of Cu^{2+} in plants.

*3.4 Reaction mechanisms of **RBO** to Cu^{2+}*

Job's plot and the Benesi–Hildebrand equation based on literature method[31, 37] were used to certify binding properties according to UV-Vis and fluorescence titration experiment. According to these results (Figure S4), the stoichiometry between Cu^{2+} and **RBO** was determined to be 1 : 1 in the complex and the apparent association constant of the **RBO**-Cu^{2+} interaction was approximately 2.26×10^6 M^{-1} [38], which were consisted with the results from the Mass Spectra, where the molecular ion peak were changed to 652.36 in the presence of $Cu(ClO_4)_2$ (Figure S9, Figure S10). The ring opened in **RBO** to form the **RBO**-Cu^{2+} was further confirmed by ^1H NMR (Figure S7) where the shape of the proton peaks got wider after addition of Cu^{2+}, owing to the paramagnet effect of Cu^{2+} in nature. Meanwhile, the corresponding ^{13}C NMR could provide some evidence further (Figure S8).

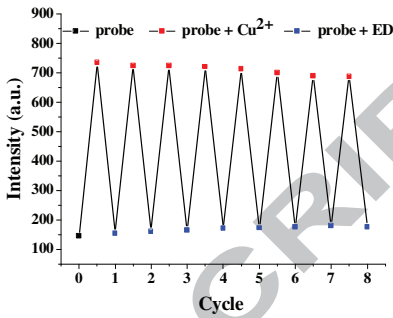


Figure 3. Fluorescence intensity changes of **RBO** on alternate addition of 2 equiv Cu^{2+} and EDTA-Na_2 (10 μM) in CH_3CN/H_2O = 1 : 1 (v/v) buffered with PBS, λ_{ex} = 540 nm.

3.5 Application in bioimaging

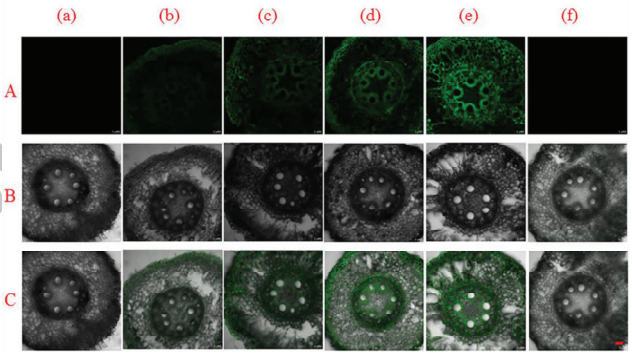


Figure 4. Confocal fluorescence images of cross section of maize roots treated with different concentrations of $CuSO_4 \cdot 5H_2O$. The seedlings pretreated with different concentrations of Cu^{2+} after 6 days were incubated with **RBO** (1 mM) for 4 h (a) 0 μM, (b) 10 μM, (c) 100 μM, (d) 500 μM, (e) 1000 μM, (f) testing (e) treated with 10 mM EDTA-Na_2 for 6 h and then incubated with **RBO** (1 mM) for 4 h. A: Dark field images. B: Bright field images. C: Merged images. (green; λ_{ex} = 514 nm, λ_{em} = 530-580 nm; Scale bar, 100 μm).

In order to explore the Cu^{2+}-sensing behavior of probe **RBO** in biological systems, the maize roots were selected by using the laser scanning confocal microscope for imaging the Cu^{2+}. As is depicted in Figure 4, the control had no emission indicated that various plant bio-molecules including chlorophyll, carotene, and phenolic compound didn't have influence on bio-imaging. Second groups (b-e), treated with **RBO** (1 mM) gave a sufficiently fluorescent intensity in CH_3CN/H_2O (1 : 1, v/v) buffered with PBS, pH = 7.0, and its fluorescence intensity increased in a dose-dependent manner with increasing Cu^{2+} concentrations. The third had no emission pretreated by EDTA-Na_2 (10 mM). Notably, when the concentration of Cu^{2+} amounted to 1000 μM, a high level of fluorescence was observed both in vascular cylinder and epidermal cells. The vascular cylinder plays an important role in translocation Cu^{2+} from the root to the shoot and leaf. In addition, epidermal cells are known as sites which accumulate the highest concentrations of Cu^{2+}. Therefore, these results indicated that the intense fluorescence in vascular

tissue and epidermal cells was due to the presence of Cu^{2+}. To further confirm these, the time dependent test was carried out. As is depicted in Figure S11, when the maize roots pretreated with Cu^{2+} (1000 μM) then incubated with **RBO** (1 mM), the probe of **RBO** gave a slight fluorescence emission in 2 days in vascular cylinder. With the extension of time to 6 days, the increasingly strong fluorescence emerged both in vascular cylinder and epidermal cells. All these suggested that the probe could detect internal Cu^{2+} in maize roots and the proposed method can be quite helpful for understanding how the plants take up, transport and store in the Cu^{2+}. Notably, the low concentration of Cu^{2+} (≤ 1000 μM) makes the seedlings grow healthily and exhibits a normal plant morphology (Figure S12).

4. Conclusions

In conclusion, the Rhodamine B-based probe was synthesized through a facile and economical route, which exhibited a highly sensitive and rapidly bound with Cu^{2+}. When formed a 1 : 1 complex of **RBO** and Cu^{2+}, there was strong fluorescence and eye-naked red color, and the cycle experience had also confirmed that the strong binding of them. Furthermore, this work demonstrated that the probe had good permeability and histocompatibility to cell wall and provided a useful tool for mapping and biosensing of Cu^{2+} in vascular cylinder and epidermal cells, the major storage sites of Cu^{2+} in maize roots. What's more, the low concentration of Cu^{2+} (≤ 1000 μM) makes the maize possess long and strong roots, which allows them to accumulate Cu^{2+} for a certain growth period. In the future, the excellent probe would lay a foundation for Cu^{2+} detection in other plants.

Bibliography

Ting Lv received her B.S. degree in 2015 from College of Life Science, Yan'an University, China. She is pursuing her master's degree under the guidance of Prof. Shiguo Sun in college of Chemistry & Pharmacy, Northwest A&F University. Her research interests focus on metal ion probe for ion detection and disease diagnosis.

Fengyu Liu received her Ph.D. in 2006 from the Dalian University of Technology. She is currently an associate professor in the state key laboratory of Fine Chemicals at the Dalian University of Technology. Her research is focused on the following two fields: (1) the quantitative detection of DNA oxidative damage based on electrochemiluminescence; (2) the fluorescent dye-based probes and their biological applications.

Yongqian Xu received his Ph.D. in Applied Chemistry from the Dalian University of Technology (China) in 2007. In 2008, he worked as a postdoctoral fellow at the University of Akron (USA). He is currently an associate professor at the college of Chemistry & Pharmacy, Northwest A&F University, China. His current research interests mainly focus on fluorescent chemosensors.

Hongjuan Li obtained her Ph.D. from Shaanxi Normal University (China) in 2010. She is currently an associate professor at the college of Chemistry & Pharmacy, Northwest A&F University, China. Her current research interests mainly focus on the development of functional nanocomposites and fluorescent chemosensor based on layered double hydroxide materials.

Shiguo Sun obtained his Ph.D. degree in 2003 from Dalian University of Technology and is currently a professor and doctoral supervisor in the college of Chemistry & Pharmacy, Northwest A&F University, China. His research interests include functional molecules with special light or electrochemistry properties, self-assembly chemistry of carbon nanotube/graphene material for DNA and protein sensing, drug delivery and fluorescence tracing, and electrochemiluminescence and luminescence sensors.

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References and notes

1 S. Sharma, S. Sharma, N. Upreti and K. P. Sharma, *Toxico. Enviro. Chem.*, 2009, **91**, 109-120.
2 S. Mazhoudi, A. Chaoui, M. Habib Ghorbal and E. El Ferjani, *Plant Sci.*, 1997, **127**, 129-137.
3 J. Zhang, L. Zhang, Y. Wei, J. Ma, S. Shuang, Z. Cai and C. Dong, *Spectrochim. Acta, Part A*, 2014, **122**, 731-736.
4 J. S. Valentine, P. A. Doucette and S. Zittin Potter, *Annu. Rev. Biochem.*, 2005, **74**, 563-593.
5 M. Bernal, R. Cases, R. Picorel and I. Yruela, *Environ. Exp.Bot.*, 2007, **60**, 145-150.
6 S. Oliver and S. A. Barber, *Soil. Sci. Am. J.*, 1966, **30**, 468-470.
7 C.-C. Lin, L.-M. Chen and Z.-H. Liu, *Plant Sci.*, 2005, **168**, 855-861.
8 B. Hotzer, R. Ivanov, T. Brumbarova, P. Bauer and G. Jung, *FEBSJ*, 2012, **279**, 410-419.
9 L. B. Pena, A. A. Mendez, C. L. Matayoshi, M. S. Zawoznik and S. M. Gallego, *Plant physiol. Biochem.*, 2015, **87**, 115-123.
10 H. Teisseire and V. Guy, *Plant Sci.*, 2000, **153**, 65-72.
11 B. Halliwell and J. M. Gutteridge, *Biochem. J.*, 1984, **219**, 1-14.
12 D. H. Atha, H. Wang, E. J. Petersen, D. Cleveland, R. D. Holbrook, P. Jaruga, M. Dizdaroglu, B. Xing and B. C. Nelson, *Environ. Sci. Technol.*, 2012, **46**, 1819-1827.
13 S. M. Ruzsa and J. G. Scandalios, *Biochemistry*, 2003, **42**, 1508-1516.
14 W. Zhang, K. Lin, J. Zhou, W. Zhang, L. Liu and Q. Zhang, *Environ. Toxicol. Pharmacol.*, 2014, **37**, 348-353.
15 Y. E. Freedman, D. Ronen and G. L. Long, *Environ. Sci. Technol.*, 1996, **30**, 2270-2277.
16 A. P. S. Gonzáles, M. A. Firmino, C. S. Nomura, F. R. P. Rocha, P. V. Oliveira and I. Gaubeur, *Anal. Chim. Acta*, 2009, **636**, 198-204.
17 L. P. Singh and J. M. Bhatnagar, *Talanta*, 2004, **64**, 313-319.
18 B. Wu, Y. Chen and J. S. Becker, *Anal. Chim. Acta*, 2009, **633**, 165-172.
19 Y. Liu, Q. Su, M. Chen, Y. Dong, Y. Shi, W. Feng, Z. Y. Wu and F. Li, *Adv. Mater.*, 2016, **28**, 6625-6630.
20 D. Zhu, Y. Luo, L. Shuai, W. Xie, X. Yan, Z. Duan and W. Cai, *Tetrahedron.*, 2016, **57**, 5326-5329.
21 Y. Liu, P. Liang and L. Guo, *Talanta*, 2005, **68**, 25-30.
22 G. W. Luther, S. M. Theberge and D. T. Rickard, *Environ. Sci. Technol.*, 1996, **30**, 3640-3641.
23 S. Ishijima, A. Uchibori, H. Takagi, R. Maki and M. Ohnishi, *Arch. Biochem. Biophys.*, 2003, **412**, 126-132.
24 A. Pal, B. Bag, M. Thirunavoukkarasu, S. Pattanaik and B. K. Mishra, *RSC Adv.*, 2013, **3**, 18263.
25 B. Biswal, D. Mallick, M. Thirunavoukkarasu, R. Mohanty and B. Bag, *Sens. Actuators B: Chem.*, 2016, **232**, 410-419.
26 J. H. Lee, J. H. Lee, S. H. Jung, T. K. Hyun, M. Feng, J. Y. Kim, J. H. Lee, H. Lee, J. S. Kim, C. Kang, K. Y. Kwon and J. H. Jung, *Chem. Commun.*, 2015, **51**, 7463-7465.
27 X. Gan, P. Sun, H. Li, X. Tian, B. Zhang, J. Wu, Y. Tian and H. Zhou, *Biosens. Bioelectron.*, 2016, **86**, 393-397.
28 R. Shen, D. Liu, C. Hou, J. Cheng and D. Bai, *Anal. Methods*, 2016, **8**, 83-88.
29 Y. Zhang, Z. Liu, K. Yang, Y. Zhang, Y. Xu, H. Li, C. Wang, A. Lu and S. Sun, *Sci. Rep.*, 2015, **5**, 8172.
30 Y. Zhang, Z. Liu, Y. Zhang, Y. Xu, H. Li, C. Wang, A. Lu and S. Sun, *Sens. Actuators B: Chem.*, 2015, **211**, 449-455.
31 Y. Yuan, S. Sun, S. Liu, X. Song and X. Peng, *J. Mater. Chem. B*, 2015, **3**, 5261-5265.
32 H. Moon, J. Park and J. Tae, *Chem. Rec.*, 2016, **16**, 124-140.
33 Q. Huang, Q. Zhang, E. Wang, Y. Zhou, H. Qiao, L. Pang and F. Yu, *Spectrochim. Acta, Part A*, 2016, **152**, 70-76.
34 L. He, X. Yang, K. Xu and W. Lin, *Chem. Commun.*, 2017.

35 W. Zhang, B. Tang, X. Liu, Y. Liu, K. Xu, J. Ma, L. Tong and G. Yang, *Analyst*, 2009, **134**, 367-371.
36 S. Gharami, D. Sarkar, S. Acharyya and T. K. Mondal, *J Fluoresc*, 2016, **26**, 2113-2118.
37 H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, **71**, 2703-2707.
38 M. Hariharan, S. C. Karunakaran and D. Ramaiah, *Org. Lett.*, 2007, **9**, 417-420.

A Rhodamine B-based fluorescent probe for imaging Cu^{2+} in maize roots

A new Rhodamine B-based fluorescent probe (**RBO**) is a selective and sensitive chemosensor for Cu^{2+} than other ions. It can clearly illustrate the distribution of Cu^{2+} with the help of laser scanning confocal microscope in plant tissues.

Ting Lv[a], Yongqian Xu[a], Hongjuan Li[a] and Fengyu Liu[b, *], Shiguo Sun[a, *]

[a] *Shaanxi Key Laboratory of Natural Products & Chemical Biology, College of Chemistry & Pharmacy, Northwest A&F University, Xinong Road 22, Yangling, Shaanxi 712100, P.R. China.*

[b] *State Key Laboratory of Fine Chemicals, School of Chemistry, Dalian University of Technology, No. 2 linggong Road, Ganjingzi District, Dalian 116023, China.*