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Enhancement of tomato resistance to *Tuta absoluta* using a new efficient mesoporous silica nanoparticle-mediated plant transient gene expression approach

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ABSTRACT

Due to their biodegradability and biocompatibility, mesoporous silica nanoparticles (MSNs) are employed as DNA nanocarriers in the following novel transient gene expression approach in tomato. The synthesized MSNs were analyzed in size, shape and charge, using FE-SEM, TEM, and Zetasizer, respectively. The tomato plants were then transformed with the MSNs containing pPZP122:35S:*GUS* (pDNA-MSNs) *via* three innovative methods including spray and injection the solution into the abaxial surface of leaves and injection into the shoot. The RT-PCR expression of *GUS* in combination with the histochemical GUS assay results show that pDNA-MSNs entered the cells and undergo the processes of transcription and translation. Subsequently, to investigate the efficiency of the newly developed methods, *cryIAb* gene through pPZP122:35S:*cryIAb*-MSNs was transferred into the tomatoes to control *Tuta absoluta.* The related molecular and bioassay analysis confirmed the transcription and translation of *cryIAb* in association with an enhanced resistance against *Tuta* in the tomatoes. Accordingly, for the first time, MSN-mediated gene transient transformation was successfully performed under *in vivo* condition, and the pDNA-MSN injection into the lower surface of leaves was recognized as the best approach. Since this system is biocompatible and saves time and energy consumption; it could be utilized as a new and more efficient technique in crop genetic engineering.

1. Introduction

During the past two decades, nanotechnology has been significantly employed in various applied sciences such as drug delivery, tissue engineering and environmental cleanup (Villaseñor and Ríos, 2018). This technology can be used across all scientific research subjects including food, medicine, electronics, energy, environment and agriculture. However, regarding this technology, just a few studies are available in plant biology research and, therefore, new approaches are required to let it move ahead. Recently, more attention is paid to the use of nanotechnology in agriculture, especially in smart pesticide delivery. Several researchers have investigated the effectiveness of nanoparticles on plant growth, germination, *etc.* For instance, Nair et al. (2011) evaluated the uptake and phytotoxicity effects of nonporous silica nanoparticles (~ 25 nm) on the seedlings of rice. They clearly showed that the concentration of up to 50 mg L^{-1} had no negative effect on seed germination. Moreover, among various nanomaterials, mesoporous silica nanoparticles (MSNs) have attracted more attention due to their biodegradability, biocompatibility, lack of toxic effects on plants and mammalian systems, and their role as a micronutrient involved in plant growth, regulation and stress.

The conventional transient expression systems including protoplast transformation (Sheen, 2001), particle bombardment (Leckie and Stewart, 2011) and agroinfiltration (Schweizer et al., 1999) have certain disadvantages, such as use of expensive pieces of equipments, transfer of unprotected DNA, and damage the target cells in particle bombardment method; hard protoplast isolation and regeneration in protoplast transfection; time-consuming and health risk associated with *Agrobacterium* in agroinfiltration technique. Therefore, transient MSN-dependent gene expression system is considered as an appropriate alternative approach for stable transformation methods in association with a rapid assessment of gene function (Takata and Eriksson, 2012). Torney et al. (2007) demonstrated that the gold-capped MSNs can deliver protein and DNA into the isolated tobacco mesophyll protoplast

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under *in vitro* condition. Chang et al. (2013) were also able to transfer the foreign gene into *Arabidopsis* intact root cells using MSNs (50 nm) under *in vitro* condition. In the previous studies, either plant protoplasts or roots have been used to transfer DNA-MSN under *in vitro* conditions (Chang et al., 2013; Sun et al., 2014; Torney et al., 2007). Those approaches require grand costs and special conditions due to isolation protoplast or sterile culture. The current research was conducted to deliver pDNA-MSN into deeper tissues of intact plants *via* stomata and shoot in *in vivo* conditions.

Pest attack is considered as one of the most important problems in agriculture, which results in the reduction of crop yield and quality. Tomato (*Solanum lycopersicum*), a dicot model for our new transient expression approach, is a favorite host for a large number of pests such as insects, fungi, bacteria and viruses. In recent years, *Tuta absoluta* has caused serve damages to products such as tomato, potato, colored peppers and eggplant (Salama et al., 2015). A significant damage to tomato crops has been reported in South America in the 1990s (Barrientos et al., 1998). In particular, the *T. absoluta* was first reported in Iran in 2009 and it was widespread in most of the regions in Iran and causes serve losses to tomato crops (50–100%) (Baniameri and Cheraghian, 2011). In the most studies, various pesticide chemical compounds have been used in order to control *T. absoluta* (Campos et al., 2014; Galdino et al., 2011; Pereira et al., 2014). Although using pesticide is a quick way to reduce pests, but it causes serious health side effects for human and the environment (Keifer et al., 1996). Therefore, employing novel technologies such as new transient expression systems, which result in significantly reduce utilization of pesticides could be very useful.

In this study, we have examined pDNA-MSN delivery into deeper tissue of intact plant *via* stomata and shoot under *in vivo* condition. The goals of this research were firstly to assess the new transient gene expression method by using the MSNs carrying pPZP122:35S:*GUS*, and secondly to evaluate the potential of employed pPZP122:35S:*cryIAb*-MSN to enhance the resistance of tomato to *T. absoluta* using the current developed transient gene expression approach. Our results indicate that the newly transient expression system can be used to transfer the foreign genes into plants, following transcription and translation in the target plant tissues. This simple and rapid approach includes some advantages such as more safety, saving costs, time and energy and using independent equipment over the other transient transformation approaches. Moreover, it could provide valuable information for plant genetic engineering for future related researches.

2. Materials and methods

2.1. Construction of pPZP122:35S:GUS and pPZP122:35S:cryIAb

In order to investigate the potential of functionalized MSNs for delivering pDNA into the tomato plants, *GUS* was used as a reporter gene. To provide a reporter construct, pPZP122 expression vector (Hajdukiewicz et al., 1994) was used due to its smaller size (8Kb), compared to pBI121 (14 kb) expression vector (Chen et al., 2013). pPZP122 (without *GUS* and *cryIAb* genes), pBI121 (containing *GUS* gene) and pCIB4421 (containing *cryIAb* gene) plasmids were first purified by plasmid extraction kit (Thermo Fisher Scientific, Lithuania) according to the manufacturer's instruction. In order to construct pPZP122:35S:*GUS* and pPZP122:35S:*cryIAb*, *Bam*HI and *Eco*RI DNA fragments carrying the *GUS* and *cryIAb* genes were cut-out from pBI121 and pCIB4421 (Koziel et al., 1993), respectively. The obtained fragments were cloned into pPZP122 separately at the *Bam*HI-*Eco*RI sites following transformation of *Escherichia coli* DH5α (Fig. 3A). For screening of colonies containing the desired insertions, several approaches including the colony PCR, vector digestion by *Bam*HI and *Eco*RI were applied (Thermo Fisher Scientific, Lithuania). The PCR program using *GUS* and *cryIAb* selective primers (Table A.1) was 5 min at 95 °C followed by 30 cycles of 1 min 95 °C, 1 min at the appropriate primer annealing temperature (Table A.1) and 1 min at 72 °C, with a final extension of 10 min at 72 °C. The confirmed plasmids containing desired genes (*GUS* and *cryIAb*, separately) were subsequently sequenced (Bioneer Inc., South Korea). The sequences were checked using Chromas version 2.13 (Technelysium Pty Ltd, Tewantin Australia). The nucleotide sequences of the *GUS* and *cryIAb* genes afterward were confirmed using BLASTn (Altschul et al., 1990) tool in NCBI.

2.2. Synthesis of the functionalized MSNs

The synthesis of the functionalized MSNs was carried out using the modified method of Hussain et al. (2013). In brief, the buffer solution containing 1.74 *g* of NaOH, 10.2 *g* of monopotassium phosphate (KH_2PO_4) in 1.5 L of deionized water (pH = 7.2) was prepared. To obtain a homogenous solution of the surfactant, 3.71 *g* cetyltrimethylammonium bromide (CTAB) was dissolved in 100 mL of the above buffer at 30 °C and 550 rpm. Then, 1.86 mL of tetraethyl orthosilicate (TEOS) was added drop-wisely and stirred at 550 rpm for 8 h during which a white sediment formed. The solution was centrifuged at 9000 rpm for 20 min and the pellet washed with 30 mL ethanol for three times to remove excess template and TEOS. The MSNs were resuspended in a solution containing 100 mL of ethanol and 1 mL of HCl with constant stirring at 550 rpm and 60 °C for 16 h. The samples were centrifuged and washed with ethanol and deionized water for several times. The final products were dispersed in absolute ethanol and stored at room temperature. Aminopropyl triethoxysilane (APTES) was used to functionalize the MSNs. 20 mg of the above synthesized MSNs were resuspended in 20 mL of dimethylformamide (DMF) and 100 µL of APTES was added into the solution. The suspension was kept at room temperature for 24 h to eliminate the unreacted APTES. The functionalized MSNs were washed with ethanol three times and dispersed in phosphate-buffered saline (PBS, pH = 7.4) and stored at room temperature for subsequent use.

2.3. Characterization of MSN-APTES

To investigate the functionalized MSNs size and morphology, the FE-SEM images (Mira 3-XMU, Czech Republic) with an accelerating voltage of 15 kV and the TEM images (Zeiss, Germany) by 100 kV were obtained. The Zetasizer 3000 HS (Malvern, UK) has been used to investigate the synthesize MSNs zeta value. The pore size distribution was evaluated using BJH (Barrett-Joyner-Halenda) method. The Specific surface area and the pore volume (Brunauer-Emmett-Teller and Barrett-Joyner-Halenda methods) were calculated using Belsorp-Mini II, Gemini 2375 (BEL Japan Inc.,Osaka, Japan). Small-angle X-ray scattering (SAXS) was recorded for phase identification of the MSNs by applying PANalytical X'Pert MPD instrument operating at 40 kV and 40 mA with Cu Ka (k¼1.5406 Å) as X-ray source.

2.4. PDNA-MSNs binding and assessment of their stability

To obtain the optimal binding ratio of the plasmid to MSNs, 1 µg of pPZP:35S:*GUS* plasmid was mixed with various amount of the functionalized MSN at a mass ratio of plasmid DNA to MSN (1:50, 1:60, 1:70, 1:80, 1:90 and 1:100) at room temperature and 250 rpm for 2 h. Five µL of each solution was loaded onto 1% agarose gel including naked plasmid DNA as the control. The intensity of the band was calculated by imageJ software (Rasband, 1997). To assay *in vitro* stability of the DNA-MSN, micro tubes containing 1:100 ratio of pPZP122:35S:*GUS* to MSN were maintained at room temperature for 24, 48 and 72 h and then the amount of DNA in the aqueous phase was measured and compared to control (naked plasmid) using Nanodrop machine (Thermo Scientific, USA).