EFFICACY OF TITANIUM DIOXIDE NANOPARTICLES IN BANANA MICROPROPAGATION AND THEIR EFFECT ON DNA CHANGE

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Micropropagation techniques are used widely worldwide to save time and cost through banana propagation process. Mitigation of microbial contamination of banana tissue culture as well as promotion of multiplication rate through banana micropropagation is main target of the specialist to enhance the economical values of banana trading market.

Nanoparticles (NPs) have a positive role in plant biotechnology including biomolecule delivery, genetic transformation, regulation of different stages of growth in plant tissue cultures, increasing somaclonal variation, enhancement of secondary metabolites etc. (Kokina and Plaksenkova, 2022). On the other hand, although NPs already have a wide range of applications in all life fields, aspects of nanotoxicity need to be studied in detail. Titanium dioxide nanoparticles (TiO_2NP) have many aspects of application in biotechnology. One of their applications is killing or growth inhibition of bacteria through tissue culture. Safavi (2014) added different amounts of TiO_2NPs to tissue culture media of potato to study their antimicrobial activity and he found that TiO_2 had a good potential for removing bacterial contaminants in plant tissue culture procedures.

Genotoxicity describes the property of chemical agents that damage DNA within a cell causing mutations. Genotoxicity induced by nanoparticles in plants is still poorly understood (Remédios *et al*., 2012). Genotoxicity of Titanium dioxide nanoparticles (TiO_2NPs) taken up by meristematic cells of *Allium cepa* on DNA had been studied by Filho *et al*., (2019). They found severe cellular and DNA changes in a concentration-dependent manner (10, 100, and 1000 mg/L). They summarized that TiO_2NPs can damage the genetic material of plants and plants displayed defense mechanisms against the

deleterious effects of these NPs (Filho *et al*., 2019). However, according to our knowledge, application of TiO_2NPs in banana tissue culture and their genotoxicity on banana DNA had not been reported to date.

Molecular markers are used to detect polymorphisms that exist among individuals in the population for specific regions of DNA (e.g., RAPD, ISSR, etc.) as well as to study the effect of nanoparticles on plant DNA modification (Abdellatif *et al*., 2016). RAPD technique is one of the methods that can be used to detect DNA change or modification, and thus it could be used to study genotoxicity (López-Moreno *et al*., 2010). Liu *et al*., (2012) performed RAPD technique to study DNA damage and mutations in the Arabidopsis shoots treated with cadmium nanoparticles and Abdellatif *et al*., (2016) used RAPD to study the effect of silver nanoparticles on eggplant DNA modification. Effects of AgNPs on the cell ultrastructure and genome integrity of green pea were also measured by ISSR marker (Labeeb *et al*., 2022) and the genotoxic effect of cadmium and lead supplied in a laboratory trial, was investigated in the moss *Sphagnum palustre* by ISSR molecular markers (Sorrentino *et al*., 2017).

In this study, the effect of different concentrations of titanium dioxide nanoparticles on stability of banana DNA through micropropagation was studied using molecular markers (i.e., RAPD and ISSR markers).

MATERIAL AND METHODS

Titanium Nanoparticles (TiO2NP) preparation and treatment

Titanium Nanoparticles were purchased as powder material from Nano-Gate company (Nasr City, Cairo, Egypt, https://nanogate-eg.com). The titanium nanoparticles used in the study were in white powder, spherical shape, anatase phase, less than 15nm, well assembled, purity~ 99.9% (Fig. 1A) with average size of less than 15 nm (Fig. 1B). Sterilized distilled water was used to prepare the different concentrations of the TiO2NP (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 and 30 mg/L).

Culture establishment and maintenance

Disease-free banana shoot tips were obtained from matured field grown banana plants of the commercial cultivar (Grand Nain). The shoot tips were taken to the traditional drying room (25-30ºC) as soon as cracked. According to Singh *et al*. (2010), shoot tips explants (4-5 cm in length) were sterilized by using 80 % Clorox (NaOCl at 5.25%) containing 2 drops of tween-20 for 30 minutes then sterilized by immersion in 0.2% $HgCl_2$ (Mercuric chloride) solution for 5 minutes. Sterilized shoot tips were then rinsed with sterilized distilled water three times under Laminar Air Flow, then shoot tip explants were cultured on MS starting medium (Murashige and Skoog, 1962)

supplemented with Titanium Nanoparticles (TiO_2NPs) at different concentrations (0, 1, 2, 3, 4, and 5 mg/l) as preliminary experiment with three replicates of each treatment to test the effect of nanoparticles on reducing the contamination of the cultured explants. After three weeks of the incubation under darkness, data were recorded as a number and percentage of free contamination explants, browning percentage. After the preliminary experiment, the main experiment was carried out using five replicates of each treatment on MS starting medium supplemented with Titanium Nanoparticles (TiO_2NPs) at different concentrations (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 and 30 mg/l). The media were autoclaved at 121ºC at 1.2 kg/cm2 for 20 minutes and then left for one week at room temperature to exclude contaminated media used for culture.

Data recording and statistical analysis

Each treatment contained five replicates and each replicate contained one explant. Cultures were incubated in the growth room for 3 weeks then subcultured every three weeks. Response of shoot tips including number of shoots and shoot length (cm) were recorded after three weeks of incubation and then were sub cultured. After another three weeks, fresh weight (g/plant), number of roots and root length (cm) were recorded.

In completely randomized design (CRD), JMP IN 7 software (Lehman *et al*., 2005, SAS institute Inc.) was used to accomplish the analysis of variance (ANOVA) of the collected data. The means of the treatments and the control were compared by the Student's Least Significant Difference (LSD) value of the different recorded data.

- **Molecular analysis**

DNA extraction

The multiplicated shoots of each treatment were sub-cultured to the corresponding concentration for three weeks and then were used for DNA isolation. DNA was extracted from normal banana shoots (Grand Nain cultivar) as well as the shoots propagated on media containing different concentrations of titanium dioxide nanoparticles, TiO_2NPs (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 and 30 mg/L). DNA was extracted to study the effect addition of different concentration of titanium nanoparticles in the nutrient artificial medium through micropropagation on banana DNA stability. DNA was extracted using i-genomic Plant DNA Extraction Mini Kit (iNtRON Biotechnology Inc., Korea) according to their manufacturer instructions. The extracted DNA solutions were adjusted at 25ng/µl as working solution and stored at -20ºC until use.

RAPD analysis

Eleven RAPD 10-mer random primers (Table 1) and eleven ISSR primers (Table 2) were used in this study to discriminate between the DNA pattern of banana plantlets propagated on normal nutrient medium and on medium containing different concentrations of titanium

dioxide nanoparticles (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 and 30 mg/L).

RAPD-PCR reactions were carried out in 15 µl volumes containing 50 ng of template DNA (2 µl of 25 ng/µl), 1.5 µl of the primer, 4 µl nuclease free ddH_2O and 7.5 µl of master mix solution (i-Taq, iNtRON Biotechnology Inc., Korea). PCR amplification was performed for 35 cycles at 95ºC for 1 minute, 32ºC for 1 minute and 72ºC for 1 minute. The program was preceded by a denaturation step at 95ºC for 5 minutes and followed by an elongation step at 72ºC for 5 minutes. The PCR products were separated into 1.5 % ethidium bromide-stained agarose gels and were photographed on gel documentation system.

ISSR analysis

ISSR analysis was conducted using reaction mixture volume of 15 µl contained: 7.5 µl of 2X PCR Master mix solution (i-Taq, iNtRON Biotechnology Inc., Korea), 1.5 µl from the primer (10 µM/ml), 2 µl DNA and 4 µl nuclease free ddH_2O. The PCR program was performed for 32 cycles of the following steps: denaturation at 95ºC for 50 seconds, annealing at 48ºC for 40 seconds and extension at 72ºC for one minute. The previous PCR program was preceded with denaturation step at 95ºC for five minutes and followed by final extension step at 72ºC for three minutes. After completing the PCR reaction, samples were separated on 1.5% agarose gel electrophoresis and a photo was taken using digital camera of the gel documentation system.

Statistical analysis

The total number and polymorphic number of amplified fragments produced from both RAPD and ISSR analyses were accounted, and the polymorphic information content (PIC) was calculated according to Anderson *et al.,* (1993) using the following simplified formula:

$$PIC_i = 1 - \Sigma p_{ij}^{2},$$

Whereas $_{pij}$ is the frequency of the $_{j}^{th}$ pattern for the primer, $_{i}^{th}$ summed across all patterns for the primer. Similarity coefficient matrices were calculated for both RAPD markers and ISSR markers using DIC similarity algorithm (Sokal and Sneath, 1963). Phylogenetic dendrogram was constructed using the UPGMA method (Unweighted Pair-Group Method with arithmetical algorithms Averages (Sneath and Sokal, 1973). All the above-mentioned analyses were performed using the NTSYS PC2.1 software (Rohlf, 2000).

RESULTS AND DISCUSSION

Tissue culture traits

Contamination and browning in tissue culture of banana

The analysis of variance of the contamination and browning traits showed highly significant differences among treatments (Table 3). No significant differences were obtained among the replications which indicate the reality of the results.

Using a concentration of 5 mg/L of nanoparticles TiO_2NPs gave the least percent of contamination (0%), while the control treatment showed the highest percent of contamination after three weeks of shoot tips culture. Taking into consideration that all the growth conditions inside the growth room are the same, using 5mg/L of TiO_2NPs as supplement to the tissue culture media reducing the contamination and browning produced through the growth period (Table 4).

Micropropagation traits of banana

According to the analysis of variance of the micropropagation traits of banana cultivar Grand Nain treated with different concentrations of nanoparticles TiO_2NPs, highly significant differences were found among the different treatments for all recorded traits, while the differences among replications were not significant for all traits except for the root length trait (Table 5).

Comparisons of the treatments means depending on the least significant differences (LSD) in terms of the micropropagation traits of the banana cultivar Grand Nain showed that treatment of 6mg/L of nanoparticles TiO_2NPs gave the highest significant values for all studied traits (Table 6). On the other hand, the higher concentrations of nanoparticles (i.e., 10, 20, 30 mg/L) showed the lowest significant values for the most traits in comparing to the other treatments. The control treatment, however, gave medium values (Table 6).

Molecular Experiment

1. Molecular pattern

The amplified fragments of RAPD primers were separated into 1.5% agarose gel electrophoresis (Fig. 2). All the amplified primers showed differences in the number and size of the amplified fragments except for the primer OPA03. Most detected differences were noticed between the control plant and the plantlets produced from the media containing the different concentrations of titanium oxide nanoparticles (Fig. 2). The most obvious different pattern for the control sample (Grand Nain) was noticed in the pattern of the primers OPB10, OPB12, OPC07, OPC15 and OPD03. The pattern of the control sample seems different indicated the effect of titanium oxide nanoparticles (TiO_2NPs) on the DNA of the banana plants when added to the tissue culture media.

The amplified fragments of ISSR primers were separated on 1.5% agarose gel electrophoresis (Fig. 3). Most of the amplified primers showed differences in the amplified fragments. The results of the ISSR patterns were not so far from the results of the RAPD results. The pattern of the control sample differed from the pattern of the other samples in the primers HB09, HB10, UBC812, UBC814, UBC818, UBC840 UBC842 and UBC842 (Fig. 3).

2. Primers polymorphisms in the amplified amplicons

The result of RAPD marker revealed that 66 fragments were amplified from eleven primers, 43 fragments of them were polymorphic. The total number of amplified fragments from each primer varied and ranged from three fragments (for primer OPN20) to nine fragments (for primer OPE20, Table 1). The total number of polymorphic bands for the RAPD primers ranged from two fragments (for primer OPN20) to six fragments (for primers OPB12 and OPE20). The primer OPA03 did not produce any polymorphic bands and consequently its polymorphic information content was zero. The polymorphic information content (PIC) of the RAPD primers ranged from 0.61 (for primer OPE15) to 0.84 (for primer OPB12) with an average of 0.61 overall for the RAPD primers (Table 1).

The total number of amplified fragments of ISSR marker was 39 fragments from eleven primers, 27 of them were polymorphic and the total number of bands for each primer pair ranged from three to five bands (Table 2). The polymorphic fragments for ISSR primers ranged for each primer from one band for the primers 14A and 44B to four bands for the primer UBC848. The percentage of polymorphism for the ISSR primers ranged from 25% for the primers 14A and 44B to 100% for the primers HB09 and UBC840 and the polymorphic information content (PIC) ranged from 0.38 for the primers 14A and 44B to 0.75 for the primerUBC812 with an average of 0.59 (Table 2).

3. Cluster analysis of molecular markers

According to cluster analysis of the RAPD data, the control sample was separated from the other samples at a level of similarity of about 50%. The nearest sample to the control was the sample obtained from the concentration 1 mg/L TiO_2NPs (Fig. 4). The other samples were separated at a level of similarity of more than 75% into three clusters. The first cluster included the samples taken from the concentrations 2, 7, 20 and 30 mg/L, while the second cluster contained the samples produced from the concentrations 3, 9, 4 mg/L and the third cluster included the samples originated from the concentrations 8 and 10 mg/L of titanium dioxide nanoparticles (Fig. 4). The samples produced from the concentrations 6 and 5 mg/L titanium dioxide nanoparticles were separated apart from the above-mentioned clusters, respectively. The most related samples according to this cluster analysis were those produced from the concentrations 20 and 30 mg/L (at similarity level of 91%).

At similarity level of about 57 %, the control Grand Nain sample was separated from the other samples (the samples taken from the different concentrations of titanium dioxide nanoparticles) according to the ISSR cluster analysis (Fig. 5). The other samples which were taken from the different concentrations of titanium diox-

ide nanoparticles (TiO_2NPs) were distributed into four clusters (from up to down of the dendrogram) at level of similarity 94 %. The first cluster (from the above) consisted of included the samples taken from the concentrations 1, 2 and 8 mg/L, while the second cluster contained the samples produced from the concentrations 3, 6, 5 and 9 mg/L and the third cluster included the samples originated from the concentrations 4 and 10 mg/L of titanium dioxide nanoparticles. The fourth cluster contained the samples produced from the concentrations 7, 30 and 20 mg/L (Fig. 5). The most related samples according to the ISSR cluster analysis were the samples originated from the concentration 7 and 30 mg/L.

Titanium dioxide nanoparticles (TiO_2NPs) are beneficial and have many applications in our life. One of the applications of TiO_2NPs in scientific research is to mitigate and minimize microbial contamination (as antimicrobial agent) in plant tissue culture (Mandeh *et al*., 2012). Our study tried to answer the question if the application of titanium nanoparticles safe (on the plant genetic material; DNA) to be used as a supplement in the artificial media through micropropagation of the banana plants (Grand Nain variety). To accomplish this purpose, a micropropagation experiment of banana cultivar Grand Nain has been done with different concentrations of TiO_2NPs (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 and 30 mg/L) and different traits indicating their response to the nanoparticles concentrations were analyzed. Moreover, samples of shoots (from different concentrations of titanium dioxide nanoparticles) were used for molecular study of DNA change by means of RAPD and ISSR markers.

According to the analysis of micropropagation and percent of contamination in the culture traits, it was clear that TiO_2NPs decreased the contamination and browning percentage to the minimum degree concentration (0%) at a concentration of 5 mg/L TiO_2NPs. In addition, all micropropagation traits were improved obviously at a concentration of 6 mg/L of nanoparticles TiO_2NPs comparing to the control and the other concentrations of TiO_2NPs. These results support the result of Safavi, (2014) where he reported that titanium dioxide nanoparticles had a good potential in removing bacterial contamination from potato tissue cultures. Zakharovaa and Gusev (2019) attributed that possibility of using TiO2 nanoparticles as bactericidal and fungicidal drugs for sterilization of explants during clonal micropropagation of plants for several factors: photocatalytic activity, particle size, concentration, morphology, and surface modification. Comparable results were reported by Dumani *et al*. (2022) on Paulwonia micropropagation and by Mandeh *et al*., (2012) on barley tissue culture.

According to our study, application of different concentrations of TiO_2NPs on the artificial media caused DNA change, although most of concentrations minimized the microbial contaminations and improved the micropropagation characteristics in banana micropropaga-

tion. It seems that this is the first report investigating the effect of TiO_2NPs on DNA stability in banana ever. However, some other studies investigated the effect of TiO_2NPs on DNA damage of different crop plants. For example, Filho *et al*., (2019) noted severe cellular and DNA damage in onion using different concentrations of TiO_2NPs (10, 100, and 1000 mg/L). also, Pakrashi *et al*., (2014) treated root tips of onion (*Allium cepa*) with TiO_2NPs with different concentrations (12.5, 25, 50, 100 mg/mL) and they observed distinctive chromosomal aberrations including chromosomal breaks and sticky, multipolar, and laggard chromosomes, and micronucleus formation. Do *et al*., (2018) used silver nanoparticles in banana tissue culture and they noticed improvement of shoots growth after twenty days cultured with 1 ppm nano silver, with 8.4 times multiplication and total chlorophyll content (2.05 mg/g-1). In contrast, Vidyalakshmi *et al*., (2017) reported that lack of toxicity of AgNP on banana tissue cultured plants as confirmed in their study offers opportunities to explore the development of NP based formulations for the enhancement of productivity of economically important plants like banana. Mahmoud *et al*., (2020) used Silicon nanoparticles to mitigate oxidative stress of in vitro-derived banana (Musa acuminata 'Grand Nain') under simulated water deficit or salinity stress. Their results revealed that SiO_2NPs application can improve chlorophyll content, induce K+ uptake, modulate Na+ levels and decrease cell wall damage in the treated plants comparing to the untreated plants under abiotic stress.

CONCLUSION

Applications of titanium dioxide nanoparticles (TiO_2NPs) as supplement in the artificial media through banana tissue culture (Micropropagation) enhance shoot proliferation (with concentration of 6 mg/L) and reduce microbial contamination (with a concentration of 5 mg/L). However, the produced banana plantlets (regenerated plants) produced different patterns of DNA (amplified by both RAPD and ISSR markers). The results of the clustering analysis showed that application of TiO_2NPs in banana micropropagation causes DNA change. According to the references, this change could be epigenetic and/or genetic changes and including both single mutations and chromosomal alteration aberration. Although using of TiO_2NPs improve production of banana plantlets through micropropagation, their use in tissue culture should be used carefully.

SUMMARY

Titanium dioxide nanoparticles (TiO_2NPs) minimize contamination and enhance proliferation and multiplication of banana through micropropagation. The effect of TiO_2NPs on banana tissue culture (micropropagation cultivar Grand Nain) as well as on banana DNA was studied using different concentrations (i.e., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20 and 30

mg/L). DNA was extracted from the regenerated plantlets along with the normal plant. TiO_2NPs (5 mg/L) decreased the contamination and browning percentage of banana cultures to the minimum degree concentration (0%). In addition, all micropropagation traits (ten traits) were improved obviously at a concentration of 6 mg/L of nanoparticles TiO_2NPs comparing to the control and the other concentrations of TiO_2NPs. Eleven primers of each RAPD and ISSR markers were used to study the genetic diversity and consequently the effect of TiO_2NPs on banana DNA change. According to cluster analysis, the normal plant was separated from the other treatments at a level of similarity of 48% (for RAPD markers) and 57% (for ISSR markers). The nearest plant to the normal one was the plant obtained from the concentration 1 mg/L then 2 mg/L TiO_2NPs. The amplified fragments differed in normal micropropagated banana from samples obtained from the TiO_2NPs treatments. This means that application of nanoparticles TiO_2NPs in banana tissue culture causes DNA change which could be genetic or/and epigenetic and consequently, could or could not affect the final product of banana plants. It could be concluded that DNA change is affected by application of nanoparticles in plant tissue cultures. This is the first report discussing the effect of TiO_2NPs on DNA change of the tissue culture-propagated banana. Although nanoparticles are beneficial in plant tissue culture micropropagation and in minimizing bacterial contamination in tissue culture, there are some issues with plant DNA change and precursors limitations that should be taken in consideration before deciding to use them.

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Table (1): RAPD primers sequences, polymorphism, and their polymorphic information content (PIC) used to study titanium dioxide nanoparticles TiO_2NPs effect on banana DNA through tissue culture.

Primer name	Sequence (5-3)	Amplified bands			PIC
		Total	Polymorphic	%Polymorphism	
OPA03	AGTCAGCCAC	2	0	0	0
OPB10	CTGCTGGGAC	6	4	66.7	0.78
OPB12	CCTTGACGCA	8	6	75	0.84
OPC07	GTCCCGACGA	8	5	62.6	0.78
OPC15	GACGGATCAG	5	5	100	0.72
OPD02	GGACCCAACC	5	3	60	0.72
OPD03	GTCGCCGTCA	6	5	83	0.78
OPE15	ACGCACAACC	7	3	42.8	0.61
OPE20	AACGGTGACC	9	6	66.7	0.82
OPH17	CACTCTCCTC	7	4	57	0.74
OPN20	GTAACCAGCC	3	2	66.7	0.67
Total & Average		66	43	65.2	0.61

Table (2): ISSR primers sequences, polymorphism, and their polymorphic information content (PIC) used to study titanium dioxide nanoparticles TiO_2NPs effect on banana DNA through tissue culture.

Primer name	Sequence (5-3)	Amplified bands			PIC
		Total	Polymorphic	Polymor-phism%	
14A	CTCTCTCTCTCTCTCTTG	4	1	25	0.38
44B	CTCTCTCTCTCTCTCTGC	4	1	25	0.38
HB-09	GTGTGTGTGTGTGTGTGC	3	3	100	0.67
HB-10	GAG AGA GAG AGA GAGACC	3	2	66.7	0.44
UBC812	GAGAGAGAGAGAGAGAA	4	3	75	0.75
UBC 814	CTCTCTCTCTCTCTCTCTA	4	3	75	0.63
UBC 818	CACACACACACACACAG	3	2	66.7	0.44
UBC 840	GAGAGAGAGAGAGAGAYT	3	3	100	0.67
UBC 842	GAGAGAGAGAGAGAGAYG	5	3	60	0.72
UBC 843	CTCTCTCTCTCTCTCTCTRA	3	2	66.7	0.67
UBC 848	CACACACACACACACARG	5	4	80	0.72
Total & Average		39	27	67.3	0.59

Table (3): Analysis of Variance of contamination and browning in tissue culture of banana cultivar Grand Nain as a response to TiO_2NPs treatments.

Source	DF	% Contamination	% Browning
Treatment (TiO2NPs)	5	2678.57**	2678.57**
Reps	2	0 n.s	0 n.s

** highly statistically significant, * statistically significant, n.s Not statistically Significant.

Table (4): Significant comparison of contamination and browning in tissue culture of banana cultivar Grand Nain under different concentrations of TiO_2NPs.

Treatment	% Contamination	% Browning
Control	100 A	100 A
1 mg/L	75 B	75 B
2 mg/L	50 C	50 C
3 mg/L	50 C	50 C
4 mg/L	25 D	25 D
5 mg/L	0 E	0 E
LSD	25	25

Levels not connected by same letter are significantly different.

Table (5): Analysis of Variance of micropropagation traits of banana cultivar Grand Nain as a response to TiO2NP treatments.

Source	DF	Root length (cm)	Shoot length (cm)	No. of Shoots	No. of Roots	Fresh weight (g/plant)
Treatment (TiO2NPs)	12	13.99**	31.20**	39.59**	0.86n.s	0.242**
Reps	4	3.41*	2.10n.s	0.83n.s	0.87n.s	1.7^{-33} n.s

** highly statistically significant, * statistically significant, n.s Not statistically Significant

Table (6): Significant comparison of micropropagation traits of banana cultivar Grand Nain under different concentrations of TiO2NPs.

Level	Root length (cm)	Shoot length (cm)	No. of Shoots	Fresh weight (g/plant)
Control	2.5 B	4.7 DE	21.4 D	0.15 I
1 mg/L	3.3 A	4.1 EFG	128 B	0.50 F
2 mg/L	2.5 B	4.8 CDE	170 A	0.50 F
3 mg/L	2 BC	4.8 CDE	145.6 AB	0.50 F
4 mg/L	1.7 CDE	4.2 EF	149 AB	0.60 D
5 mg/L	1.8 CD	5.7 B	141 B	0.80 B
6 mg/L	1.3 DEF	9 A	156 AB	1.0 A
7 mg/L	1.3 DEF	5.5 BC	66 C	0.80 B
8 mg/L	1.5 CDEF	5.1 BCD	36 D	0.80 B
9 mg/L	1.4 DEF	3.5 FG	28 D	0.70 C
10 mg/L	1.2 EF	3.4 G	28 D	0.55 E
20 mg/L	1.1 FG	3.5 FG	13 D	0.28 G
30 mg/L	0.6 G	3.7 FG	9.4 D	0.20 H
LSD	0.6	0.8	29	0.05

Levels not connected by the same letter are significantly different.

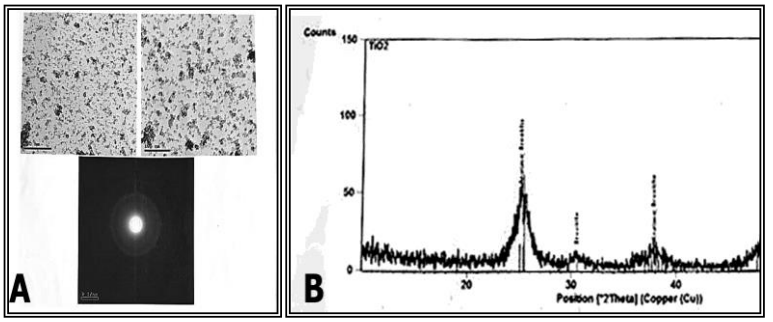

Fig. (1): TEM images (A) and XRD Spectrum (B) of prepared TiO_2NP used in the study synthesized by NanoGate Company.

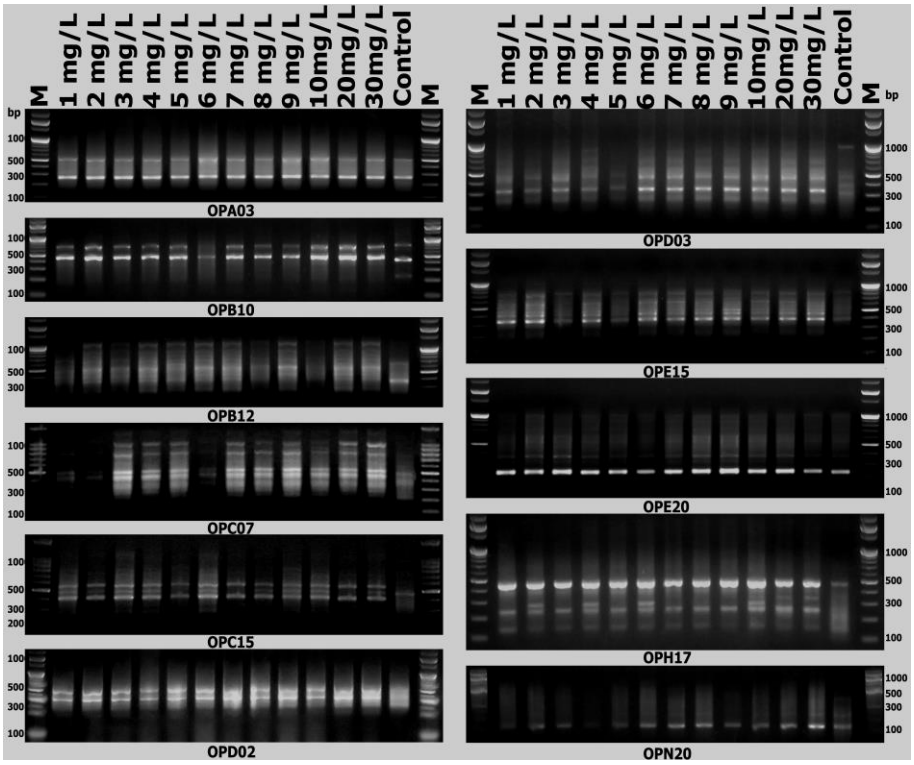

Fig. (2): PCR products of RAPD primers of banana cultivar Grand Nain (Control) and its plantlets propagated on media containing different concentrations of titanium dioxide nanoparticles TiO_2NPs and separated on 1.5% agarose gel electrophoresis.

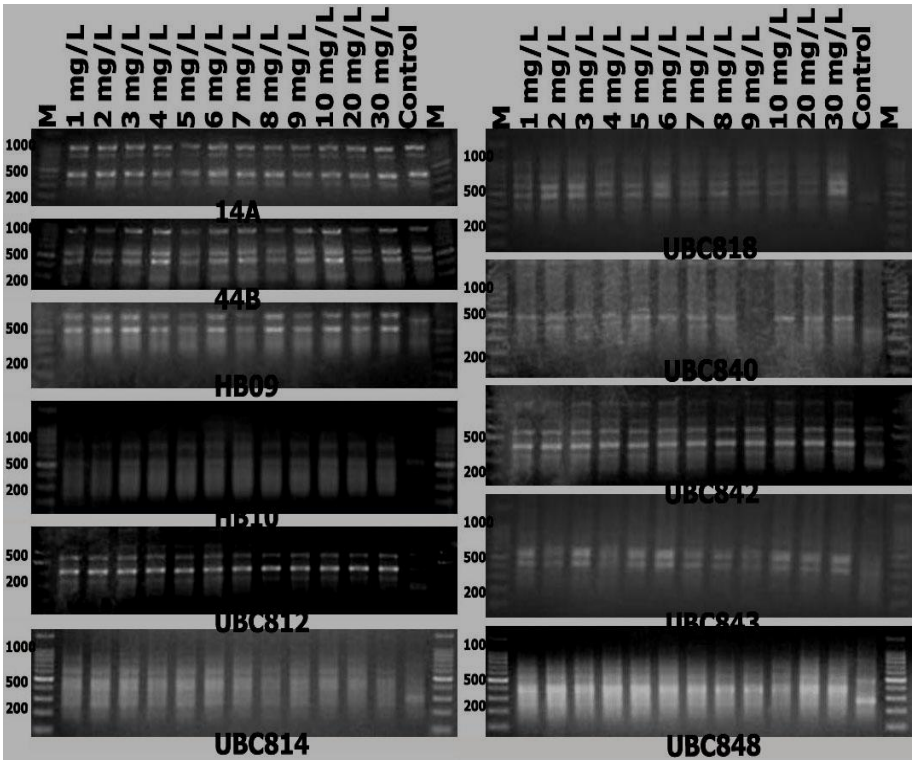

Fig.(3): PCR products of ISSR primers of banana cultivar Grand Nain (Control) and its plantlets propagated on media containing different concentrations of titanium dioxide nanoparticles TiO_2NPs and separated on 1.5% agarose gel electrophoresis.

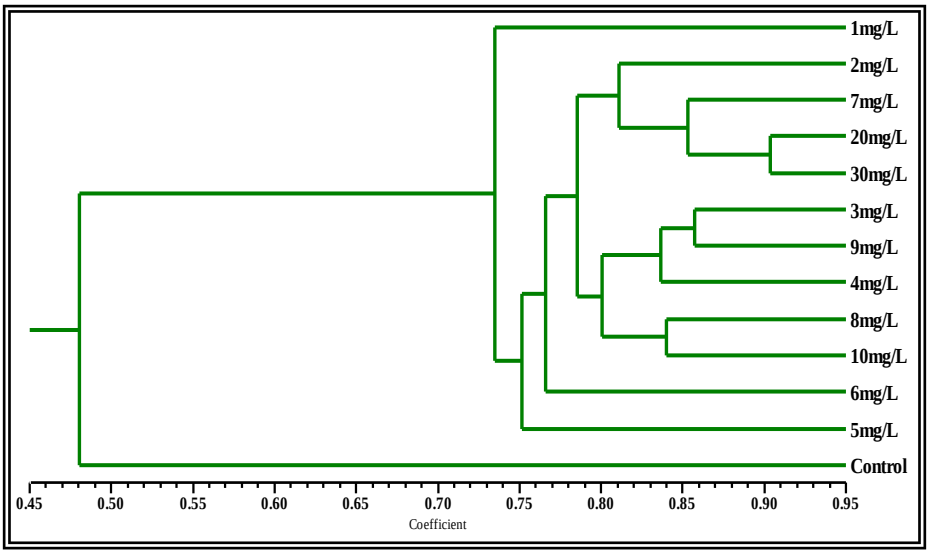

Fig. (4): Cluster analysis of banana cultivar Grand Nain (Control) and its plantlets propagated on media containing different concentrations of titanium dioxide nanoparticles TiO_2NPs using DIC coefficient of RAPD data and UPGMA clustering method.

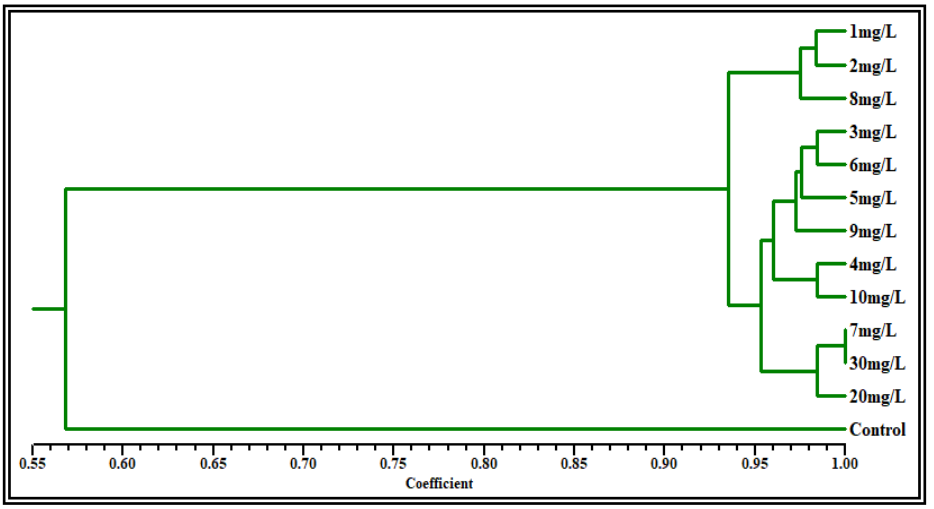

Fig. (5): Cluster analysis of banana cultivar Grand Nain (Control) and its plantlets propagated on media containing different concentrations of titanium dioxide nanoparticles TiO_2NPs using DIC coefficient of ISSR data and UPGMA clustering method.