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Gold Nanoparticles, A Novel Application in Identification of Plants

Yaser Ghazi[1], Dr. Fatemeh Haddadi[1]*, Dr. Hossein Kamaladini[1]
[1]Department of Biology, Faculty of Sciences, University of Zabol, Zabol, Iran
*Corresponding E.mail: haddadi.biotech @ yahoo.com

Abstract: *In recent years, a great tendency toward using diagnostic tests which are based on DNA has expanded. Thus, DNA- recognition biosensors have been created which can facilitate DNA identification. These DNA- oriented identification systems work based on hybridization of a target DNA with its complementary probe that can be performed in solution or on a solid surface. In this study, Zabol mildew melons were used as the model plant. After identify a specific sequence was of the species Cucumis melo L. Using NCBI and BLAST sites using the probe designed for specific sequencing, Identification of plant was performed using a probe attached to gold nanoparticles to observe color variation of gold nanoparticles in presence of the target molecules. In addition, the hybridization of the probes with target molecules was evaluated at a wavelength of 400 to 700 nm, so that maximum change was observed in the wavelength range of 550 to 650 nm. The results of this study showed that using detectors attached to gold nanoparticles is a more specific and rapid way to detect than the biochemical and molecular techniques .It can also be achieved spending lower costs.*

Keywords: *Biosensor, Blast site, Cucumis melo L., NCBI site, Zabol mildew melons*

INTRODUCTION

cucumismelo L. with 2n = 2x = 24 chromosomes has various farming groups, including melon, cantaloupe, and cucumber collarbone that belong to the Cucurbitaceae family. With regard to the distribution of wild melons in Kirk monograph (Kirkbride, 1993), it seems that Africa is the first center of this diversity and India, Iran, Afghanistan, and China are respectively the second sources of this diversity. Nadine (Naudin, 1859), French botanist, has classified melon variety into ten subgroups. This classification was revised by Munger and Robinson (Munger and Robinson, 1991) with respect to the three-part name. The latest classification offered by Peter *et al* (Pitrat *et al*., 2000) was based on identifying various identical features in the subgroups. There are plenty of methods for estimating genetic variation within species such as morphological and molecular markers. However, in some cases, markers such as AFLP and SSR are preferred in studies of genetic variation, yet RAPD marker has got its own place in many molecular research such as assessment of genetic diversity due to reasons such as lack of need for basic information about the sequence of genomic DNA for designing primers, small amounts of genomic DNA, simplicity and speed tests (Weising *et al*.,2005). So far, several studies have been carried out using morphological and molecular RAPD markers to investigate polymorphism in the regimes of melon germplasm.

During the last decade, great advances have occurred in Nano methods to identify molecules, and most attempts have focused on designing biosensors which are useful for accurate, sensitive selective, and functional detection of biological molecules. Today, nanoparticles are vastly used in biosensors to detect

nucleoid acids and proteins. Since these particles are as tiny as Nanos and have adjustable physical and chemical properties (such as electrical, electrochemical, optical, and magnetic features), they can be used as a suitable option marker for replacing other color molecules to diagnose molecules (Pal, 2004).

As a marker, Nanoparticles can increase sensitivity, speed, and flexibility of biological tests in measuring the presence or activity of materials. In one hand, because a little volume of the sample is required is used in these particles, in some methods which are designed based on nanoparticles, the basic need of the sample to propagate the measured material (e.g. PCR) is removed. Another advantage of these particles is their performance in recognizing microorganisms, cancer tissues, and the like both in vivo and in vitro (Poy *et al.*, 2016).

Due to their optical and thermal properties, gold nanoparticles with dimensions of 3-100 nm are good markers in designing biosensors. The sensitivity of this molecular detection method is 100 times more than common fluorescence spectrometry methods. DNA identification systems are often based on the molecular detection ability of single-stranded oligonucleotide probes which act as hybrid supplements with a single- stranded target molecule. The oligonucleotide probes usually have a reporter group that are linked covalently and produce radioactive, electrochemical, florescent, or color signals. Colorimetric systems which are used for detection are extraordinarily attractive, since 1) they do not have any security issues, 2) they are monitored easily, and 3) they are relatively cheap (Rosi and Mirkin, 2005).

Materials and methods

In first phase, after collecting mildew plant, it was detected using Cucurbit family identification key extracted from Flora Iranica.

DNA extracted from plant leaves

Dellaporta's method with a few modifications, was used for extracting DNA from leaves of plants. Initially, 0.2 g of leaf tissue as raw powder using liquid nitrogen and was transferred to a 1.5 ml sterile tube. Next, 600 ml extraction buffer was added to the tube. Then, the tube was placed for 15 minutes in a 65 °C water bath. 200 ml 5M potassium acetate and 300 ml phenol and 300 ml chloroform were added to the tube. The tube was centrifuged for 10 minutes with 13000 rpm. The top phase was carefully transferred to a new 1.5 ml tube, 700 ml of cold isopropanol was added, and it was centrifuged for 10 minutes at the previous round. Ethanol was discarded and 30 ml 70% ethanol was added to the sediment. Then, it was centrifuged for 2 minutes with 10000 rpm. ethanol was discarded and the tube was placed with the door open at room temperature to evaporate the remaining ethanol. In the final step, 30 ml of TE buffer was added to the deposit (DNA) was added and it was kept at -20 °C (Dellaporta, 1981).

Determining concentration and quality of extracted DNA

The spectrophotometer was used to determine the concentration and purity of extracted DNA from. Initially, the spectrophotometer was calibrated and with 50 ml of distilled water as a blank solution, it was scaled nil. Then, 2 µl of extracted DNA solution was mixed with 48 ml of water (dilution 1 to 25) and was cast in the tube. DNA concentration was determined at a wavelength of 260 nm. In order to determine the DNA optical density, the ratio of light absorption was determined at a wavelength of 260 to 280 nm. Light absorption equals one at a wavelength of 260 nm and is the same as 50µg of double- stranded DNA in 1 ml of distilled water.

ynthesis of gold nanoparticles

Gold nanoparticles were synthesized using sodium citrate (Turkevich *et al.*, 1951; Frens, 1973). Then, the generated gold nanoparticles were kept in the dark and at room temperature.
In order to evaluate the morphology of the synthesized gold nanoparticles, two methods were used: electronmicroscopy of transmission and analysis of absorption spectrum of gold nanoparticles using a spectrophotometer.

Using gold nanoparticle probe to confirm the recognize of specefic gene

In order to confirm the transfer of recognize of specefic gene in melon plant, the following protocol was used (Qi and Li, 2009). 2µl DNA was mixed and vertexed with 10 µl phosphate buffer 0/02 M. 5 ml of each probe and some NaCl 0.25 M were first added to the tubes resulted from the additional reaction mixture, then they were vortexed and spinned. The tube was placed for 5 minutes at a temperature of 94 °C to strain DNA. Then, be hybridized between the probe and DNA and after cooling at room temperature, 120 ml of gold nanoparticles was added to it. Finally, after observing the desired color variation, its ansorption rate was read by spectrophotometer. After cooling at room temperature, 120 micro liters of gold particles is added to create stronger connections between the DNA and the probe. As soon as the change in the color of its absorption is observed, it is read by a spectrophotometer. To determine the specificity of the designed markers, different plants were used as the negative controls.

RESULTS:

The results showed that the plant is a part of *Cucumis melo L.* species.

DNA extraction results

Analysis results of spectrophotometry

To determine the concentration of extracted DNA, the amount of light absorption at 260 nm was measured. Wavelength absorption (A_{260}) which is usually equal to 1, is equivalent to 50 µg of DNA in the two monofilaments per ml. In order to determine the purity of extracted DNA, the ratio of light absorption to its length at a wavelength of 260 nm to 280 nm was used. In a pure sample DNA, the absorption ratio A280 / A260, is equal to 1.8 to 2. The lower value indicates a protein contamination while rates higher than 2 stands for large amounts of RNA in the samples. Moreover, in case of A260 / A230, if it is more than 2, it will indicate phenol contamination in the sample. The results of extracting the Cucumis melo DNA represent the extracting boiling method. The ratio of absorption for the extracted samples in the boiling method is 2. Agarose gel was also used to check the quality of the extracted DNA. Presence of a band indicates the safety and quality of DNA .

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Analysis of synthesized gold nanoparticles using an electron microscope

Synthesized gold nanoparticles are the result of regeneration of gold salt with trisodium citrate with regenerative properties. The taken Images indicate spheric and uniform state of the synthesized gold nanoparticles (Figure 1 and 2). Figure 2 shows the image of gold nanoparticles taken by TEM.

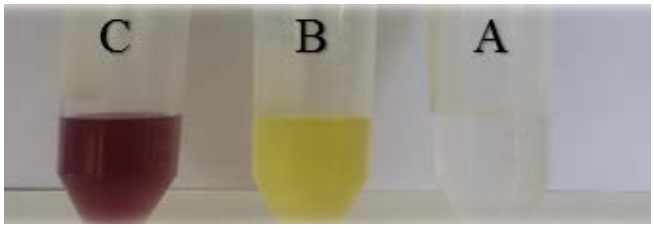

Figure 1: Synthesis of gold nanoparticles A) sodium citrate B) solution of gold colloids C) Gold nanoparticles synthesized

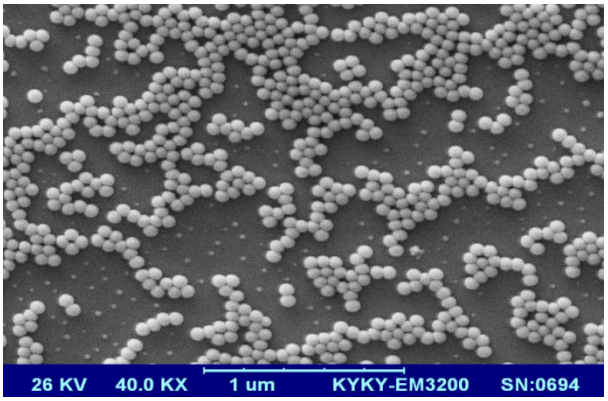

Figure 2: Image of gold nanoparticles synthesized by trisodium citrate taken by TEM, as is evident in the picture, all synthesized gold nanoparticles are spherical.

FT-IR analysis results

FTIR spectrum of the synthesized gold nanoparticles is shown in Figure 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 highlight 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.

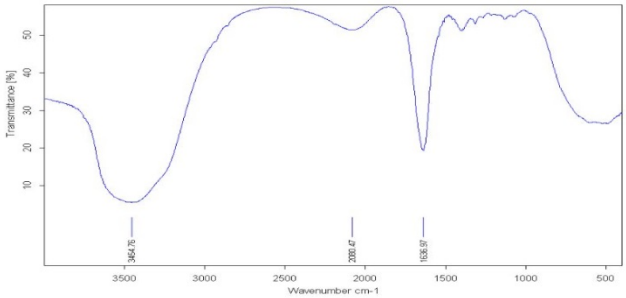

Figure 3: FT-IR spectrum of synthesized gold nanoparticles

Absorption spectrum of gold nanoparticle synthesis

The maximum absorption (OD) of nanoparticles synthesized after viewing the cherry-red color was achieved with a spectrophotometer at a wavelength of 530 nm (Figure 4).

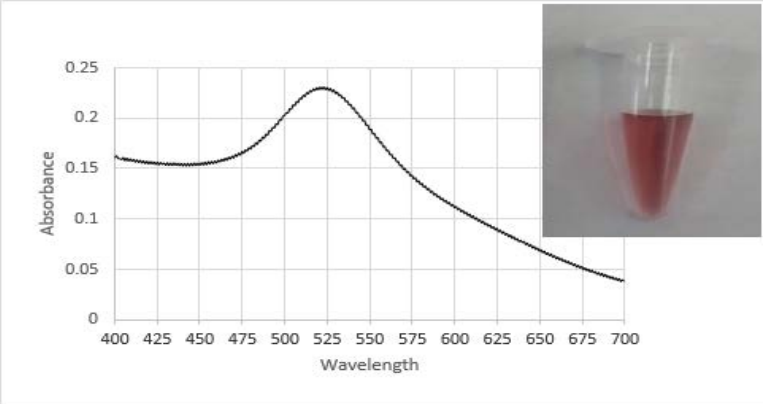

Figure 4: absorption spectrum of gold nanoparticles. As shown in the picture, the maximum absorption of gold nanoparticles occurred at the wavelength of 530 nm.

Identify and verify the specificity of candidate genes

After selecting the Nucleutide sequences using NCBI (Figure 5), alignment of these genes in the NCBI BLAST site were to be examined in its specificity (Figure 6). Then, using the appropriate probe was designed Primer3Plus site (Table 1).

Cucumis melo unplaced genomic scaffold, ASM31304v1 scaffold00383, whole genome shotgun sequence

NCBI Reference Sequence: NW_007546648.1

GenBank Graphics

>gi|655231652|ref|NW_007546648.1| Cucumis melo unplaced genomic scaffold, ASM31304v1 scaffold00383, whole genome shotgun sequence

TATATATATATATATATATATATATATAATTTCAATTCCATATGGATTATTTTATTTCTAAATTATT
TTG
AAAATTAAATCCATCTATAGAGTGATAATTTGTATCTTAACAAATAATTATTTCTTAAAACCTCAG
TGAA
TGACCAACTTAATTTCCACATAAGCATGATGGCATCATTATATTTCATTAAAAAGAGTACCAAGC
AAGGA
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GTAT
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TACAA
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GCGATC
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GCGTAT
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AAGGTTCAAAAAAATTCTATAGTTTTCTTGAAGAAGTGAACTGGTTGCGTGCAGGTGATGTTTTC
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GTTT
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CAAT

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ATAA
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CCTA
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AGGAA
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AAAGT
GAGATGTCAAATCAATTCCCCCAATCCCCACCTCCTCTCTCTCTCTCTCCTCCTCTCTAATTCT
CAAT
GAAA

Figure 5: Nucleutide sequences selected using NCBI and BLAST sites and sequence of Probe designed using Primer3Plus site.

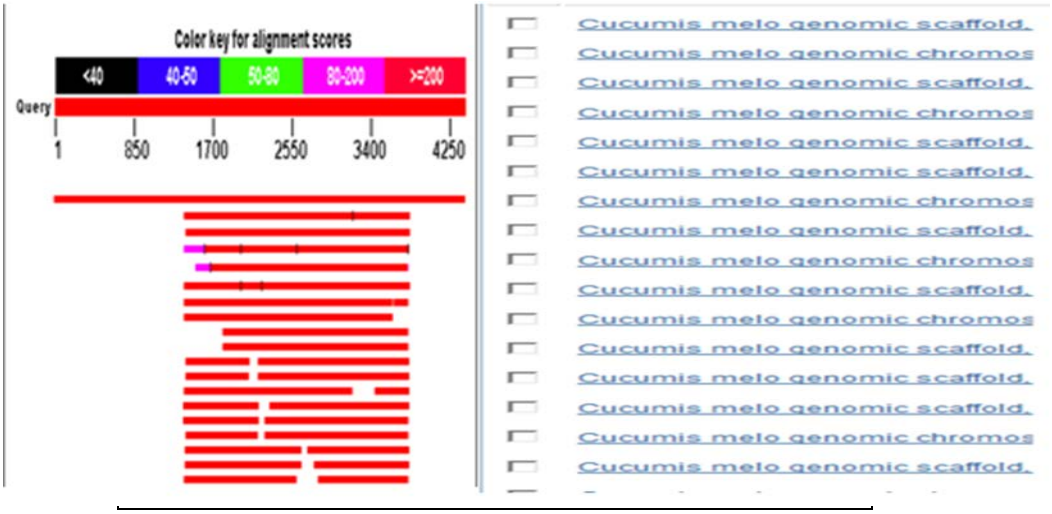

Figure 6: result of Blast for Confirmation of specify of selected sequence

Table 1: sequence of Probe designed for connecting the target DNA

GATGGGAAGCAATGTGTGGAA
CCAATTGATTGTGAATATCAT

Connect the probe to genomic DNA

The results from the reaction of different plant genome DNA as a negative control probes were selected in the presence of gold nanoparticles did not cause any change in the reaction solution. In addition, it changes the whole for different plant species as well as significant changes were found in the range of 525 to 535 nm were observed that a slight change may be due to the presence of DNA, probes and other materials in the reaction (compared to nanoparticles of pure) is (Figure 7).

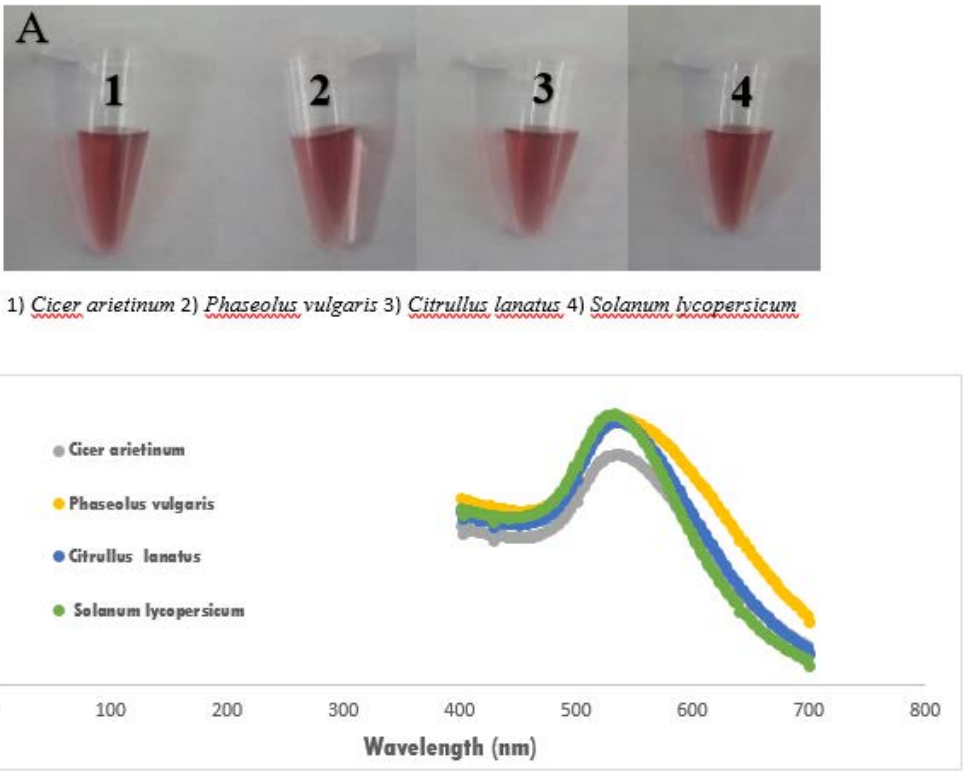

Figure 7: result of changes in colorimetric (A) and wavelength (B) for negatives controls.

Results of probes bound to genomic DNA

The results of aggregation of gold nanoparticles and changes of their color or wavelength in presence of a probe for gene and genomic DNA are in accordance with Figure 8 indicating DNA probe's connection with genomic DNA.

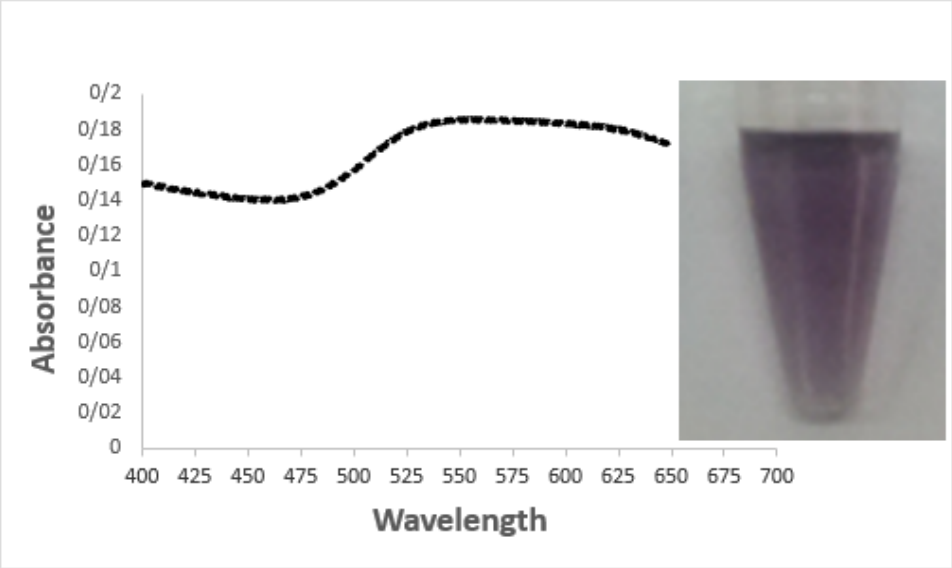

Figure 8: result of changes in wavelength and colorimetric changes for probe connection to the target sequence

Discussion

Systematic specialists have several detection means among which the most important is the double- branch plug which offers the users a series of choices of mutually exclusive mode and parallel mode (single- couple options). Flora and single- images are other botany means used for plant identification. Flora is a description of the plant life occurring in a particular region containing diagnostic keys, descriptions, and pictures. In contrast, a single image is a comprehensive systematic investigation of a specific taxonomic group including its keys, descriptions, and pictures. Herbariums are other plant identification tools which play an important role in plant detection (Joud *et al.*, 2003).

There are different methods such as morphological and molecular markers which are used for estimation of genetic diversity in the genetic species. Although in some cases, markers like AFLP and SSR are preferred in the studies of genetic diversity, RAPD marker has preserved its own position in most molecular studies such as evaluation of genetic diversity mainly because it does not need initial information in DNA genome sequencing for designing the primer. Also, it needs a little genome DNA, and the test can be done easily and quickly (Weising et al., 2005).

In comparison with other methods like biochemical and molecular detection or PCR- oriented methods, modern Nano biosensor methods have more specificity and sensitivity. Designing primers in using molecular methods such as multiplex PCR has some limitations such as nonspecific amplification and unwanted bonds, low efficiency of DNA amplification in the selected template or lack of PCR produce, and mutation formation which is the result of error in nucleotides' bonds. However, using biosensors and Nano biosensors can remove this limitation mainly due to its optical properties. Two pieces of Oligonucleotide can be designed side- by-side as a marker for the markers connected to gold nanoparticles. It can detect more than 400 open specificities which are near 100%, so the possibility that identical points attach each other on another genome is almost zero, even if there is a different open pair, because by using the gold nanoparticles, even a single-variation can be noticed by color variation and absorption spectrum.

Using gold nanoparticles' probe optimized factors such as temperature, size, shape, and density of salt. The size and shape of the particles are controlled by Trisodium citrate's density which acts as a reducing agent. It is basically based on the ability of single- stranded markers and their stabilization according to Van der Waals bonds and electrostatic forces on the surface of the gold nanoparticles and their resistance against sedimentation in presence of the electrolytes like NaCl. Negative loads of citrate ions present on the surface of these nanoparticles and in the solution prevent gold nanoparticles' sedimentation, but in presence of salt solution, they convert and sediment. In presence of the target DNA and by performing hybridation reaction, the markers join their supplements in presence of saline and phosphate buffer, and by creating sediments, their colors change from red to blue.

The method of using gold nanoparticles which is easy to perform and has high specificity owns a high potential in clinical uses. The major characteristics of this system are its ease, lack of need to use complicated detection tools, time- saving, and continuous sensitivity and specificity. Despite its similarities to other molecular detection systems, its sensitivity depends on the availability of enough DNA for interacting with gold nanoparticles. In addition to its ability to identify Staphylococcus epidermises bacteria, the method can be used for any other sample which requires detection of a fragment(s) of DNA.

Some of the advantages of this detection methods used in this research are absence of gold nanoparticles in high temperature and marker hybridation with the target DNA without any spatial limitation, removal of PCR phase, use of genomic DNA, removal of toxic and expensive materials such as Ethidium bromide and agarose, no need to use costly laboratory instrument and florescent markers, lower detection costs in any reaction (than other detection methods), rapid detection (in less than 600 minutes), and its performance in doing tests in any research laboratory. Observation of the results of this detection method which is based on gold nanoparticles was in line with the results of a great number of the published papers as well as the relevant studies (Shawky *et al*., 2010, Ali *et al*., 2012, Qi and Li, 2009, Khalil *et al*., 2014). Gold nanoparticles have higher specificity than PCR method since it is possible for the primers to attach other points or due to removal and addition, proliferate different parts of the desired fragment, but while using the markers attached to gold nanoparticles, there is little possibility of removal and addition in the point where the probe is designed. Therefore, in this study, the observed specificity of the markers for identifying the desired fragment was 100 %. Ali et al. in their research used this method to detect pig DNA and reported the specificity and sensitivity as 90-95% and 6ng/ L in the sample, respectively and stated that it can detect within an open pair limit (Ali *et al*., 2011). Likewise, Deng et al., used unchanged gold nanoparticles like the performed protocol to identify Anthrax bacterium using PCR produce (Deng *et al*., 2013). Khalil *et al*., also detected Acinetobacter baumanni using genomic proliferation and connection probe to the target molecule, and then added gold nanoparticles. Using each protocol requires optimization of factors such as gold nanoparticles' temperature, size, and shape as well as the probe content, and salt density. In this method, first, the gold nanoparticles were heated with the connection temperature and then, were added to the produce to prevent instability of the nanoparticles under high temperatures. Moreover, using different ratios of Oligonucleotide in presence of suitable salt density should be considered (Khalil *et al*., 2014).

The results of this research showed using markers attached to gold nanoparticles for detection purposes has higher speeds and specificity than the biochemical and molecular methods and is less costly, too. Based on the protocol expressed in the study, the requited time for detecting the target genome is less than 1 hour which is much less than the traditional methods and PCR. It also needs no toxins like Ethidium bromide, and because of using plant genome, the proliferation phase of the desired fragment by PCR is removed. These experiments can be performed with little care and initial optimization and with simple equipment at the research centers and they do not need any specific machinery.

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