Int. J. Bio-Inorg. Hybr. Nanomater., 5(3): 159-165, Autumn 2016

Providing a mathematical model for measuring the expression of GUS gene was transferred temporarily through xylem vessels using RT-PCR and probe Gold nanoparticles

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Received: 19 April 2016; Accepted: 22 Jun 2016

ABSTRACT: Gene transfer to plants and the production of transgenic plants with various purposes, such as improving the performance and quality, resistance to pests, diseases, etc., and of great importance are carried out Gene transfer to plants performs to evaluate the transient and permanent gene expression. Transient expression is quick, easy and simple and is not influenced by position effect compare to the permanent expression. Due to the advantages of Agrobacterium-mediated gene transfer methods, including simple, inexpensive, and accurate and being natural, in this study, development of a new method of Agrobacterium-mediated gene transfer to plants through the wooden vessel Transient Expression of GUS gene using RT-PCR method and the gold nanoparticle probes. However, the use of detectors attached to gold nanoparticles to detect biochemical and molecular techniques in the specificity and high speed and lower costs can be performed. The intron GUS reporter gene plasmid pCAMBIA1305.2 of Agrobacterium-mediated plant transformation was tested and the results of gene expression between them were analyzed using a mathematical model. The results of this study showed that gene transfer through the stem and the stem xylem vessels associated with two factors, so that the square of the radius of the stem directly and inversely proportional to its length.

Keywords: *Gold nanoparticles; GUS gene; Mathematical model; RT-PCR; PCAMBIA1305.2 plasmid*

INTRODUCTION

Production of transgenic plants with various purposes such as improving yield and quality, resistance to pests, diseases, etc. are carried out (Kumar, *et al.*, 2003, Fischer, *et al.*, 2004) Because gene transfer and regeneration of higher plants laborious, time consuming and expensive (Trieu, *et al.*, 2000), known as the GUS gene, is widely used in plant molecular biology (Jefferson, *et al.*, 1987). For analysis of GUS gene promoters, factors influencing the promoters as regulators of tissue, hormone regulators, etc. is used to wound and Optical regulators. As well as temporary and permanent expression of these genes in the genome, gene transfer methods to plant and study the mechanism of transmission is through Agrobacterium (De Block, *et al.*, 1984). RT-PCR is a method of PCR. This technique is commonly used in molecular biology to measure RNA expression (Freeman, *et al.*, 1999).

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Numerous applications of this type of biosensor in detecting DNA, gene analysis, as well as in criminal cases are the rapid identification of pathogenic agents. The DNA based identification systems, based on a target DNA hybridization probe is the complementary N that can be performed in solution or on a solid surface. This method also can be used for DNA sequencing (Vo-Dinh, and Cullum, 2000). The tool must be able to detect low concentrations of DNA and identifying mutations in DNA is point mutation. Full compliance with the probe target sequences, leading to stable double strand DNA in throughput, while the presence of one or more open-mistake, and decrease the double strand DNA, leading to a change in throughput and signal point mutations identified in this way would-be (Li, *et al.*, 2005). Gold nanoparticles of different sizes of particles that are one nanometer to several hundred nanometers (Liu, *et al.*, 2005) Gold nanoparticles, the chemical, physical and optical characteristic in relation to the size, shape and surface charge indicated (Azzazy and Mansour, 2009). Very small size of the nanoparticles makes them able to react in time to the biological molecules (Vaseghi, *et al.*, 2013, Azzazy, *et al.*, 2006, Jain, 2005).

MATERIALS AND METHODS

GUS gene transfer and RNA extraction

GUS gene transfer through Agrobacterium transformation method was used tumefaciens through xylem vessels. First, from a single colony containing plasmid of Agrobacterium tumefaciens pCAMBIA1305.2 liquid culture in LB liquid medium for 16 hours in a shaking incubator at a temperature of 28 °C heat was far rpm 160. The bacteria grown in 5.1 ml sterile tubes were split (1 ml per tube). The test plants were cut and the suspension of bacteria to bacteria through the xylem vessels to reach leaves. The experiment took 4 hours. The number of leaves as a control (negative control) was placed in distilled water at the same time (Schob, *et al.*, 1997). RNA extraction using GeneAll kit to ensure the quality and quantity of extracted RNA concentration and OD size-measured by optical spectroscopy and also samples was observed on 2% agarose gel.

CDNA synthesis

For cDNA synthesis step 2-Steps RT-PCR kit (Product Code: RTPL 12) Vivantis company of Malaysia were used.

GUS gene expression using RT-PCR confirmed

An RT-PCR for mRNA sequence GUS and actin genes of interest from NCBI (National center for biotechnology information) and then according to the primer sequences were designed using software Oligo7. F-GTCGTGATCGACCAGACTCC and R-GCTCACCCACGAAGTTCTCA Continue to check the quality primers were designed from the site IDT. Finally, to confirm the specificity of the primers were designed from the NCBI Blast Primer site.

The synthesis of gold nanoparticles

Gold nanoparticles were synthesized using sodium citrate (Turkevich, *et al.*, 1951, Frens, 1973). In order to evaluate the morphology of the synthesized gold nanoparticles, three methods were used: TEM, analysis of absorption spectrum of gold nanoparticles using a spectrophotometer and using of FT-IR analysis.

GUS reporter gene expression using gold nanoparticle probes GUS gene sequence using the NCBI database accession number AF354046.1 probs identification and exon regions were designed F- GCC TCG TTG GCG ATG CTC CA and R- CGC CCT CTT CCT CAG TCG GC. Probes designed for head-to-tail were together (TGGAGCATCGCCAACGAGGCGGC-GACTGAGGAAGAGGGCG). The detector oligonucleotide create pieces with a length of 40 virgin and the probability that a randomly in the genome of the 40 other indicator bacteria, pathogens or other organisms hybrid virus be zero indicates that is the specificity of the assay to approve the GUS gene transfer.

In order to confirm the expression of GUS gene to plant melons using gold nanoparticle probes of the method used for the detection of telomeric DNA (Qi, *et al.*, 2009). The amount of RNA with 10 mM phosphate buffer without NaCl necessary amount of the mixture and Vertex, Then, 5 ml of each of probs and amount of 0.25 M NaCl added to the rolls of the reaction mixture, then the vortex and spin. Tubes for 25 minutes at a temperature between the probe and RNA hybridization 65°C in order to be taken and after

cooling at room temperature to make the connections between RNA and probe, 120 ml of gold nanoparticles added discoloration was observed after the absorption was read by a spectrophotometer.

RESULTS AND DISCUSSION

RNA extraction result

The RNA was extracted using agarose gel Total RNA was extracted according to what was explained in the third quarter. The image below shows an example of the Total RNA extraction Over 85% of total cellular RNA rRNA or ribosomal RNA to form filler. Total RNA was extracted in the process of doing so 18s rRNA and 28S rRNA bands should be observed (Fig. 1) and the low rate of mRNA and tRNA for them to stretch or weak bands in the gel-are displayed. Hence with two bands in each extraction can be 28srRNA 18srRNA and to ensure accuracy of the extraction. The absorption ratio between 8 / 1-5 / 1, respectively, and the RNA concentration was estimated to average 230 micrograms per ml.

Evaluation and verification of gold nanoparticles

In order to evaluate the morphology of the gold nanoparticles synthesized from the use of TEM, FT-IR and examine the absorption spectrum of the gold nanoparticles using a spectrophotometer was used. GUS gene expression using gold nanoparticle probes confirmed Study of the synthesis of gold nanoparticles using electron microscopy. Synthesized gold nanoparticles of gold salt trisodium citrate reduction is

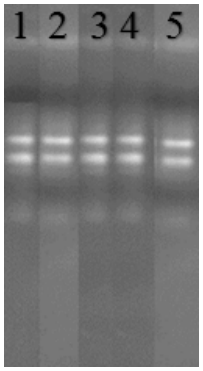

Fig. 1. Total RNA extraction results for five different plants on 2% agarose gel 1) *Cucumis melo* 2) *Cicer arietinum* 3) *Phaseolus vulgaris* 4) *Citrullus lanatus* 5) *Solanum lycopersicum*

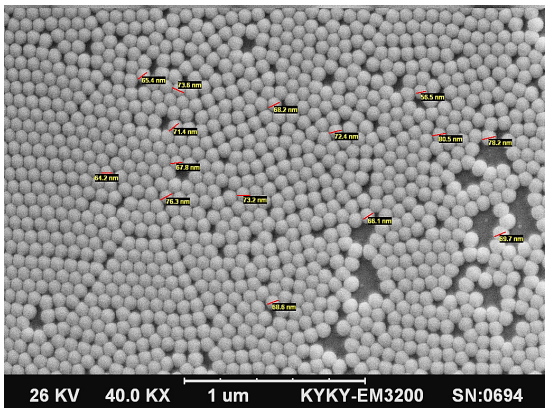

Fig. 2. The image gold nanoparticles synthesized by trisodium citrate and by transmission electron microscopy at a magnification of 140,000, as is obvious in the picture synthesized gold nanoparticles are spherical and assembly is not observed.

achieved with regenerative properties. Images indicating globular and uniform gold nanoparticles with a size of about 20 nm were synthesized (Fig. 2). Fig. 2 shows the image of gold nanoparticles by TEM.

FT-IR analysis results

FT-IR spectrum of the synthesized gold nanoparticles is shown in Fig. 3. Despite the relatively broad absorption, within the range of 3443 cm^{-1}, the presence of hydroxyl functional groups is confirmed. Aliphatic C-H bonds create severe peaks in the range of 2850-3000 cm^{-1}. Existence of these peaks is evident in the range of 2925 cm^{-1} in the structure of synthesized gold nanoparticles. The average peak, 1624 cm^{-1} is related to stretching vibration C=O. Respectively, peak observed in 1398 cm^{-1} represent stretching vibration of the peak and 620 cm^{-1} represents C-H of the bending alkaline (Logaranjan, *et al.*, 2012). These results high-

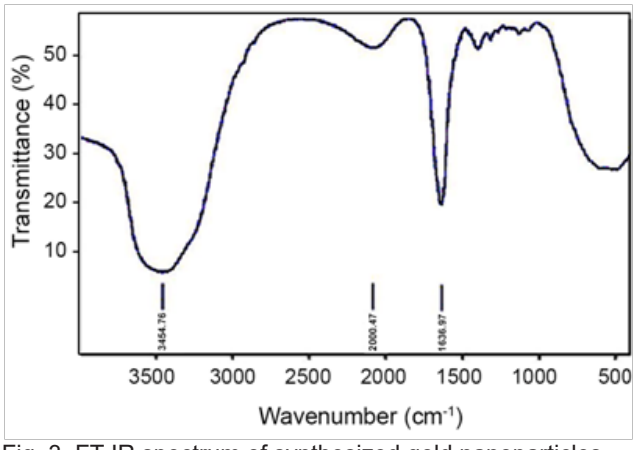

Fig. 3. FT-IR spectrum of synthesized gold nanoparticles

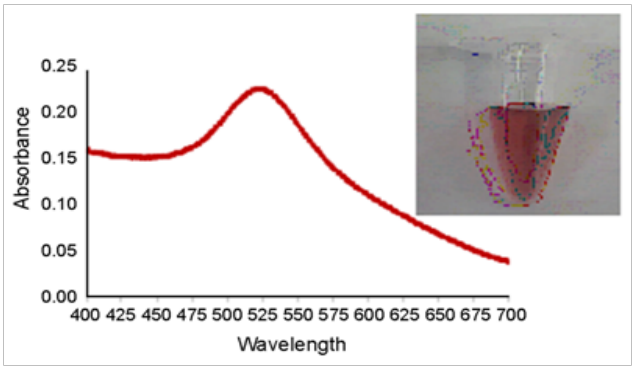

Fig. 4. The absorption spectrum of the gold nanoparticles, As the Figure shows, the maximum absorption at a wavelength of 528 nm gold nanoparticles

light the presence of hydroxyl and carbonyl functional groups of trisodium citrate molecules present on the surface of the nanoparticles which play an important role in reduction of Au^{+} ions and result in stability of nanoparticles.

Absorption spectrum of the gold nanoparticles the maximum absorption (OD) nanoparticles synthesized after observing cherry-red color with a spectrophotometer at a wavelength nm 528 (Fig. 4).

RT-PCR analysis for different plants

RT-PCR analysis for plants melons, watermelons, tomatoes, peas and beans infected with Agrobacterium for 5 minutes bp600 band showed the GUS reporter gene (Fig. 5).

The results of using gold nanoparticle probes for GUS gene expression in different plants the results of the aggregation of gold nanoparticles and changes color in the presence of oligonucleotide and RNA them in accordance with modified nucleotides into RNA oligo is the connection represents Table 1.

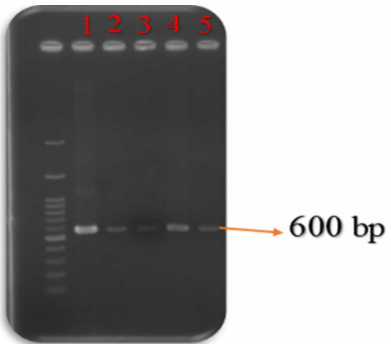

Fig. 5. RT-PCR analysis for different plants 1) *Cucumis melo* (2 *Cicer arietinum* 3) *Phaseolus vulgaris* 4) *Citrullus lanatus* 5) *Solanum lycopersicum*

Table 1: results from the aggregation of gold nanoparticles and changes color in the presence of oligonucleotide and RNA species

Plant species	Within shoot radius (mm)	Gold nanoparticles results
Cucumis melo	1.1	
Solanum lycopersicum	1.0	
Phaseolus vulgaris	1.0	
Citrullus lanatus	2.0	
Cicer arietinum	0.9	

The results of using gold nanoparticle probes to investigate the expression of GUS gene in the plant melons. The results of the aggregation of gold nanoparticles and changes color in the presence of oligonucleotide and RNA them in accordance with modified nucleotides into RNA oligo is the table 2 represents connection.

Study of gene expression in plants with two probes attached to gold nanoparticles using RT-PCR showed that the gene expression within stem and stem length will depend on two factors, so that the expression of

Table 1: results from the aggregation of gold nanoparticles and changes color in the presence of oligonucleotide and RNA species

Plant species	Within shoot radius (mm)	Gold nanoparticles results
Cucumis melo	2.8	
Cucumis melo	2.1	
Cucumis melo	1.8	
Cucumis melo	1.5	
Cucumis melo	1	

stem directly proportional to the cross-sectional area and length it inversely.

The following equation shows the gene expression is G, A is the area of the stem, and L represents the length of different plants.

$$G = \frac{A}{l}$$

A is equal to the radius squared shoot multiplied the π

$$A = \pi r^2$$

Since the π for all plants is a constant value A is equal to the square of the radius of the stem

$$A = r^2$$

When the stem length (L) has been constant for all plants G is equal to the square of the radius r ^ 2 or the stem. Study of gene expression in plants with two probes attached to gold nanoparticles using RT-PCR showed that the gene expression within stem and stem length will depend on two factors, so that the expression of stem directly proportional to the cross-sectional area and length it inversely. The following equation shows the gene expression is G, A is the area of the stem, and L represents the length of different plants.

$$G = \frac{r^2}{l} \qquad G = r^2$$

Transgenic plants with different purposes, such as creating new traits in plants, pest control and used as a bioreactor for the production of recombinant proteins are cheap and with high added value. Productions of recombinant proteins in plants are superior to other systems and their main advantage is their low cost, the cost of 2 to 10 percent system cost microbial and mammalian expression system is 1.0 percent (Giddings, 2001). Another benefit of the high potential of plant expression system which produces recombinant antibodies unlimited, vaccines, growth factors and enzymes respectively (Fischer, *et al.*, 2004). Also present in a public reception for the production of recombinant proteins in plants has been (Obregon, *et al.*, 2006). Gene transfer to plants by various methods that put these methods into two categories: direct and indirect. The most widely used and popular methods of gene transfer into plants through Agrobacterium gene transfer method and the gene gun (Veluthambi, *et al.*, 2003). Gene gun method significant disadvantages such as high cost complexity of the death of the target cells and DNA of target cells and cannot cross this technique in normal laboratories and the least used facilities. Agrobacterium gene transfer methods through benefits such as simplicity, high accuracy and the ability to transfer DNA fragments larger than 150 kb major disadvantage of these methods and the inability to transfer genes into important crop plants

that are monocots (Veluthambi, *et al.*, 2003), is of course, the development and use of new vectors of gene transfer Acetosyringone monocots crops such as wheat, rice, corn and barley using Agrobacterium is done. Given the time-consuming transfer of genes into plants through Agrobacterium in the study of a new method of gene transfer to plants through Agrobacterium tumefaciens was used. This method than Agro-interferation that require a vacuum system, it is much easier. In this study, all the conditions for being infected with the bacterium Agrobacterium tumefaciens leaf area were taken into consideration. DNA was extracted from leaf and only where there is no contact with the Agrobacterium was done and then PCR was performed to establish false positive responses in PCR avoided. The study also tested the plants that were infected with Agrobacterium, plant or leaves in the same condition in distilled water as a control and were negative. The study of vector PCAMBIA1305.2 with GUS reporter gene containing intron and is expressed only in plant cells, which is still a lack of response from the reporter gene in bacteria caused by false-positive population. The CaMV35S promoter in the vector that induces the expression of genes in plant cells is strong and fit. The promoter and NOS terminator in the vector and the expression of the target gene are the cause polyadenylation (Kumar, *et al.*, 2003).

CONCLUSIONS

The results of this study showed that gene transfer through the stem and the stem xylem vessels associated with two factors, So that the square of the radius of the stem directly and inversely proportional to its length and because the same plants during the shoot was selected for gene expression proportional to the square of the radius r2 or the stem.

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