Pluronic F68 - capped SiO_2 nanoparticles are compatible with the wheat rhizosphere

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Abstract

Pluronic F68 was not phytotoxic to wheat (*Triticum aestivum*); a root-applied dose at 14 d enhanced shoot mass and length for 36 d - harvested plants. Root applications of fluorescein - labelled F68 enhanced fluorescence of nuclei in epidermal cells in the root's elongation zone and root hairs. SiO_2 NPs are other possible delivery vehicles for plant beneficial compounds. Fluorescent aggregates from rhodamine - SiO_2 NPs formed on root but not shoot surfaces. These core NPs, when capped with silica containing fluorescein labelled F68, showed emissions under Fourier Resonance Energy Transfer spectral analyses from rhodamine and fluorescein. Their application to roots caused trichomes on shoots to fluoresce from both dyes suggesting root to shoot NP-transfer. Fluorescent aggregates also occur in patches on the surfaces of sand grains present in the rhizosheath. These results suggest that root applications of composite NPs could act as delivery vehicles to boost plant growth.

Key words: F68, SiO_2 NPs, wheat, fluorescence

Impact Statement

Plant productivity must be increased for food security as the world's population rises. Novel strategies to deliver compounds beneficial for plant production include nanoparticles (NPs) as delivery vehicles. This paper addresses plant responses to composite NPs formulated with Pluronic F68 and SiO2 NPs. Already established is the beneficial value of the SiO2 NPs as a source of Si and positive effects on plant growth of F68, as confirmed in this paper for wheat. Such composites also could carry other plant beneficial products. The composites were derived by deposition of new surface layers of silica containing F68 to be deposited around SiO2 cores. The composite NPs became associated with wheat root surfaces as well as sand grains associated with the roots. The finding that trichomes in the shoots of wheat exposed through the roots to fluorescently labelled F68 – SiO2 NPs become enhanced in fluorescence suggest uptake and transfer through the vasculature can occur of intact NPs. These findings indicate that the NPs become located in the plant at sites appropriate for delivery of beneficial products to improve plant vigor.

Introduction

Agriculture is facing challenges in provision of foods for the increasing global population. Supply of plant boosting materials, such as growth promoters, protectants against stress, pesticides and nutrients in nano-sized particles is actively pursued. The triblock copolymer, Pluronic F68 (F68), could be valuable] because it stabilizes nanoparticles (NPs) [1] and is regarded as safe (GRAS) by the FDA [2]. Pluronic F68 has low toxicity to plants [3] and aquatic life [4], important because of likely transportation from soil applications in run off. At field applied concentrations (0.01-1% w/v), positive effects from F68 include stabilizing membrane structure [5,6], protecting rice against freezing temperatures [7,8], increasing growth in citrus [9,10] enhancing plant regeneration [11,12], as well as heightening intake and transfer of oxygen to the plant [13,14]. In this paper we first examined phytotoxicity in wheat (*Triticum aestivum*). Applications with SiO_2 NPs were pursued because these NPs are beneficial to plants [15,16]. Nanoparticles labelled with rhodamine in the SiO2 core and with fluorescein conjugated to F68 within newly deposited silica capping layers were generated to follow the fate of the NPs in wheat tissues and on sand particles in the growth matrix.

Materials and Methods

Generation and characterization of novel capped NPs with fluorescently labelled SiO_2 NPs and F68

Solid fumed silica SiO_2 NPs, Aerosil 200 NPs (Evonik Industries) were labelled with rhodamine B (rho) by soaking in aqueous 10^{-6} M rhodamine for 24 h following methods by Hartlen et al. [17]. The labelled rho – SiO_2 particles retained from filtering through 0.45 μm Whatman filter paper were rinsed with double distilled water (dd H_2O) until the filtrate had no rhodamine fluorescence. The rho - SiO_2 NPs were stored in the dark as a dry powder. F68 was labelled with fluorescein by the procedures of Rapoport et al. [18] using carboxy-2'7'-dichlorofluorescein (CDC-fluorescein. The reaction mixture was dialyzed (Spectra/Por ® 1 Dialysis Membrane, MWCO: 6-8 kD, Spectrum Labs) against extensive changes of dd H_2O and the fluorescein F68 (fF68) solution was stored protected from light. Passage of a suspension of fF68 micelles through a PD-10 column; showed all fluorescein in the effluent to be associated with the F68 micelles.

Capping of rho - SiO_2 NPs with new silica layers, with or without F68, followed procedures of Wang et al. replacing F127 with F68 [19]. In brief, rho - SiO_2 NPs (80 mg) suspended in 4 mL dd H_2O were mixed with L-arginine (18 mg) dissolved in 10 mL dd H_2O. Some particles were only coated with extra silica, termed srho-SiO_2 NPs. For coating with F68, F68 (146 mg) containing 10% m/m fF68 was added to generate fF68-rho - SiO_2 NPs. Mixtures were heated to 60 °C and 0.9 mL tetraethyl orthosilicate (TEOS) added with stirring to cap the seed particles for 24 h. After cooling to room temperature, particles were pelleted by centrifugation for 45 min at 20,000 g before their suspension in dd H_2O. This process was repeated twice more until there was no fluorescence of the supernatants as measured using a Synergy 4 Fluorometer (BioTek Instruments). The NPs were characterized by FRET analysis using the same instrument. The stock suspensions of capped SiO_2 NPs (~20.78 mg/mL SiO_2) were stored in the dark at room temperature.

Zeta potential analysis was performed with a calibrated Brookhaven Instrument Zeta Plus for suspensions of the novel NPs in dd H_2O. Hydrodynamic radii were measured using a DynaPro Nanostar dynamic light scattering (DLS) instrument (Wyatt Instruments) with bovine serum albumen as the standard. Capped and uncapped NPs were imaged using a Nanoscope III Bioscope (Digital Instruments, Inc.) atomic force microscope (AFM) in tapping mode using a TAP300AL-G tip (Budget Sensors).

Fluorescence was observed with a Nikon microscope fitted with relevant filters for excitation at 484 nm (CDC-fluorescein) and 560-580 nm (rhodamine B)

Plant studies

Wheat was grown in sterilized sand in a growth chamber using the procedure of Potter et al. [20]. Five nonsterilized wheat seeds, cultivar Juniper from a 2020 harvest, were planted per pot and three replicate pots per treatment were prepared for three separate trials. Water was added to maintain wetted pot mass of 135 g above pot dry mass. Pots had ½ strength Hoagland's nutrient solution added at 7, 14, 21 and 28 d to supply complete mineral nutrition. At 14 d treatments of F68 (0.4 mg /pot) or a natural osmolyte, glycine betaine (GB, 90 mg/pot), were added dissolved in the nutrient solution. The control plants only received Hoagland's solution. At 36 d, shoot lengths were measured from the top of the coleoptile to the tip and excised at this point to measure fresh mass. Tissue was oven-dried at 60 °C for 48 h. Data was analyzed statistically using "SAS on Demand for Academics for MS Windows" [21].

The presence of fF68, srho-SiO_2 NPs or fF68-rho-SiO_2 NPs in tissues and sand grains used younger plants from wheat grown in wetted sand with these amendments for 7 d. Control plants were raised without any additions. Fluorescent microscopy of sand grains associated with the harvested root, or the surfaces of roots and shoots was conducted.

Results and Discussion

F68 is not phytotoxic but increases shoot fresh mass growth in young wheat seedlings

Application of F68 at 14 d to wheat growing in a sand matrix confirmed the low phytotoxicity of F68. The F68 increased the fresh mass (Fig. 1 A) and lengths (Fig. 1 B) of shoots for 36 d-old watered plants by 9-10%. Increases in shoot mass and length was not observed with GB applications although GB, but not the Pluronic, increased shoot water content (Fig. 1 C). These finding suggest that growth improvement with F68 involved mechanisms independent of cell water content.

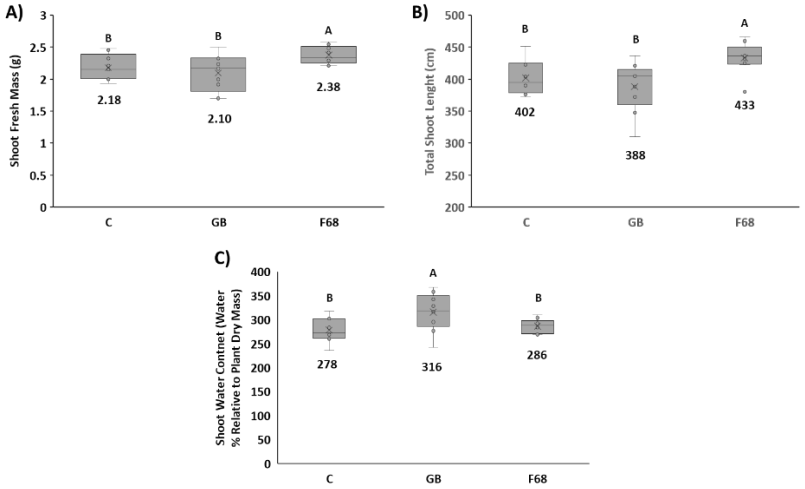


Figure 1. Effects on wheat of adding one dose at 14 d of F68 or glycine betaine compared with no additions, control (C), for plants harvested at 36 d. Data are means of three independent studies, each with three replicates/treatment where each replicate is a pot containing five wheat seeds. The letters above each data box show statistical variance at $p = 0.05$ for treatments versus control.

Characterization of F68 - coated SiO_2 NPs

Coating of the SiO_2 NPs with silica, with or without fF68, altered charge, shape and size (Table 1, Fig. 2). Hydrodynamic radii increased for srho-SiO_2 NPs from 12 to 27 nm with retention of spherical shapes (Fig 2). With fF68 in the new silica layers diameter increased, and particles were deformed spheres and showed more aggregation (Fig. 2a versus 2b). The surface potential was less negative when for NPs capped with fF68.

FRET analyses (Fig. 2c) for fF68-rho SiO_2 NPs showed the emission maxima of fluorescein (max 530 nm) from F68 and rhodamine B (max 578 nm) from the core NPs. Electron donation from f-F68 enmeshed in capping layers, increased intensity of rhodamine emission, 35,000 relative fluorescence units (RFUs) to 90,000 (Fig. 2 c). This signal enhancement did not occur with mixes of free dyes in solution. These analyses confirmed that seed SiO_2 NPs could be capped with fresh silica layers containing fF68 while allowing the rhodamine B label to remain detectable.

Table 1. Size and surface potential of seed SiO_2 NPs, srho - SiO_2 NPs, and fF68 – rho - SiO_2 NPs'.

NP Type	Size (nm)	Zeta Potential (mV)
Seed SiO_2	12.0 ±2	-47.5
srho - SiO_2	27.0±5	-45.9
fF680 - rho- SiO_2	55.6±17	-32.1

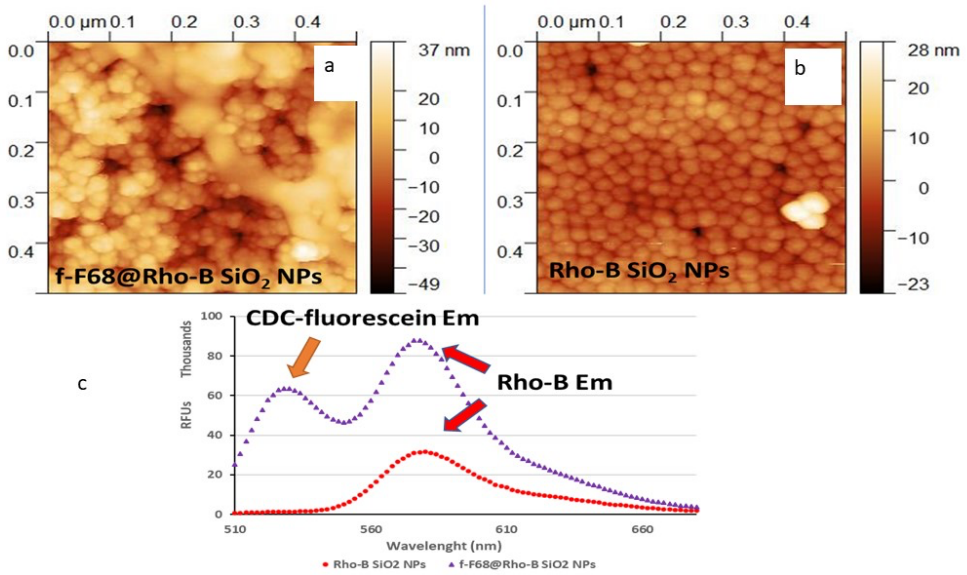
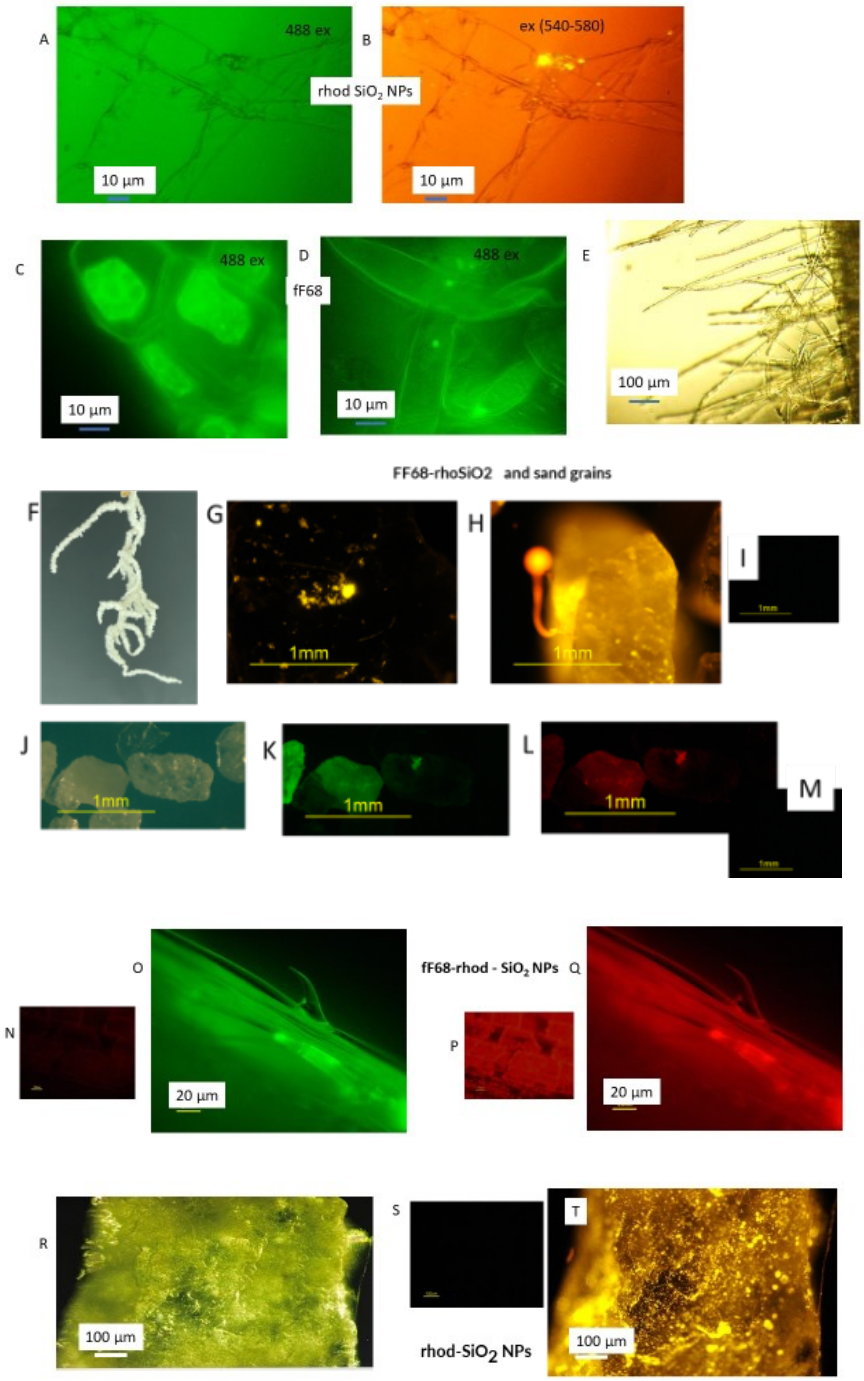


Figure 2. AFM images of a) fF68 - rho-SiO_2 NPs versus b) srho - SiO_2 NPs. The bars provided indicate particle height (nm). Image (c) shows the FRET spectra from fF68 -rho-SiO_2 NPs versus the silica capped srho - SiO_2 NPs.

Interaction of F68 and SiO_2 NPs in wheat and the rhizosphere

The interactions of fF68, with and without SiO_2 NPs, with plant tissues were explored by fluorescence microscopy of 7 d-old wheat (Fig. 3). The root hairs formed in a zone above the root cap, are important in root absorption. Trapping of srho - SiO_2 NPs by root hairs(Fig. 3 A and B) occurred. Sorption of free fF68 enhanced fluorescence in nuclei of root epidermal cells (Fig. 3 C) and root hairs (Fig.3 D); Fig. 3 E is the bright field image of root hairs of a control root. Fig. 3 F shows the wheat root enwrapped in particles of sand to form the structure called a rhizosheath; root hairs and root- and microbiome - secreted products in the rhizosphere allow rhizosheath formation. Figs. 3 G through M illustrate that fF68-rho - SiO_2 NPs were deposited on rhizosheath sand grain surfaces. Fig. 3 H demonstrated that the fF68- rho - SiO_2 NPs bound and became internalized by fungi colonizing the sand grain. The inoculum likely was from the array of microbes, including fungi present on the seeds. Thus, microbial colonies could aid in trapping the labelled NPs. Images J-M confirmed patches of labelled NPs on sand grain surfaces with both fluorescein- (K) and for rhodamine- (L) fluorescences visible.

Images N-T compare fluorescence from surfaces of shoot and root for wheat grown with fF68-rho - SiO_2 NPs. Images N-O show enhanced fluorescence for both dyes in the leaf epidermal structure, the trichome. Trichomes labelling may indicate internalization and transportation of the NPs; trichomes are known sites for silicification. Shoot surface deposits of srho – SiO_2 NPs were not present in contrast to the many patches observed on the root surface when excited for rhodamine fluorescence shown in image Q. Image R shows a brightfield image of the roots surface.



Legend for Fig. 3

Images A and B of collapsed root hairs from 7 d-old wheat seedling roots grown with srho - SiO_2 NPs show agglomerated NPs as black masses with 488 nm excitation whereas excitation at 540-580 nm revealed bright rhodamine fluorescence. Images C and D are from plants exposed to soluble fF68 with enhanced fluorescence of large nuclei of root surface cells in the elongation zone as well as in the root hairs. E is a brightfield image of the root hairs.

Image F shows the rhizosheath formed on the wheat root and image G is from a rhizosheath - sand grain showing fluorescence expected from bound aggregates of fF68- rho – SiO_2 NPs. Image H showed fluorescence from a fungal colonist (mycelium and spore) on another sand grain. Image I is a rhizosheath sand grain from wheat grown without NPs viewed with rhodamine excitation. Images J, K and L are the same sample imaged under brightfield (J) and with excitation for fluorescein (K) and rhodamine (L) fluorescence. No fluorescence exists for rhizosheath sand grains grown without NPs.

Images N through T are surface views for shoots (N - O) and roots (P - T). Images N and P from control plants grown without NP lack the fluorescence seen when fF68-rho - SiO_2 NPs were present. These NPs resulted in fluorescence from both fluorescein (O) and rhodamine (Q) for a leaf surface trichome. No surface labelling was observed for the shoots (Q) in contrast to bright fluorescent sites on root surfaces, T, from growth with s-rho - SiO_2 NPs.

Conclusions

Pluronic F68 promoted wheat growth under watered conditions and this finding stimulated production of fluorescently - labelled SiO_2 NPs coated with F68. Capping with F68 in newly added silica layers increased NP diameter, reduced the negative surface charge and deformed the spherical shape. Use of the labelled SiO_2 NPs led to novel findings of interactions with wheat. Patches of labelled NPs with and without F68 were detected on the root surface, root hairs and sand grains associated with the surface in the rhizosheath. Because a fungal colonist on the sand grain was labelled, microbial colonization on root and sand surfaces may function in trapping NPs. Exposure of root to fF68 in solution revealed fluorescein accumulations for root epidermal cell nuclei including the root hairs. Transport through the xylem to the shoots may explain both rhodamine and fluorescein signals for trichomes on the leaf surface for plants grown with fF68-rho - SiO_2 NPs. These finding show that such NPs could function as delivery vehicles to plant tissues when supplied through the root.

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Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interests

The authors have no conflicts of interest.

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