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Short communication

Effects of the physical state of nanocarriers on their penetration into the root and upward transportation to the stem of soybean plants using confocal laser scanning microscopy

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ABSTRACT

We determined whether nanocarriers can penetrate into plant roots and be transported upward, from the root to stem, as well as studied the effect of the physical state of the lipid matrix of the nanocarriers on their penetration and transportation in plants. Firstly, solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and lipid-based nanoemulsions (NE) with similar characteristics (particle size, polydispersity index, and zeta potential) were successfully prepared by the combined method of hot homogenization and sonication, with beeswax as a solid lipid, corn oil as a liquid lipid, and Nile Red as a fluorescent active-ingredient. Penetration of nanocarriers into the roots and their transportation to the stem were visualized using confocal laser scanning microscopy. The images of vertical sections illustrated that NE penetrated into the root and was transported upward at a rate faster than did NLC and SLN, because of its relatively higher flexibility. While it took only 1 day for NE to penetrate into the center of the root and be transported upward to up to 4 cm of the stem, it took 3 and 6 days, respectively, for NLC and SLN to achieve the same. This study provides an important basic background required to generate a new generation of pesticide formulations, where pesticides will be encapsulated in nanocarriers, which in turn will be embedded into a patch that will be stuck on the root or stem. This would minimize pesticide loss, resulting in higher commercial profit and better environmental protection.

1. Introduction

Pesticides play an important role in agriculture, as they help prevent crop loss caused by insect pests (Pimentel, 1995; Gilden et al., 2010). However, less than 0.1% of the applied pesticides actually reach the target pests (Pimentel, 1995; Wang and Liu, 2007), the remainder released to the surrounding environment, causing toxicity in the ecosystem including humans (Kromer et al., 2004; Covaci, 2006; Arias-Estévez et al., 2008). Root and stem-eating pests are the most difficult targets that need to be controlled, because the larvae of these pests enter the plant and bore through the plant from the inside. Moreover, the conventional methods for the management of root and stem-eating pests, by applying pesticides externally (such as by the spraying method), not only cause phytotoxicity and death of beneficial insects (for example: honey bees) when applied at a high dose but also show very low effectiveness in controlling the target pest. This is because such pesticides cannot efficiently penetrate through the cuticle (in the case of hydrophilic pesticides) or epidermis, cortex, and endodermis (in the case of hydrophobic pesticides) (Connel and Miller, 1984; Gordh, 2011; Tapparo et al., 2011). Therefore, a novel

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strategy for the management of root- and stem-eating pests is necessary.

Nanocarriers, with their small size (10–1000 nm), high surface area to volume ratio, high payload of pesticides, high photoprotection capacity, and controllable release of encapsulated pesticides (Bang et al., 2009; Mishra et al., 2010; Nguyen et al., 2012, 2013), have been used to form safer pesticide formulations and maintain the desired target effects for a long time. As a result, they help to avoid repetitive pesticide applications or the need for higher doses, as well as reduce the risk of water contamination (Frederiksen et al., 2003; Nguyen et al., 2013). However, thus far, there have been no studies related to the application of pesticide nanocarriers to protect plants from root- and stem-eating pests. In particular, the effect of the physical state of the lipid matrix of nanocarriers on their penetration through the roots and further transportation through the stem has not been studied.

With the aim to confirm whether nanocarriers (solid lipid nanoparticles [SLN], nanostructured lipid carriers [NLC], and lipid-based nanoemulsions [NE]) can penetrate into the root and subsequently transport to the stem, as well as to study the effects of the physical state of the lipid matrix of the nanocarriers on the penetration and transportation rate, NE, NLC, and SLN with almost similar characteristics (particle size, polydispersity index [PDI], and zeta potential) were prepared by the combined method of hot homogenization and sonication (Nguyen et al., 2012). The penetration and transportation of the nanocarriers in soybean plants were evaluated and compared using confocal laser scanning microscopy.

2. Materials and methods

2.1. Materials

Soybean seeds (*Glycine max* (L.) Merrill) were purchased from a local market and planted at the Laboratory of Biopolymers Engineering, Korea University (Seoul, Korea).

Lecithin from soybean (Junsei Chemical, Tokyo, Japan) and Tween-80 (Samchun Pure Chemical, Seoul, Korea) were used as surfactants. Beeswax and corn oil (Sigma-Aldrich, St. Louis, MO, USA) were used as solid lipid and liquid lipid, respectively. Nile Red (Sigma-Aldrich, St. Louis, MO, USA) was used as a fluorescent active-ingredient. All other chemicals were of analytical grade.

2.2. Preparation of nanocarriers

The nanocarriers (SLN, NLC, and NE) were prepared using a combined method of hot homogenization and sonication (Nguyen et al., 2012). Briefly, lipids (3%, w/w) (beeswax, or corn oil or a mixture of beeswax and corn oil [60:40, w/w]) and Nile Red (0.05%, w/w) were heated to 85 °C, followed by mild stirring to dissolve the Nile Red. At the same time, a mixture of soybean lecithin and Tween-80 (1.5%, w/w) (in 1:1 ratio, w/w) was added to distilled water and stirred at 85 °C for 45 min. The surfactant mixture was poured into the melted lipid containing Nile red. The resulting solution was homogenized using an Ultra-Turrax® T 25 basic homogenizer (IKA®-Werke, Staufen, Germany) at 19,000 rpm for 5 min followed by sonication using a VCX 750 ultrasonic processor (Sonics & Materials, Newtown, CT, USA) at 20 W for 8 min. The resulting nanoemulsion was dispersed in 4 °C cooled water at a ratio of 1:2 (v/v) with stirring at 500 rpm to form the nanocarriers (NE, NLC and SLN).

2.3. Measurement of particle size and zeta potential

Mean size, PDI, and zeta potential of the nanocarriers were determined using a nanoparticle size analyzer (Malvern Zeta sizer, Nano Z-S; Malvern Instruments, Malvern, UK). Measurements were carried out at 25 °C with a detector angle of 90°.

2.4. Plant material and growth conditions

Firstly, soybean seeds were sterilized in 3% sodium hypochlorite for 3 min and rinsed 5 times with distilled water. The sterilized seeds were subsequently placed on sterile moist cotton in a sterile Petri dish and incubated at 26 °C under 16 h light/8 h dark cycle for germination using Multi-cooling incubator (Jisico, Seoul, South Korea). After 5 days of incubation, soybean seedlings of uniform size at the two-leaf stage (not embryonic leaves) were selected and transferred to Murashige and Skoog (MS) broth and allowed to continue growing in a multi-cooling incubator. After 9 days, soybean young-plants (grown from seedlings) of similar size were selected for further experiment.

2.5. Penetration and transportation of nanocarriers in soybean plants

Equal volumes of the nanocarriers were added to 2× MS broth to form 1× MS broth containing nanocarriers. The selected soybean young-plants were placed in the above medium (only the root was dipped into the medium; the stem was not in contact with the medium) and incubated in the multi-cooling incubator under the same conditions as those described above for 1, 3, and 6 days. At defined time intervals, the young-plants were withdrawn and washed carefully with distilled water. Immediately after washing, the root and stem at a distance of 4 cm above the root–stem junction (approximately 4 cm above the level of the MS broth containing the nanocarriers) were cut and fixed in paraffin wax. Vertical 50-μm-thick sections of the root and stem samples were cut using a rotary microtome (model 5062; SLEE Medical GmbH, Mainz, Germany). Penetration of the nanocarriers containing Nile Red into the roots and their upward transportation into the stem were observed using confocal laser scanning microscopy (LSM 5 Exciter, Carl Zeiss. Jena, Germany). The root and stem sections were inspected under a 20× objective lens (EC Plan-NEOFLUAR 20×/0.5 M27) with the mode to remove plant autofluorescence (excitation wavelength was 543 nm [25% of laser power]; emission filter was LP 560). Confocal laser scanning microscopic images were recorded and further processed using LSM Image Browser software, ver. 4.2.0.121 (Carl Zeiss Microimaging, Gottingen, Germany).

3. Results and discussion

3.1. Preparation of NE and CH-NE

SLN, NLC, and NE were first emulsified at 19,000 rpm for 5 min to form pre-emulsions, followed by sonication for 8 min to reduce particle size. By adding the resulting pre-emulsions (after sonication) into 4 °C cooled water at a 1:2 (v/v) ratio, the respective nanocarriers were formed depending on the lipids used. Particularly, SLN and NE were formed when only beeswax and only corn oil, respectively, was used as the lipid. NLC was obtained when the

Table 1
Characteristics (particle size, PDI, and zeta potential) of NE, NLC and SLN formulations. Data are mean ± standard deviation (n = 3).

Nanocarriers	Mean particle size (nm)	PDI	Zeta potential (mV)
NE	158.5 ± 0.2	0.215 ± 0.002	−48.5 ± 0.6
NLC	167.8 ± 1.7	0.219 ± 0.011	−46.5 ± 0.2
SLN	168.2 ± 0.7	0.209 ± 0.002	−48.2 ± 0.7