J Fluoresc (2011) 21:2057–2068
DOI 10.1007/s10895-011-0904-5

ORIGINAL PAPER

Uptake of FITC Labeled Silica Nanoparticles and Quantum Dots by Rice Seedlings: Effects on Seed Germination and Their Potential as Biolabels for Plants

Remya Nair · Aby C. Poulose · Yutaka Nagaoka · Yasuhiko Yoshida · Toru Maekawa · D. Sakthi Kumar

Received: 6 February 2011 / Accepted: 20 May 2011 / Published online: 14 June 2011
© Springer Science+Business Media, LLC 2011

Abstract The use of fluorescent nanomaterials with good photostability and biocompatibility in live imaging of cells has gained increased attention. Even though several imaging techniques have been reported for mammalian cells, very limited literatures are available for nanomaterial based live imaging in plant system. We studied the uptake ability of two different nanomaterials, the highly photostable CdSe quantum dots and highly biocompatible FITC-labeled silica nanoparticles by rice seedlings which could provide greater opportunities for developing novel *in vivo* imaging techniques in plants. The effects of these nanomaterials on rice seed germination have also been studied for analyzing their phytotoxic effects on plants. We observed good germination of seeds in the presence of FITC-labeled silica nanoparticles whereas germination was arrested with quantum dots. The uptake of both the nanomaterials has been observed with rice seedlings, which calls for more research for recommending their safe use as biolabels in plants.

Keywords Fluorescent silica nanoparticles · Quantum dots · Germination of rice seeds · Photobleaching · Biolabeling in plants

Introduction

Nanomaterials have found several applications in medicine and agriculture. Eventhough much works have been carried out using fluorescent nanoparticles and quantum dots as imaging agents in animal cells, only very limited works have been reported regarding the successful uptake and use of quantum dots and fluorescent nanoparticles in live plant tissues. *In vivo* visualization of nanomaterials is important for their successful use as delivery vehicles in plants.

Successful internalization of FITC conjugated silica nanoparticles [FITC-SNPs] into mammalian cells and their use as cellular markers have already been reported [1, 2]. However reports regarding the application of these nanomaterials in plants are very limited. Phytotoxic studies are important for the successful application of any nanoparticulates to plant system. It was reported that two commercial silica nanoparticle suspensions showed some toxicity to the unicellular green algae, *Pseudokirchneriella subcapitata* and the toxicity had happened not by the uptake of nanoparticles but due to cell wall-nanoparticle interactions [3]. In another study it was observed that under moderate and high concentrations of SNPs of around 20 nm diameter the chlorophyll contents of the green algae *Scenedesmus obliquus* was decreased significantly [4]. However experiments with Changbai larch seedlings showed improved seedling growth and seedling quality when they were soaked in nanostructured silicon dioxide solution of different concentrations [5]. Upon the entry of nanoparticles into plant system by overcoming the cell - wall barrier different plants respond differently to them and hence the behavior of nanoparticles inside the plant system is really unpredictable. Recently several works have reported showing the uptake and translocation of different nanoparticles in various types of plants [6]. Successful use of honeycomb mesoporous silica nanoparticle (MSN) system for transporting DNA and chemicals into isolated plant cells and intact leaves provided a breakthrough in

R. Nair · A. C. Poulose · Y. Nagaoka · Y. Yoshida · T. Maekawa · D. S. Kumar (✉)
Bio-Nano Electronics Research Center, Graduate School of Interdisciplinary New Science, Toyo University, Kawagoe, Saitama 350-8585, Japan
e-mail: sakthi@toyo.jp

plant nanotechnology [7]. However the use of fluorescent nanomaterials for live imaging in plants is a novel criterion that calls much for future research. Such an approach is vital for the visualization of various physiological processes, for monitoring transgene expression etc. Only very limited works have been done for the use of fluorescent nanomaterials as biolabels in plants. *In vivo* imaging of the uptake of nanocrystals showing photon upconversion by plant roots has been recently reported [8].

Quantum dots (QDs) are novel fluorescent nanomaterial having immense applications in *in vivo* imaging. They provide excellent photostability with broad absorption spectra and narrow emission spectra, and hence overcome several drawbacks of organic dyes and other fluorophores. Several research works have already been reported regarding their applications in *in vivo* cell tracking in animals, cell-surface protein interactions and other biomedical applications [9–11]. However major challenge is the cytotoxicity of these semi conductor nanocrystals along with its ecotoxicological effects. Several mixed reports came regarding the toxicity of quantum dots to mammalian cells [12, 13], however its effects on plants are least studied. Studies on the interaction of quantum dots with algal cells showed inhibited photosynthetic activity of algae and it was observed that their adsorption to algal cells got increased with corresponding increase in the dosage of QDs [14]. There was not any internalization of QDs by the algal cells due to their thick cell wall and the lack of endocytosis in algal cells. However in plant cells the uptake of fluorescent quantum dots by sycamore cultured cells and their colonization within cytoplasmic vesicles was reported as a confirmation of the presence of fluid phase endocytosis in plants [15]. QDs for live imaging in plant system was first reported for understanding the mechanism of interaction between the pollen tube and female tissue during reproduction by utilizing them as bio-labels for the localization of a pistil protein at the growing pollen tube tip [16]. QDs for in situ hybridization analysis was also tested for investigating their signal stability and intensity in plant chromosome analyses and it was reported that QDs were more suitable for immunolabeling of tissue sections than the conventional detection systems, however not good for improving the sensitivity of in situ hybridization on plant chromosomes [17]. Imaging of microtubules in tobacco BY-2 cells was achieved with silica-coated QDs coupled to anti-tubulin antibodies using Trojan Peptoids as the delivery vehicles into tobacco cells [18]. Very recently transport of MAA-coated QDs into maize seedling roots with the help of a cuticular penetrating surfactant have reported, thus suggesting the possibility of using them for live imaging in plant system [19].

For understanding the effects of various nanomaterials in plant system, it is essential to study their phytotoxic effects. In our work we studied the phytotoxicity of two fluorescent nanomaterials, FITC labeled silica nanoparticles [FITC-SNPs] and MPA linked CdSe quantum dots [MPA-CdSe] by investigating their effects on rice seed germination. Quantum dots are highly photostable in nature however cytotoxic effects are the major limiting factor for their wide scale applications in life sciences. SNPs are highly biocompatible with reduced phytotoxicity and ecotoxicity. To make it fluorescent we coupled FITC with SNPs and such a coupling could reduce the photobleaching property of FITC thus providing increased photostability. We also studied the uptake efficiency of QDs and fluorescent SNPs by rice seedlings that could provide great support of their use as biolabels in live plants.

Experimental Methods

Synthesis and Characterization of Water-Soluble MPA Linked CdSe Quantum Dots and FITC-SNPs

Oleic acid-CdSe quantum dots (OA-CdSe) were synthesized according to the procedure of Boatman et al. [20], made soluble in chloroform and stored in airtight bottles with aluminium foil covering. Stable water-soluble quantum dots were then prepared by phase transfer method [21, 22] with mercaptopropionic acid (MPA). The optical characterization of OA-CdSe QDs and water-soluble MPA-linked CdSe QDs was carried out using Shimadzu UV-2100PC/3100PC UV visible spectrophotometer (UV–vis spectrophotometer). QD solutions were taken in quartz cuvettes and absorption was measured. Electron microscopic studies were carried out by JEM-2200-FS Field Emission Transmission Electron Microscope [TEM] at an accelerating voltage of 200 KV for studying the internal structure of QDs. The sample was dropped on carbon film coated 200 mesh copper grid and then vacuum dried in a dessicator before examination. FITC-SNPs were synthesized based on reported procedures [23, 24] and their characterization was carried out using TEM and UV-visible spectrophotometer. Thermogravimetric analysis of bare SNPs and FITC-SNPs was carried out using Shimadzu DTG-TG 60 H Apparatus. For TGA analysis both the samples were heated from room temperature to 1,000° C and maintained at that temperature for 10 min. For investigating the leaking of FITC from SNPs aqueous solution of fluorescent SNPs were ultrasonicated for 1 h followed by centrifugation at 6,000 rpm for 15 min. The supernatant was then removed and the sedimented SNPs were again subjected to ultrasonication for 1 h followed by centrifugation. The supernatant was again removed and the fluorescence of the sedimented nanoparticles was checked with fluorescence microscope.

MPA-CdSe QDs and FITC-SNPs on the Germination of Rice Seeds

For studying the phytotoxic effects of water soluble QDs rice seeds were placed in 3 different concentrations of MPA-CdSe QDs (1 ml QDs+0.5 ml double distilled (dd) H_2O, 0.5 ml QDs+1 ml dd H_2O and 0.25 ml QDs+1.25 ml H_2O) and the seeds in dd H_2O alone was taken as the control. Similarly FITC-SNPs were taken at a concentration of 50 μg/ml of H_2O, and SNPs alone at 50ug/ml as the standard and dd H_2O as the control. Before keeping them for germination the seeds were surface sterilized with sodium hypochlorite and washed several times in deionized water. The seeds were then transferred to nanoparticle solutions for germination. The treated seeds were kept under controlled condition for germination and those seeds showing the emergence of either radicle or plumule were considered as germinated.

Water Soluble QDs and FITC-SNPs as Bioimaging Agents

For studying the *in vivo* imaging ability of QDs and FITC-SNPs, 4 days old rice seedlings were dipped in QDs solution and FITC-SNPs solution. After 4 h one set of seedlings was taken out from both the nanoparticle solutions and washed thoroughly with 1X PBS buffer (pH=7.4) and deionized water for 15 min and then placed in ddH_2O until they were subjected for imaging. Again after 8 h another set of seedlings was removed from both the nanoparticle solutions followed by washing as mentioned above and kept in ddH_2O until imaging. Rest of the seedlings was allowed to be in nanoparticle solutions overnight, and then washed thoroughly with PBS buffer and deionized water. The uptake of QDs and FITC-SNPs by rice seedlings was imaged with Nikon Eclipse TE2000-U Fluorescent Microscope. For sample preparation the seedlings were washed again in deionized water and then fresh shoot tissues were sectioned longitudinally and transversally by hand cut. The sections were then placed on micro cover glass for imaging. The images were recorded using Nikon Digital Sight DS-U1 camera. Photobleaching experiments were conducted for FITC-SNPs treated samples at a regular interval of 10 min for understanding the photostability of the fluorescent nanoparticles in the seedlings and comparison was done with seedlings treated with FITC alone.

Results and Discussion

Characterization of MPA-CdSe QDs and FITC-SNPs

TEM images of MPA linked CdSe QDs [MPA-CdSe] (Fig. 1A) and FITC-SNPs (Fig. 1B) show the internal structure of the nanomaterials. It is clear from the TEM image that MPA linked water soluble QDs is stable with good monodispersity. The UV-visible absorption spectrum of OA-CdSe in $CHCl_3$ and water soluble MPA linked QDs [MPA-CdSe] are shown in Fig. 2A. The UV-visible absorption spectra showed an absorption peak at 530 nm for both OA-CdSe and MPA-CdSe, which makes clear that the QDs maintained their size on water solubility in which the inner crystal structure has not changed and only ligand exchange has happened. Figure 2B shows the absorption spectrum of FITC-SNPs on comparison with pure FITC. FITC alone showed a broad absorption peak whereas the FITC on conjugation with silica nanoparticles showed a narrow absorption peak with a little red shift for maximum absorption. Figure 3 shows TGA curve of bare silica nanoparticles (SNPs) and FITC-SNPs. The increased weight loss from 12.54% for SNPs to 23.65% for FITC-SNPs was attributed to FITC functionalized with SNPs. Dye leaking experiments were also conducted for investigating any leakage of FITC molecules from SNPs on dissolution in water. Figure 4a shows the fluorescent microscopic images of the as synthesized FITC-SNPs and FITC-SNPs after dye leaking experiments respectively. The supernatant obtained after 2 h of ultrasonication followed by centrifugation showed no fluorescence (Fig. 4b), which confirmed that there was no leakage of fluorescein compound from SNPs and hence such fluorescent nanoparticles could be successfully used for bioimaging in plants.

Phytotoxic Studies with QDs and FITC-SNPs

Phytotoxic studies are essential for understanding the effects of various nanomaterials to plant system. Prior to the application of water soluble QDs and FITC-SNPs as *in vivo* imaging agents in plants, their effects on rice seed germination were studied.

Effects of QDs on the Germination of Rice Seeds

For studying the phytotoxicity of quantum dots on rice plants germination assay was conducted and those rice seeds from which either radicle or plumule has emerged out was considered as germinated. On germinating rice seeds with three different concentrations (1 ml QDs+0.5 ml double distilled (dd) H_2O, 0.5 ml QDs+1 ml dd H_2O and 0.25 ml QDs+1.25 ml H_2O), it was observed that there was no germination for the seeds in quantum dot solution of higher concentrations in comparison to the control seeds germinated in double distilled water (Fig. 5). At very low concentration of QD solution, germination was observed, however further growth was arrested. Hence we conclude that prolonged application of quantum dots is not suitable for plants due to its phytotoxicity.

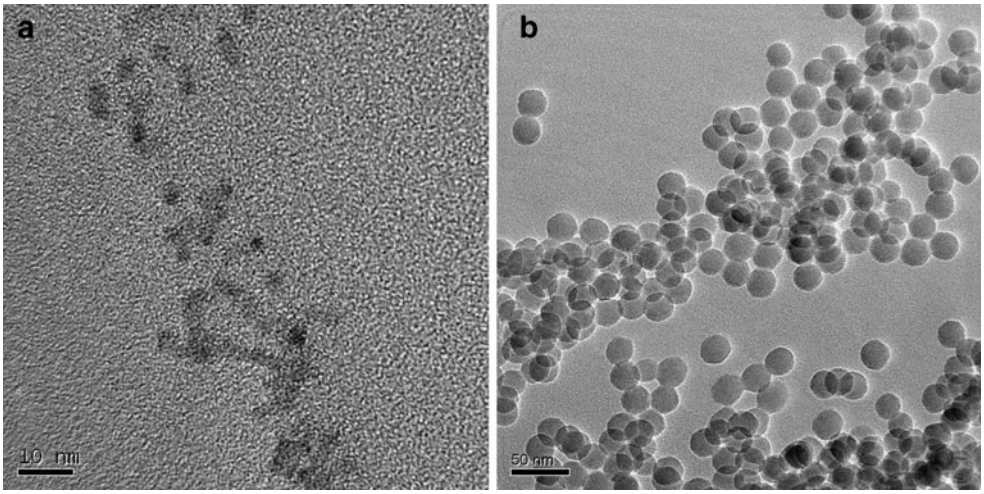

Fig. 1 TEM images of fluorescent nanomaterials. **a** TEM image of water-soluble MPA linked-CdSe QDs [MPA-CdSe] (scale bar: 10 nm). **b** TEM image of FITC-SNPs (scale bar: 50 nm)

Effects of FITC-SNPs on the Germination of Rice Seeds

We studied the germination effects of rice seeds with silica nanoparticles (SNPs) and FITC-SNPs for confirming their safe use on rice seedlings. Good germination was observed for seeds in the presence of both SNPs and FITC-SNPs, which are highly comparable with the germination rate of control seeds in distilled water. However seeds in FITC solution showed some decreased germination rate compared to control and nanoparticles' treated seeds (Fig. 6). This decrease in germination could be correlated to the hydrophobic nature of this fluorescein compound [25]. On conjugating FITC with biocompatible silica nanoparticles, there were not any detectable negative effects on the germination of rice seeds and hence such fluorescent nanoparticles are safe for various applications in plant biology.

QDs and FITC-SNPs as Biolabels for Plants

Several shortcomings of the organic dyes such as poor photostability, low brightness etc. could be overcome to a great extent by the use of fluorescent nanoparticles. Among the fluorescent nanoparticles, quantum dots and fluorescent silica nanoparticles attained special attention due to high resistance to photobleaching and high biocompatibility with less cytotoxic effects respectively.

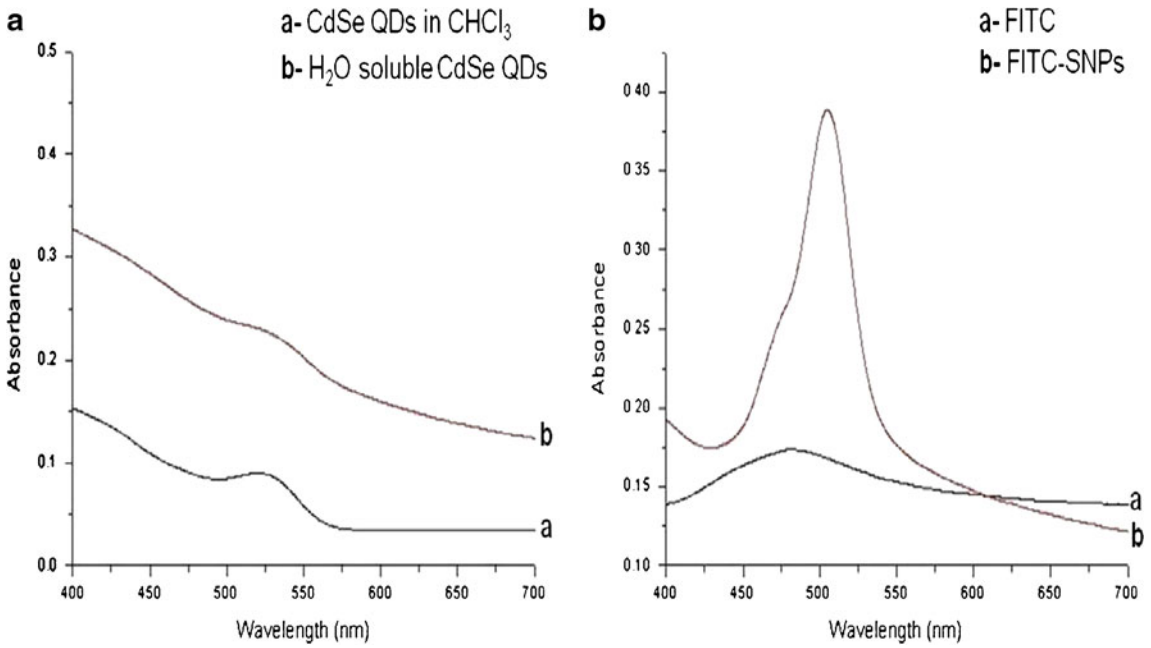

Fig. 2 UV-visible absorption spectrum of fluorescent nanomaterials. **a** UV-visible absorption spectrum of OA-CdSe in $CHCl_3$ and water soluble QDs [MPA-CdSe QDs]. **b** UV-visible absorption spectrum of FITC-SNPs and pure FITC

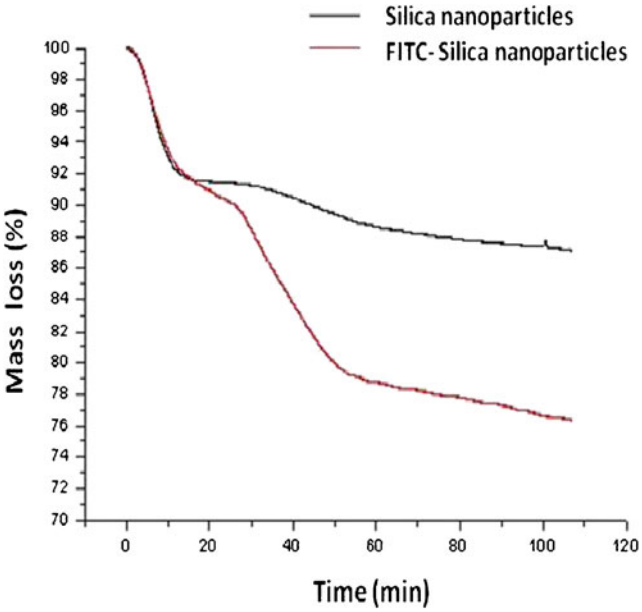

Fig. 3 TGA curve of FITC-SNPs and SNPs (*Red line* denotes the TGA curve of FITC-SNPs and *black line* denotes TGA curve for bare SNPs)

Uptake of Quantum Dots and FITC-SNPs by Rice Seedlings

We studied the uptake ability of quantum dots by rice seedlings through their roots which open up the possibilities of novel imaging techniques in plants. We observed very good adsorption of quantum dots on the root surface on dipping rice seedlings in QD solution. We also investigated the uptake ability of QDs by rice seedlings. Figure 7 shows fluorescent microscopic images of cross section of shoot (from 10 mm above the roots) of 4 day old normal rice seedlings and rice seedlings treated with quantum dots respectively. For the normal shoot at an excitation with blue light, UV and green light the emission peak is in red region, blue region and red region respectively. The synthesized water-soluble quantum dots showed a green fluorescence at an excitation with either UV or blue light. On studying the uptake of quantum dots by rice seedlings by dipping in QD solution, we observed green fluorescence of QDs in the cross section of shoot on excitation with blue light thus confirming the uptake of QDs through the roots of seedlings (Fig. 8). Even though the photostability of QDs have

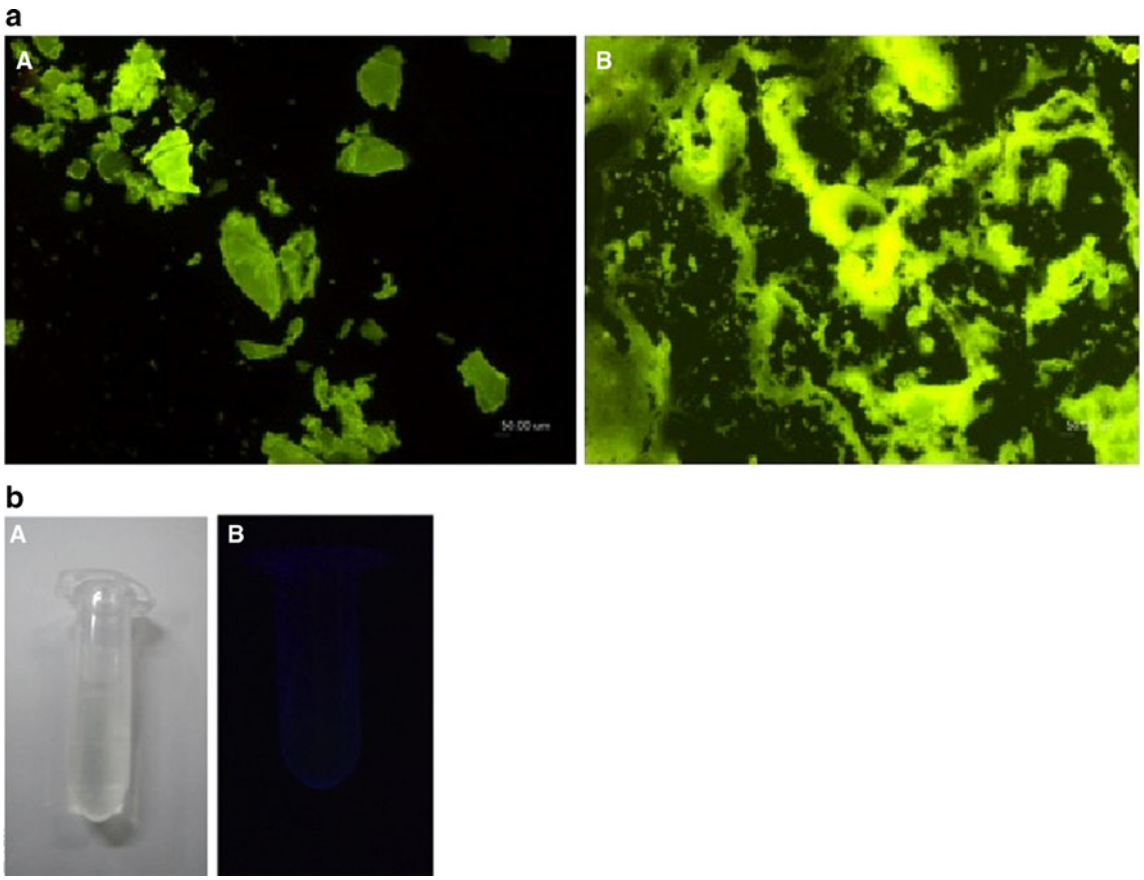

Fig. 4 **a** Fluorescence image of FITC-SNPs before and after dye leaking experiments. **A** Fluorescence image of the as-synthesized FITC-SNPs. **B** Fluorescence image of FITC-SNPs after dye leaking experiment. **b** Supernatant obtained after dye leaking experiment of FITC-SNPs under normal light (**A**) and under UV light (**B**). Fluorescence was not detected in the supernatant, which concluded that there was not any dye leakage

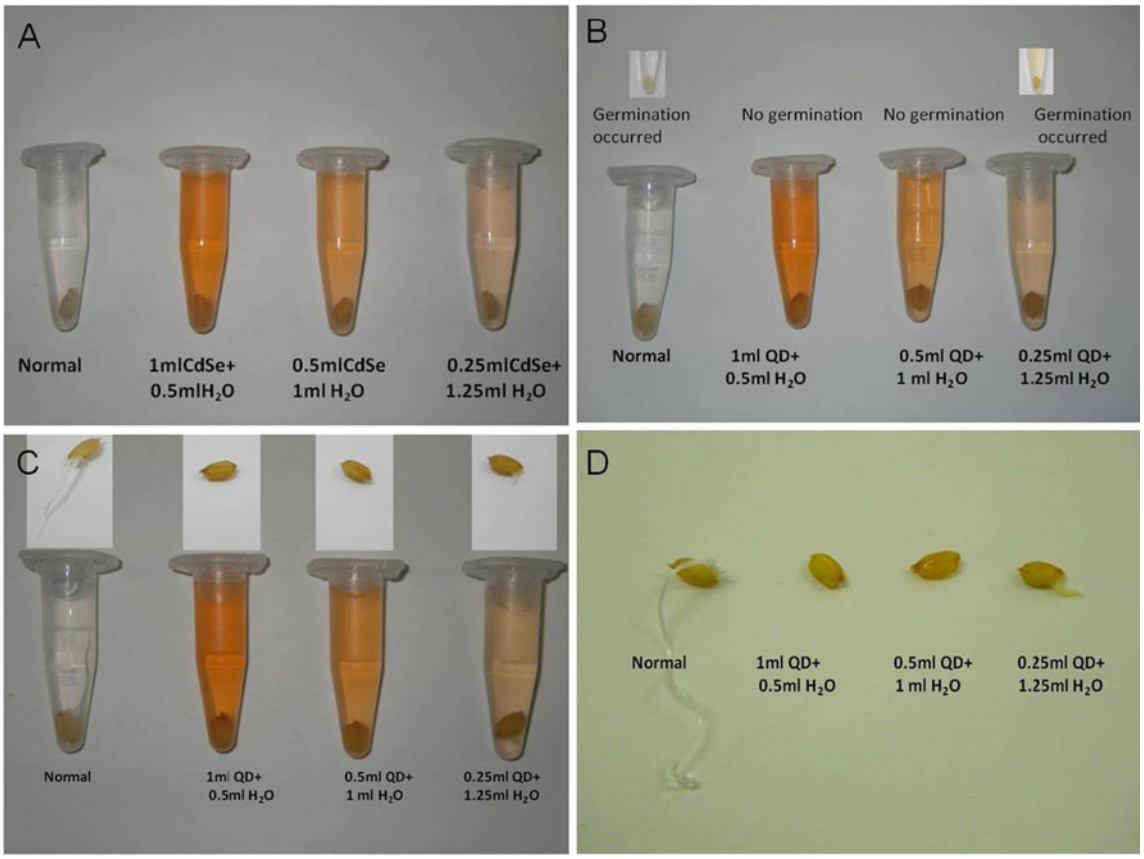

Fig. 5 Images showing the germination of rice seeds in the presence of different concentrations of MPA-CdSe quantum dots. **a** Seeds kept in QD solution of different concentration for germination. **b** Normal seeds and seeds in low concentrated QD solution showed germination after 3 days. **c** Normal seed continued to grow whereas arrested growth in the QD treated seed. Seeds in high concentrated QD solution showed no germination. **d** comparison of growth of normal seeds with QD treated seeds. (All the images were taken using Pentax Optio W80 digital camera)

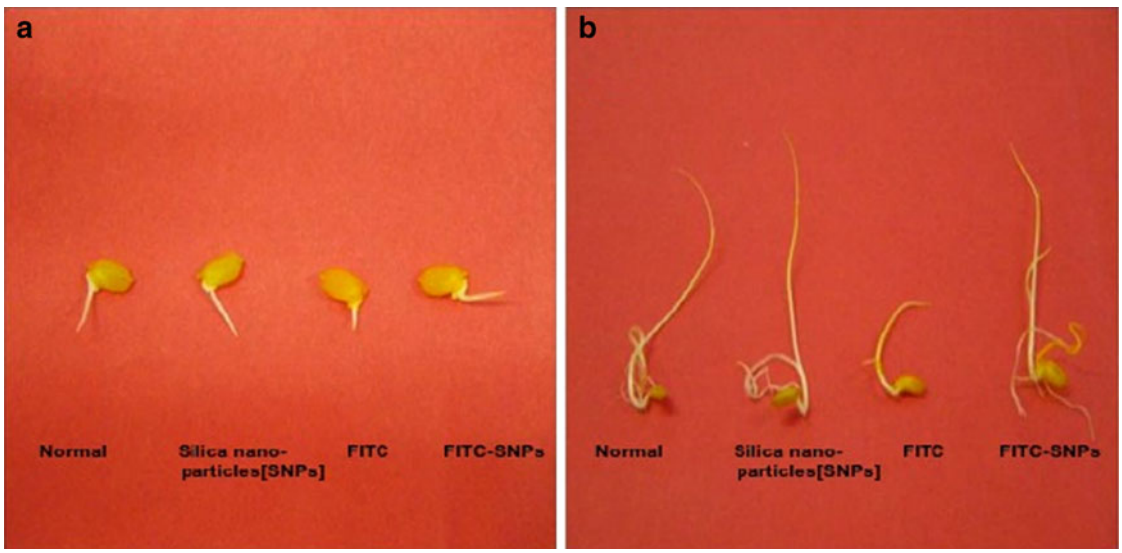

Fig. 6 Images showing the effects of Silica nanoparticles (SNPs), Fluorescein isothiocyanate (FITC) and FITC-SNPs on the germination of rice seeds. **a** Image of seeds showing germination in the presence of nanoparticles in which the germination is highly comparable with the normally germinated seed. **b** Image showing further growth of rice seedlings treated with SNPs, FITC and FITC-SNPs in comparison to normally seedling

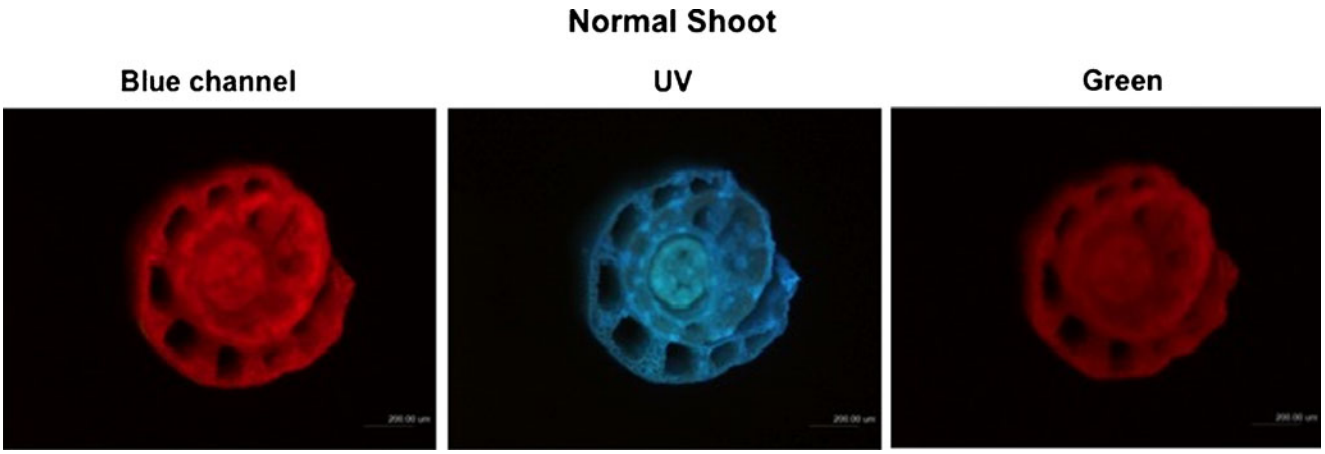

Fig. 7 Fluorescent microscopic image of the cross section (C.S.) of 4 day old normal rice seedling shoot on excitation with light of different wavelength. Scale bar: 200 micrometers

been highly appreciable and widely reported, the major concern is the toxicological effect of QDs with plants which has been mentioned above in the germination of rice seeds with QDs. Hence more studies are needed to optimize safe and effective concentration of QDs recommended for various applications in plant biology.

In this context we studied the uptake ability of FITC-silica nanoparticles, which are highly biocompatible for *in*

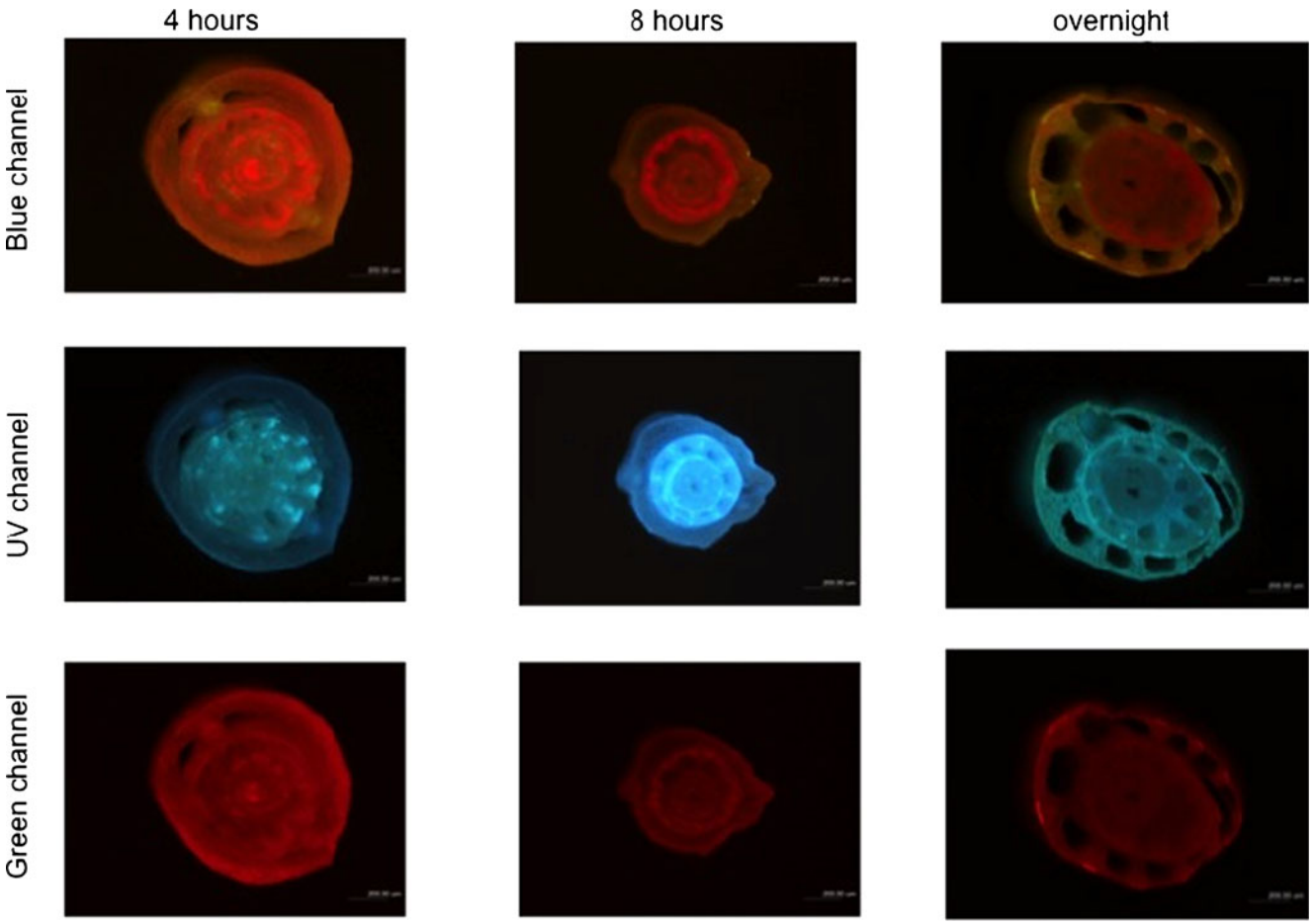

Fig. 8 Fluorescent microscopic images of C.S. of rice seedling shoot treated with quantum dots showing the uptake of quantum dots (QDs). The *green color* in the shoot on excitation with *blue light* is due to the presence of QDs. Scale bar: 200 micrometers

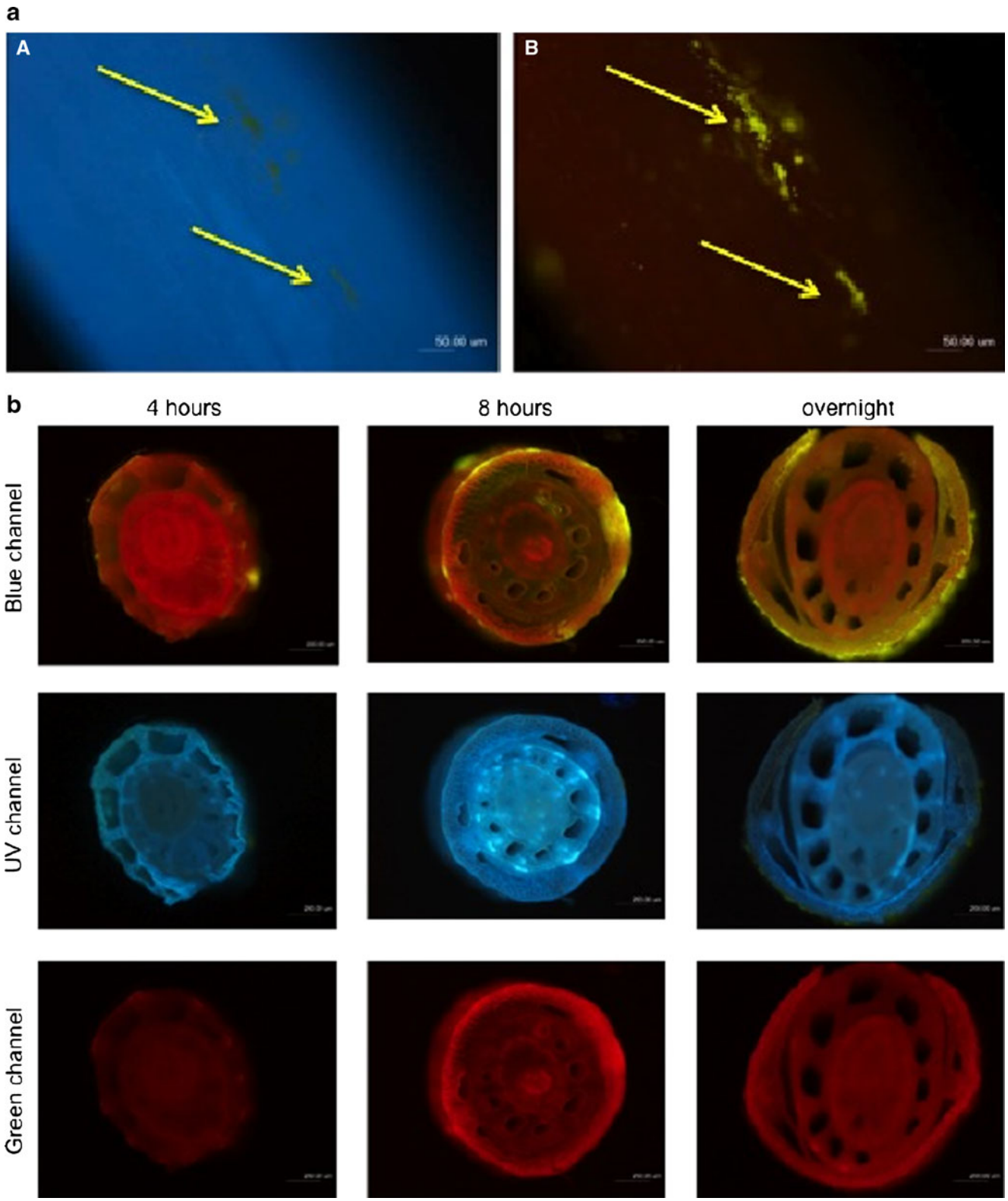

Fig. 9 **a** Fluorescent microscopic images of the longitudinal section of rice seedling shoot showing the presence of FITC-Silica nanoparticles (FITC-SNPs). **a** (A and B) is the longitudinal section of rice seedling shoot on excitation with UV light and blue light respectively. The *green color* in both the images shows the presence of FITC-SNPs. Scale bar: 50 micrometers. **b** Fluorescent microscopic images of the cross section of rice seedling shoot treated with FITC-SNPs on excitation with blue light, UV light and green light. The *green color* of seedling sections in the blue channel shows the presence of FITC-SNPs which confirmed its uptake by rice seedlings. Scale bar: 200 micrometers

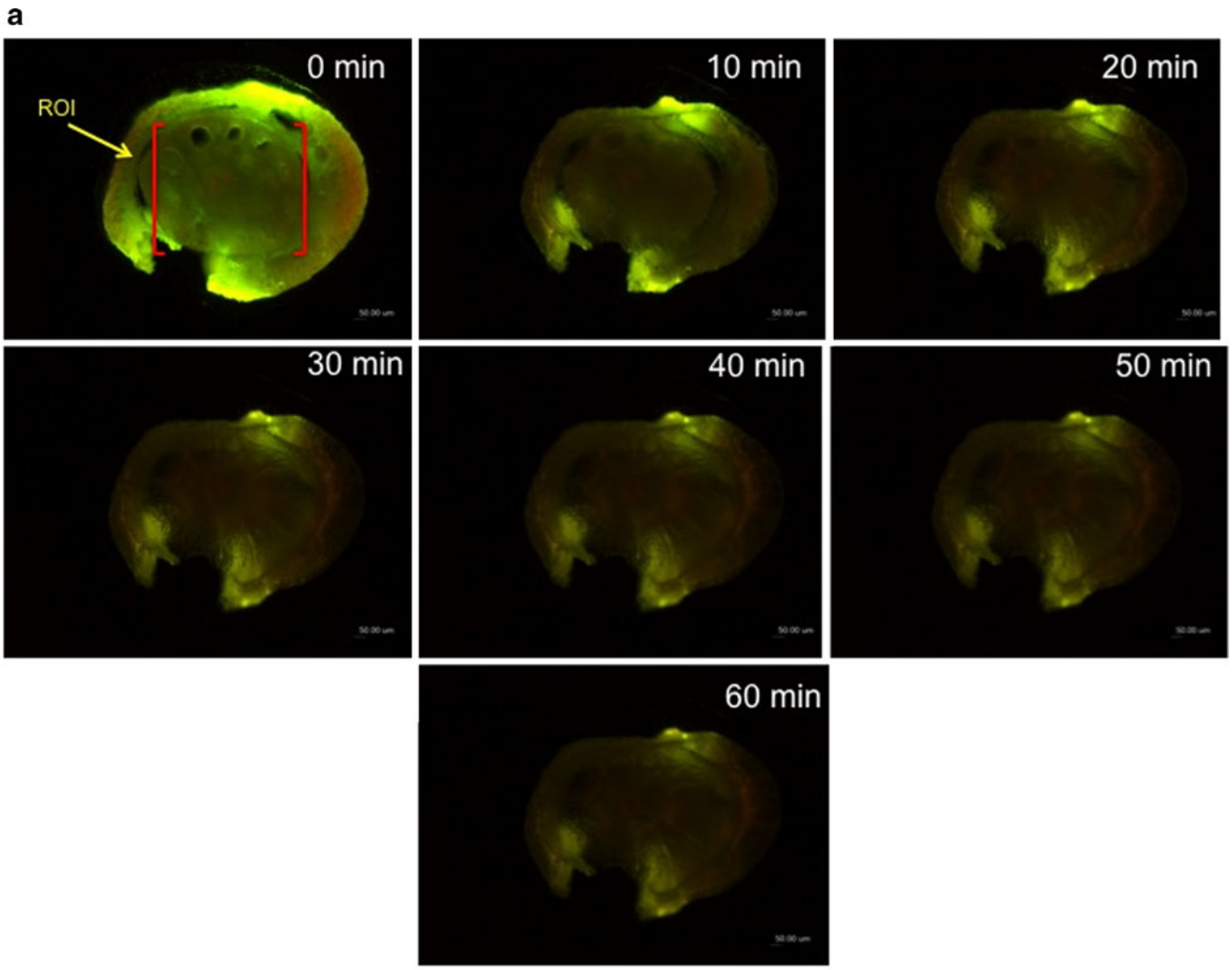

Fig. 10 **a** Fluorescent microscopy images showing the photobleaching of FITC in cross section of treated rice shoot over time (*ROI* region of interest) (Scale bar: 50 μm). **b** A] Fluorescence intensity graph of FITC treated seedling at 0 min of photobleaching. B] Fluorescence intensity graph of FITC treated seedling at 1 h of photobleaching. Red line denotes Shoot, Green line denotes FITC and blue line denotes Reference

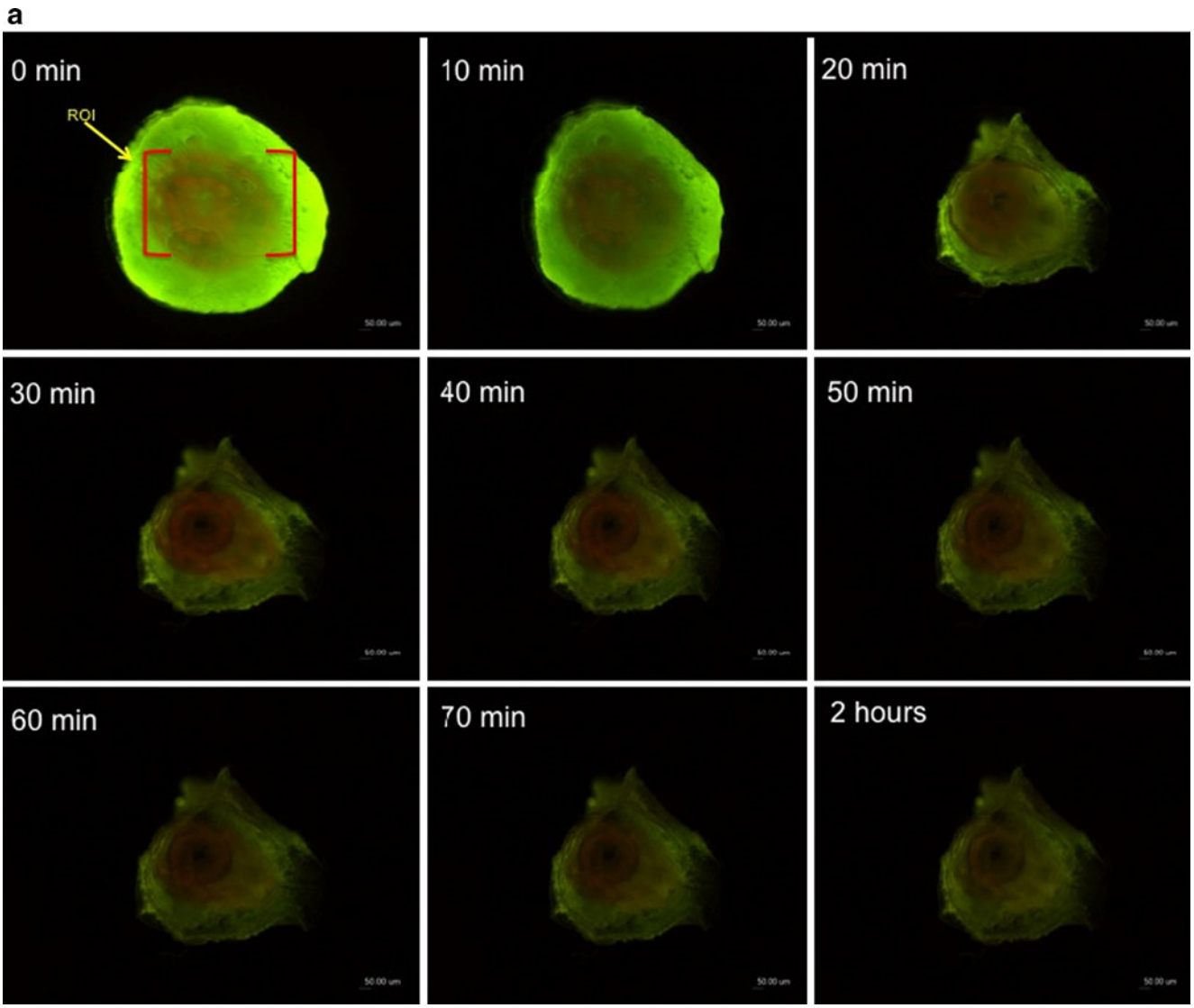

Fig. 11 **a** Fluorescence microscopic images of FITC-SNPs (Fluorescent silica nanoparticles)-treated rice seedlings showing the photostability of FITC-SNPs over time. Scale bar: 50 micrometers **b** Fluorescence intensity of FITC-SNPs in treated rice seedling shoot A] 0 hr photobleaching B] 1 hour photobleaching C] 2 h photobleaching. Red line denotes Shoot, Green line denotes FITC-SNPs and blue line denotes Reference

vivo imaging in plants. Moreover our investigation proved that both SNPs and FITC-SNPs did not pose any toxicological effects on rice seedlings (as shown in Fig. 6). We observed an upward translocation of fluorescent silica nanoparticles from the solution in which the seedlings were dipped to the shoot system. Figure 9 (a and b) shows fluorescent microscopic images of the longitudinal section of the shoot of treated seedlings showing the presence of fluorescent SNPs and the cross section of treated seedlings at different treatment time; 4 h, 8 h and overnight dipping respectively. The green fluorescence observed on excitation with blue light on the sample confirmed the uptake of fluorescent SNPs by the seedlings.

For checking the photostability of fluorescent silica nanoparticles the cross section of treated seedlings was subjected to photobleaching. For comparison the seedlings dipped in FITC solution alone were also cross-sectioned for photobleaching experiments. Figure 10 shows the photostability of seedlings treated with FITC alone. Figure 10a shows the fluorescent microscopic images of shoot cross section exposed to continuous blue light and the images captured at regular interval of 10 min for I hour. The intensity of fluorescence was analyzed using WinROOF Ver 6.3.1 Analysis Software. Fluorescent images showed that by a period of 1 h complete bleaching out has occurred in the selected region of interest (ROI). Severe reduction in fluorescence over time was analyzed with the help of analysis software as shown in Fig. 10b. More than 80% reduction in fluorescent intensity has been occurred within 1 h of photobleaching. However we found that by

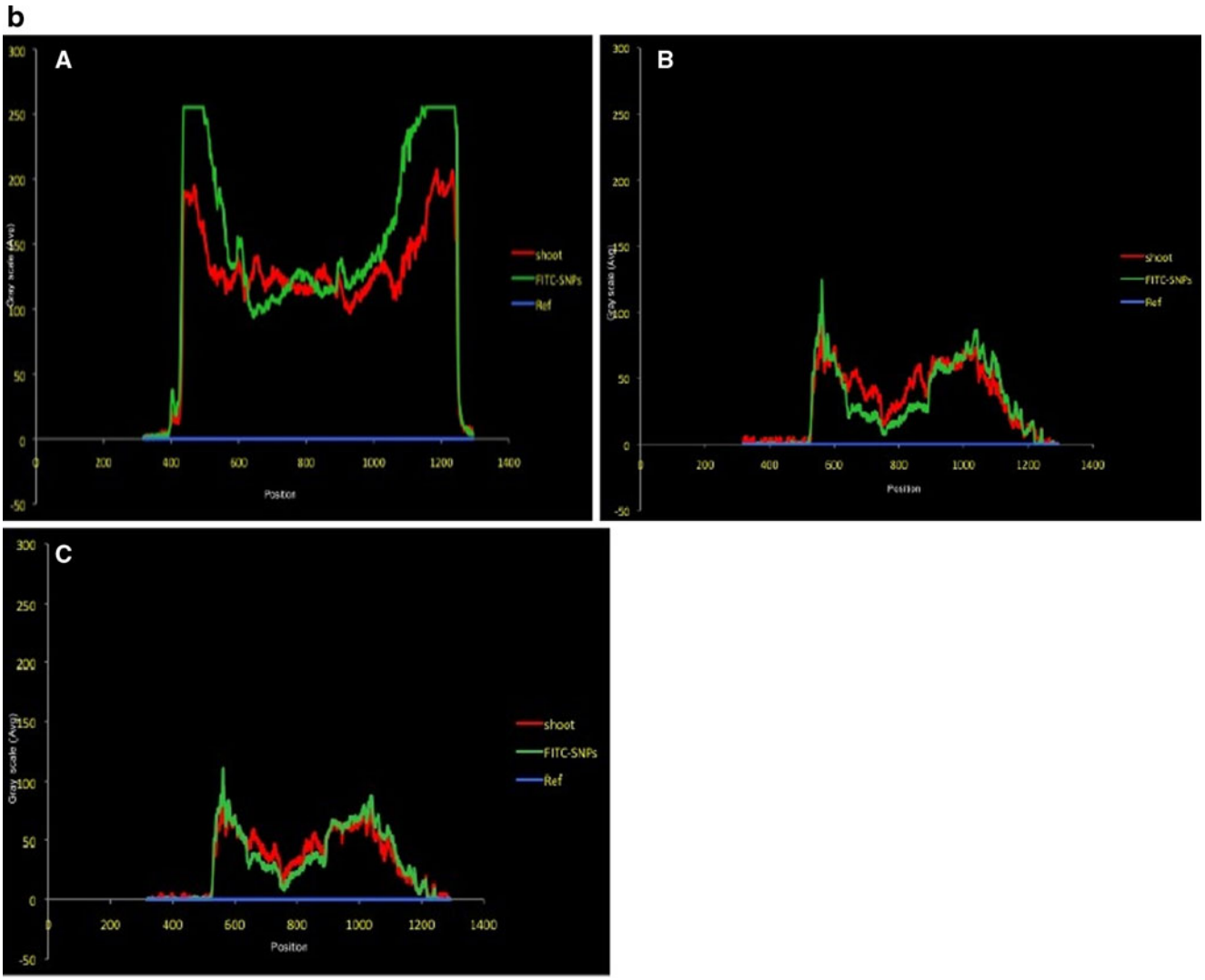

Fig. 11 (continued)

entrapping FITC molecules in silica nanoparticles we could extend its photostability. Figure 11a shows the fluorescent images of the cross section of FITC-SNPs treated seedlings showing the fluorescent images at regular interval of 10 min. The photostability of FITC-SNPs in the treated seedlings was found to be higher in comparison to FITC alone treated seedlings. This is due to the binding of FITC molecules to silica nanoparticles that reduces the dye leaking and photobleaching effects. The intensity of fluorescence in ROI was analyzed with the help of WinROOF analysis software as shown in Fig. 11b. It was noticed that 50% fluorescent intensity of FITC-SNPs could be maintained for 1 h continuous exposure to photobleaching and this intensity has almost been maintained even upto 2 h on continuous exposure to photobleaching process and this showed the photostability of these fluorescent nanoparticles in plant system.

Conclusion

Nowadays using fluorescent nanoparticles as biolabels in plants has been considered as a novel approach gaining increased demand. However greater concern is the phytotoxicity of these nanomaterials to plant system, which limits their wide scale applications. We studied the germination effects of two fluorescent nanoparticles, FITC-SNPs and QDs on rice seeds as a model phytotoxicity test. Good germination of seeds have been observed in the presence of FITC-SNPs which is highly comparable with normal germination of seeds whereas the presence of quantum dots posed some toxicological effects on rice seed germination. Effective transport of both fluorescent nanomaterials has been observed in rice seedlings through their root. However more studies are needed regarding quantum dots for optimizing their concentration at a safe level for the plants. Since FITC-SNPs have proved to be safe for plants and show

good fluorescence in rice seedlings, their use for bioimaging in plants tissues is highly recommendable. We have checked the photostability of fluorescent silica nanoparticles in the seedlings and our results proved good photostability of silica nanoparticles with less photobleaching over extended period of time. Hence our studies call for more research with different plant species for the successful wider application of fluorescent nanomaterials for live imaging of plants.

Acknowledgement Remya Nair and Aby C. Poulose thank the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan for the financial support given as Monbukagakusho fellowship

References

1. Lin Y-S, Tsai C-P, Huang H-Y, Kuo C-T, Hung Y, Huang D-M, Chen Y-C, Mou C-Y (2005) Well ordered mesoporous silica nanoparticles as cell markers. Chem Mater 17:4570–4573
2. Santra S, Yang H, Dutta D, Stanley JT, Holloway PH, Tan W, Moudgil BM, Mericle RA (2004) TAT conjugated, FITC doped silica nanoparticles for bioimaging applications. Chem Commun 24:2810–2811
3. Hoecke KV, De Schamphelaere KAC, der Meeren PV, Lucas S, Janssen CR (2008) Ecotoxicity of silica nanoparticles to the green algae *Pesudokirchneriella subcapitata*: Importance of surface area. Environ Toxicol Chem 27:1948–1957
4. Wei C, Zhang Y, Guo J, Han B, Yang X, Yuan J (2010) Effects of silica nanoparticles on growth and photosynthetic pigment contents of *Scenedesmus obliquus*. J Environ Sci 22:155–160
5. Lin B-S, Diao S-Q, Li C-H, Fang L-J, Qiao S-C, Min Y (2004) Effect of TMS (nanostructured silicon dioxide) on growth of Changbai larch seedlings. J For Res 15:138–140
6. Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Sakthi Kumar D (2010) Nanoparticulate material delivery to plants. Plant Sci 179:154–163
7. Torney F, Trewyn BG, Lin VS-Y, Wang K (2007) Mesoporous silica nanoparticles deliver DNA and chemicals into plants. Nat Nanotech 2:295–300
8. Hischemoller A, Nordmann J, Ptacek P, Mummenhoff K, Haase M (2009) In-vivo imaging of the uptake of upconversion nanoparticles by plant roots. J Biomed Nanotech 5:278–284
9. Bailey RE, Smith AM, Shuming N (2004) Quantum dots in biology and medicine. Phys E 25:1–12
10. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, Li JJ, Sndaresan G, Wu AM, Gambhir SS, Weiss S (2005) Quantum dots for live cells, in vivo imaging, and diagnostics. Science 307:538–544
11. Lidke DS, Nagy P, Heintzmann R, Arndt-Jovin DJ, Post JN, Grecco HE, Jares-Erijman EA, Jovin TM (2004) Quantum dot ligands provide new insights into erb/HER receptor-mediated signal transduction. Nat Biotechnol 22:198–203
12. Guo G, Liu W, Liang J, He Z, Xu H, Yang X (2007) Proing the cytotoxicity of CdSe quantum dots with surface modification. Mater Lett 61:1641–1644
13. Gagne F, Auclair J, Turcotte P, Fournier M, Gagnon C, Sauve S, Blaise C (2008) Ecotoxicity of CdTe quantum dots to freshwater mussels: impacts on immune system, oxidative stress and genotoxicity. Aquat Toxicol 86:333–340
14. Lin S, Bhattacharya P, Rajapakse NC, Brune DE, Ke PC (2009) Efffects of quantum dots adsorption on algal photosynthesis. J Phys Chem C 113:10962–10966
15. Etxeberria E, Gonzalez P, Baroja-Fernandez E, Romero JP (2006) Fluid phase endocytic uptake of artificial nano-spheres and fluorescent quantum dots by sycamore cultured cells. Plant Signaling Behav 1:196–200
16. Ravindran S, Kim S, Martin R, Lord EM, Ozkan CS (2005) Quantum dots as biolabels for the localization of a small plant adhesion protein. Nanotechnology 16:1–4
17. Muller F, Houben A, Barker PE, Xiao Y, Kas JA, Melzer M (2006) Quantum dots-a versatile tool in plant science. J Nanobiotechnol 4:5–10
18. Eggenberger K, Frey N, Zienicke B, Siebenbrock J, Schunck T, Fischer R, Brase S, Birtalan E, Nann T, Nick P (2010) Use of nanoparticles to study and manipulate plant cells. Adv Eng Mater 12:B406–B412
19. Hu Y, Li J, Ma L, Peng Q, Feng W, Zhang L, He S, Yang F, Huang J, Li L (2010) High efficiency transport of quantum dots into plant roots with the aid of silwet L-77. Plant Physiol Biochem 48:703–709
20. Boatman EM, Lisensky GC (2005) A safer, easier, faster synthesis for CdSe quantum dot nanocrystals. J Chem Educ 11:1697–1699
21. Xie R, Kolb U, Li J, Basche T, Mews A (2005) Synthesis and characterization of highly luminescent CdSe-Core CdS/$Zn_{0.5}Cd_{0.5}S$/ZnS multishell nanocrystals. J Am Chem Soc 127:7480–7488
22. Chan WCW, Nie SM (1998) Quantum dot bioconjugates for ultrasensitive nonisotopic detection. Science 281:2016–2018
23. Han Y, Jiang J, Lee SS, Ying JY (2008) Reverse microemulsion mediated synthesis of silica coated gold and silver nanoparticles. Langmuir 24:5842–5848
24. Zhao L, Zhao Y, Han Y (2010) Pore fabrication in various silica-based nanoparticles by controlled etching. Langmuir 26:11784–11789
25. Liu Z, Jiao Y, Wang Y, Zhou C, Zhang Z (2008) Polysaccharides-based nanoparticles as drug delivery systems. Adv Drug Deliv Rev 60:1650–1662