Physiological Assessment of Radiation and PVP/ Zn-Nanoparticles on Sour Orange Seedling

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Authors' contributions

This work was carried out in collaboration among all authors. Author NAH performed the statistical analysis, managed the analyses of the study and wrote the first draft of the manuscript. Author MS generated the Zn Nanoparticals and managed the literature searches. Author MFA was responsible for the seeds radiation, designed the study and wrote the protocol. Author TS carried out the cytological studies. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Aims: The objective of this study is evaluate the effects of both pre-sowing gamma irradiation at low dose of 0 or 20 Gy and the soaking part of radiated seeds in zinc nanoparticles (Zn NPs) solutions at rate 0, 500, 1000 and 2000 ppm on behavior and physiological changes of sour orange seedling.
Study Design: The design of the study is Two-Way Randomized Blocks.
Place and Duration of Study: The present study was carried out during two successive seasons of 2016-2017 and 2017-2018 on sour orange rootstock (*Citrus aurantium*) grown at the experimental farm of the Horticulture Institute, Giza , Egypt.
Methodology: Eight different treatments were used as follow: 1) Control, 2) Gamma radiation at 0 Gy + soaking in Zn NPs at 500 ppm. 3) Gamma radiation at 0 Gy + soaking in Zn NPs at 1000

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ppm. 4) Gamma radiation at 0 Gy + soaking in Zn NPs at 2000 ppm. 5) Gamma radiation at 20 Gy + soaking in tap water Zn NPs at 0 ppm. 6) Gamma radiation at 20 Gy + soaking in Zn NPs at 500 ppm. 7) Gamma radiation at 20 Gy + soaking in Zn NPs at 1000 ppm. 8) Gamma radiation at 20 Gy + soaking in Zn NPs at 2000 ppm.

Results: Transmission electron microscope (TEM) images showed multiple shapes and uniform distribution of Zn NPs through the polymer network and the mean size of Zn NPs ranging is 40.9 nm. Generally, the results reveal that, 20 Gy combined with 1000ppm Zn NPs treatment increased seed germination percentage and stem length. While, 20 Gy plus 500 ppm Zn NPs treatment increased stem diameter and plant pigments concentration. Protein analysis of sour orange seedling treated with gamma radiation and then Zn NPs showed that, protein groups pattern (10-20 KD) enhanced with gamma rays 20 gray alone or with Zn NPs at 500 or 1000 ppm, while the protein groups pattern above 60 KD disappeared.

Conclusion: Using gamma irradiation and then soaking part of irradiated seeds in Zn NPs solutions had significant effects on vegetative growth and root characters as well as some chemical properties of sour orange leaves. In addition, there are variable differences in the protein pattern between treated and untreated sour orange seedlings with gamma radiation and / or Zn NPs. Although, 20 Gy + 500 ppm Zn NPs treatment increased stem diameter, which is the main characteristic for the success of grafting process, the obtained results showed changes in proteins and it possible the gene structure had been changed due to this treatment.

1. INTRODUCTION

Citrus is an important genus of the family Rutaceae in the plant kingdom. Its importance is demonstrated by its wide distribution and large-scale production. It is highly prized and economically remunerative fruit [1].

Rootstocks had a substantial role in citrus industry development in the world. Rootstock utilization is necessary for solving both limiting and restricting factors of citrus production (soil, climate, disease, and pests, etc.), meeting producers and consumers demands such as productivity, earliness, shorter juvenility, fruit quality [2]. Citrus rootstocks have differently influenced in the growth and development, including yield, fruit quality, and tolerance to stress caused by biotic and abiotic factors of budded cultivars in relation to ecological conditions [3].

Sour orange (*Citrus aurantium*) has been widely used as rootstock for citrus production. Fruit quality is highly influenced by the rootstock [4]. Some citrus rootstocks seem to provide fruits with higher content of bioactive compounds, which is very interesting for a better aptitude for both fresh consumption and industry.

Gamma irradiation induced physiological changes in crop although, it is a technology with immense applications in agriculture, its potential exploitation in agriculture is limited mainly because of lack of information awareness on optimal dose of irradiation which varies depending on crop and application. Radiation mediated morphological, structural and/or functional changes in a plant are governed by the intensity and duration of the gamma irradiation [5].

The increasing tree growth in Zn treated trees might be due to active involvement of Zn in the tryptophan synthesis which is a precursor of indole acetic acid synthesis; consequently it increased tissue growth and development [6]. Sufficient level of Zn in plants promotes the photosynthesis, nucleic acid metabolism and protein biosynthesis [7].

Nanotechnology has a great potential in agriculture sector and the food industry, by improving the life quality through its applications. Nanotechnology tools are used to enhance the ability of plants to absorb nutrients. Micronutrients are important in agricultural production in terms of quantity, quality and human health. Nano magnesium, manganese, zinc, potassium compounds are in the form of nanostructures that the plant needs in a very small form compared to the available fertilizer [8].

Nanoparticles (NPs) interact with plants causing many morphological and physiological changes, depending on its properties. Nanoparticles efficacy is determined by their chemical composition, size, surface covering, reactivity, and most importantly the dose at which they are effective [9].

Nanoparticles applied to plant, may cause modifications for the gene expression of this treated plant as well as the accompanying genetic pathways that finally affect the growth and development of this plant [10]. Engineered nanoparticles are able to inter into plants cells, leaves, and also can transport DNA and chemicals into plant cells [11].

The objective of this study is report facile and novel technique for the synthesis of the PVP-capped zinc nanoparticles under the gamma-irradiation method. Also, evaluate the effect of gamma irradiation and nano-Zn on behavior as well as physiological changes of sour orange seedling.

2. MATERIALS AND METHODS

The present study was carried out during two successive experimental seasons 2016 - 2017 and 2017-2018 on sour orange rootstock (*Citrus aurantium*), planted in March at the nursery of Horticulture Research Institute.

2.1 Plant Material Source

The required seeds of sour orange were obtained from healthy mature fruits of adult trees in mid of February during 2016 and 2017 seasons. Seeds were extracted, washed and air dried, soon after fruits picking. The prepared seeds were preserved at 3ºC. Preserved until treated and sown.

2.2 Gamma Irradiation

Dry seeds of sour orange were irradiated with gamma rays at doses 0 and 20 Gy (dose rate 2.028 and 1.776 Gray/min for both seasons, respectively) at the third week of March, 2016 and 2017 using gamma-Cell ^{60}Co, National Center for Radiation Research and Technology, Atomic Energy Authority.

2.3 Preparation of Nano Zinc Particles

A detected amount of PVP was dissolved in 1 liter distilled water via magnetic stirring then adding 0.35M of $ZnSO_4$ at 70°C. After dissolving, 2.3 M of acetic acid was added and followed by the addition of glycerol. The solution was left under stirring until the appearance of pale yellow color. Finally, the solution was irradiated via ^{60}Co gamma irradiator at the irradiation dose 30 k Gy.

2.4 Characterizations

The structural and morphological characteristics of the prepared zinc nanoparticles were studied with various techniques such as transmission electron microscope (TEM) and dynamic light scattering (DLS) measurements.

2.4.1 Transmission electron microscope (TEM) measurements

Transmission electron microscopy (TEM) measurements were performed with a (JEOL, JEM 100CX, Japan) operating at 80 kV. Transmission electron microcopy (TEM) was used to find out the size of zinc nanoparticles inside the aqueous solution. To image the zinc nanoparticles on TEM, 0.01 ml of the prepared solution was diluted by adding 1 ml of distilled water followed by sonication. Approximately 10–20 µL of this solution was dropped on a 3 mm copper grid, drying at room temperature. The copper grid was inserted into transmission electron microscope.

2.4.2 Dynamic light scattering (DLS)

Zinc nanoparticles size was measured by Zetasizer Nano ZS, Malvern Instruments Ltd, United Kingdom according to Kumar and Unstedt [12], where every 0.01 ml of the prepared aqueous solution from PVP/Zinc nanoparticles was diluted with 1 ml of distilled water then sonicated and examined by DLS.

Irradiated seeds as well as the control seeds "0 Gray" were soaked in nano-Zn solutions at the rate 0, 500, 1000 and 2000ppm immediately after exposure to gamma radiation, and planted at the third week of March 2016 and 2017 in plastic bags. Each bag contained 5 seeds to represent one replicate, and each of the eight treatments was represented by ten replicates. Two months after planting, uniform and healthy seedlings were chosen and transplanted individually in black polyethylene bags with dimensions 15 x 35cm, filled with sand: peat moss (3:1) and kept under greenhouse. The planted bags were arranged in the nursery in factorial randomization completely block design with 10 replicates, 8 treatments.

The following treatments were applied:

1. Control (0 Gy + soaking in tap water, Zn NPs at 0 ppm).

2. Gamma radiation at 0 Gy + soaking in Zn NPs at 500 ppm.
3. Gamma radiation at 0 Gy + soaking in Zn NPs at 1000 ppm.
4. Gamma radiation at 0 Gy + soaking in Zn NPs at 2000 ppm.
5. Gamma radiation at 20 Gy + soaking in tap water Zn NPs at 0 ppm.
6. Gamma radiation at 20 Gy + soaking in Zn NPs at 500 ppm.
7. Gamma radiation at 20 Gy + soaking in Zn NPs at 1000 ppm.
8. Gamma radiation at 20 Gy + soaking in Zn NPs at 2000 ppm.

Agricultural practices were done as the Citrus Res. Dept. and Ministry of Agriculture recommendation in the nursery during two experimental seasons.

The following parameters were recorded:

2.5 Seeds Germination Stage

Seed germination, albino and damping off seedlings were counted in each replicate and then germination, albino as well as damping off percentages were calculated according to Hartmann and Kaster [13] as follows:

Germination (%) = (Number of germinated seeds / Initial number of seeds) x 100

Albino (%) = (Number of albino seedlings / Initial number of seedling germinate) x 100

Damping off (%) = (Number of damped off seedlings / Initial number of seedling germinate) x 100

2.6 Vegetative Growth Characters

Five leaves per plant from each replicate were picked from the middle part of plant for leaf area (cm^2) determinate. Also, stem length (cm), stem diameter (mm), and number of leaves per plant for each replicate were determined at the two experimental seasons.

2.7 Leaf Chemical Composition

2.7.1 Plant pigments

Chlorophyll a, b, total chlorophyll as well as total carotenoids were measured by spectrophotometer in fresh leaves according to Nornai [14].

2.7.2 Total phenols, total indoles and total free amino acids

Total phenols, total indoles and total free amino acids were determined in fresh leaves. Total phenols were determined according to Swain and Hillis [15]. Total indoles were determined according to Larsen [16], as well as total free amino acids were determined according to Jayaraman [17].

2.7.3 Total carbohydrates

Total carbohydrates in dry matter of leaves were determined by 3,5-dinitrosalicylic acid according to Miller [18].

2.7.4 Nutrients

N, P, K, and Zn concentrations were determined in dry matter of leaves at the end of experimental. Total N% was determined by semi-micro Kjeldahl method described by Plummer [19]. Phosphorus was estimated colorimeterically by using the chlorostannous reduced molybdophosphoric blue colour method as described by King [20]. Potassium concentration was determined by using the flame photometer. Zinc concentration was determined by atomic absorption spectrophotometer.

2.8 Root Characters

Horizontal and vertical root extension, fresh and dray weight of roots was determined for each replicate at the end of experimental season and then dry matter percentage of roots was calculated according to the following equation:

Dry matter percentage = (Fresh weight- dry weight / Fresh weight) x 100

2.9 Protein Extraction Method and Analysis

Different plant samples were collected, labeled and grounded by using liquid nitrogen using mortar and pestle, and then 1 ml of cold QB buffer was added to 1 g plant powder and mixed vigorously under cooling system. The mix was transferred to 1.5 ml microfuge tube and placed on ice. Samples Span at the highest speed in a microfuge at 4 degrees C for 15 minutes. Supernatant was transferred into new microfuge tube and centrifuged at the highest speed for 10 minutes under cooling system and resulted supernatant was transferred to new microfuge.

Samples stored in -80 degree freezer. SDS-PAGE was performed by the method described by Laemmli [21]. Proteins were analyzed on 1.5-mm thick and 15-cm long gels run in a dual vertical slab unit (Hoefer Scientific Instruments, San Francisco, CA, USA). From each sample, 25 ul of protein extract was loaded on a polyacrylamide gel. The separation gel (10 %) and staking gel (3.5%) were prepared from an acrylamide monomer solution (Roth, Karlsruhe, Germany). Protein was electrophoresed at a constant current of 30 mA through the stacking gel, and at 90 mA through the separation gel at room temperature, the gels were stained by silver nitrate [22].

2.10 Statistical Analysis

Obtained data was statistically analyzed to determine the analysis of variance and significant differences between means according to Snedecor and Cochran [23]. The multiple comparisons of means were performed according to Duncan's multiple test range [24] using COSTAT computer program.

3. RESULTS AND DISCUSSION

3.1 Zn Nanoparticles Synthesis

In this study, zinc nanoparticles [ZnNPs] was synthesized by gamma radiation. The gamma irradiation induced technique has proven to be an appropriate method for fabrication of monometallic or bimetallic nanoparticles because it produces fully reduced and highly pure metallic nanoparticles, free from by-products and reducing agents [25]. Large numbers of free electrons and solvated electrons produced during gamma-ray irradiation in colloidal solutions can reduce the metal ions into zero-valent metal atoms without using reducing agents or catalysts and their consequent side reactions [26]. In addition, hydroxyl and hydrogen radicals ($OH^\bullet$ and $H^\bullet$), induced in radiolysis of water are also strong reducing agents in aqueous colloidal solution which eventually reduced Zn^{+2} into zero-valent atoms of Zn^0 as shown in the following reactions:

$$n \bullet H_2O \xrightarrow{\gamma-rays} e_{aq}^- + H^\bullet + OH^\bullet + H_3O + H_2 + \cdots,$$

$$Zn^{+2} + 2e_{aq}^- \xrightarrow{reduction} Zn^0$$
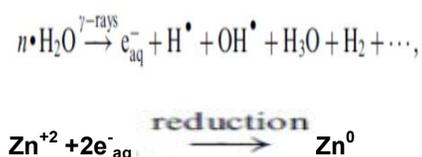
To prevent increasing particle size, a polymer is often used, either natural or synthetic, with some affinity for metals. The polymer is adsorbed on the cluster in aqueous solution and reduced surface tension. These substances also control both the reduction rate of metal ions and the aggregation process of metal atoms. It was reported that polyvinyl pyrrolidone (PVP) could stabilize colloidal particles in water and many non-aqueous solvents by adsorbing onto a broad range of materials, such as metals (e.g., gold, silver, and iron), and metal oxides (kaolinite, TiO_2, iron oxide, and alumina) [27]. Here, in this work, PVP will be used as stabilizing agent for zinc nanoparticles to prevent agglomeration formation.

3.1.1 Transmission Electron Microscope measurements (TEM)

It is clear from the Fig. 1(A) that, the zinc nanoparticles formed in the polymer networks have multiple shapes (such as spherical, pyramidal, and rhombus), and lower than 100 nanometers in size. On the other hand, as seen in Fig. 1 (B) demonstrates the corresponding size distribution of zinc nanoparticles over the polymer network. The size distribution values of zinc nanoparticles were found to be in the range over 11-98 nm. The average diameter value was 40.9 nm, which obtained by averaging the size of 9 particles detected in the TEM image [27].

3.1.2 Dynamic light scattering (DLS) measurements

The data in Fig. 2 revealed that, the dynamic light scattering (DLS) measurements of PVA/PVP hydrogel loaded by 12 mmol Ag NO_3. For this purpose a 0.01 ml of PVP/zinc nano gel solution was added to 1 ml bid stilled H_2O then examined by DLS. The particle size was found to be about 98.4 nm. DLS size ranges of Zn NPs were found to be greater than TEM size. Might be because DLS measures the hydrodynamic diameter of nanoparticles, where the amphiphilic nanoparticles were surrounded by water molecules; may be attributed to being the cause of the large size of capped formulation.

3.2 Seed Germination

It is clear from data presented in Table 1 that, pre-sowing gamma irradiation and Zn NPs soaking of sour orange seeds had significant effect on germination percentage. As, the highest values of seed germination percentage were

recorded by the 20 Gy +1000 ppm Zn NPs and 20 Gy +2000 ppm Zn NPs treatments at the two experimental seasons, when compared with control (zero Zn NPs + 0 Gy).

These results are in agreement with those obtained by Jawaharlal et al. [28] on citrus species.

In this respect, it is well known that gamma radiation effects on most components in living cells of tissues or seeds and plants, and this effect depending on several factors. The more important of these factors are moisture content and chemical constituent. Moreover, ionization also causes the production of hydrogen peroxide and free radicals, which are themselves activator, stimulator and mutagenitic through

molecular effects [29]. Low irradiation doses had significant effect on germination and growth parameters of three varieties of Chinese cabbage [30].

Also, nutrients along with water might also transport nanoparticles (NPs) and ions to the intra cellular space of seed coat and affects seed germination [31]. Enhanced germination of *Glycine max* by ZnO NPs at 1000 ppm could be attributed to increased nitrate reductase enzyme activity and enhanced antioxidant system [32]. ZnO nanoparticles concentration ranging from 500 to 1500 mg/L adversely affects seed germination, growth of *Brassica nigra* seedling and also lead to an increase in the antioxidative activities and non-enzymatic antioxidants [33].
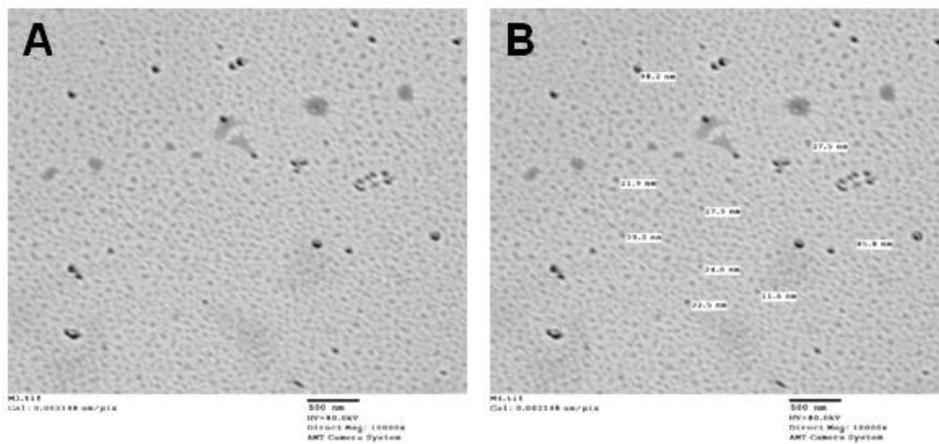
Fig. 1. (A) TEM micrograph of PVP/Zinc nanoparticles, (B) The corresponding size distribution of zinc nanoparticles
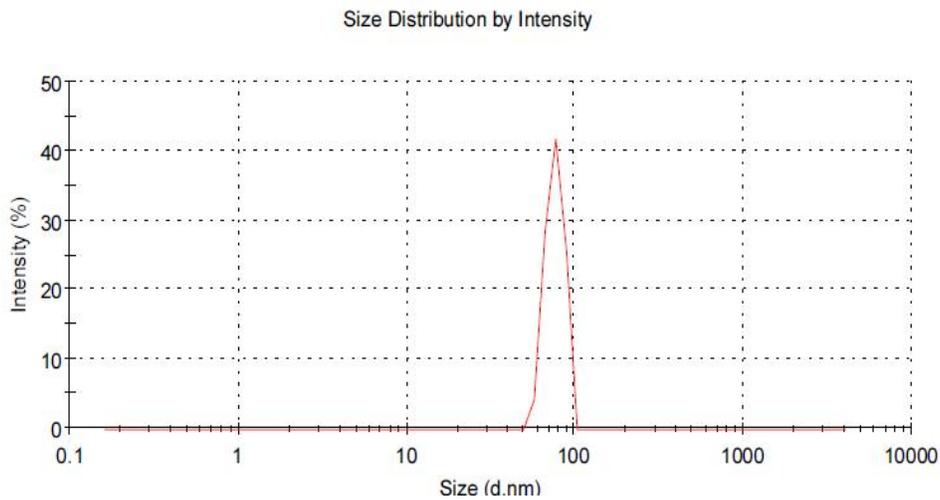
Fig. 2. DLS measurement of the zinc nanoparticles involved in diluted PVP nanogel solution

Table 1. Effect of pre-sowing gamma irradiation and nano-Zn (Zn NPs) soaking on germination, albino and damping off percentages

Treatments	Germination (%)		Albino (%)		Damping off (%)	
	1st	2nd	1st	2nd	1st	2nd
0 Gy+ 0 Zn NPs(control)	64.00 e	75.00 d	3.85 a	3. 66 a	10.99 a	19.58 a
0 Gy+ 500 Zn NPs	76.00 d	78.00 d	3.13 b	2.67 b	2.63 b	2.57 bc
0 Gy+ 1000 Zn NPs	82.00 bc	88.00 b	1.22 d	1.14 cd	1.22 c	1.14 d
0 Gy+ 2000 Zn NPs	78.00 cd	82.00 c	2.63 c	2.57 b	3.85 b	3.66 b
20 Gy + 0 Zn NPs	84.00 b	84.00 c	1.19 de	1.19 c	1.19 c	1.19 cd
20 Gy + 500 Zn NPs	94.00 a	90.00 b	1.06 ef	1.11 cd	1.06 c	1.11 d
20 Gy + 1000 Zn NPs	97.00 a	96.00 a	1.03 f	1.04 d	1.03 c	1.04 d
20 Gy + 2000 Zn NPs	96.00 a	94.00 a	1.04 f	1.07 d	1.04 c	1.07 d

Means in each column followed by the same letters did not differ at $p < 0.05$

Concerning to albino and damping off percentages the data showed that, in the tow experimental seasons pre-sowing gamma irradiation and soaking in Zn NPs decreased albino and damping off percentages. The lowest values of albino and damping off percentages were recorded by 20 Gy+1000 ppm Zn NPs, 20 Gy + 2000 ppm Zn NPs, respectively as compared with control.

3.3 Vegetative Growth Characters

The data in Table 2 showed that, there is positive relationship between the concentration of Zn NPs solution and stem length. Also, pre-sowing gamma irradiation and then soaking in Zn NPs solution enhancing stem length more than soaking in Zn NPs solution without gamma irradiation. In the two experimental seasons, the highest values of stem length were recorded by 20 Gy plus 2000 ppm Zn NPs as compared with control.

Stem length is important part of vegetative growth of citrus, if the plant has more height so there will be more number of branches and total number of leaves [34].

In this respect, the increase in shoot length in response of NPs might be nutritional behavior of particles or of dissociated ions but at non- lethal concentration [33]. The potential contribution of nano fertilizers to improving the growth, development and productivity of agricultural crops is their ability to increase both absorption and high interactivity [8]. The application of these nano- Zn fertilizers will be having high surface area and also required in very less quantity and promotes better absorption, translocation and hence reduces the Zn toxicity [35].

Concerning the stem diameter, the highest values of stem diameter was obtained by pre-sowing sour orange seeds with gamma irradiation at 20 Gy plus 500 or 1000 ppm Zn NPs respectively, at the two experimental seasons. The stem diameter of seedlings is reaching to the grafting size by 20 Gy +500 ppm Zn NPs treatment (4.49 and 4.88) as compared with control (2.36 and 2.78), respectively, in the two experimental seasons.

The major objective is increasing the growth rate of the main stem of the seedling in order to obtain a greater stem diameter for earlier grafting and budding, thereby shortening the time needed to produce a budded tree [36].

In this respect, low dose of gamma irradiation stimulates cell division, on the other hand, high-dose inhibits cell division due to free radicals and DNA system damage [37]. Effects of gamma rays on quantitative and qualitative characteristics of rice indicated that radiation dose somewhat improved growth characteristics, but with increasing dose rate, a decreasing trend is observed on the studied traits [38].

Also, although, zinc itself regulated the synthesis of endogenous plant hormones; presence of auxin also regulates local synthesis of cytokinin by controlling the expression of adenosinephosphate–iso pentenyl transferase (PsIPT) gene, which encodes a key enzyme in cytokinin biosynthesis [39]. Auxin and cytokinin also interact at metabolic level to control plant development and emergence of one part initiate to synthesize hormones for opposite one [40].

The data in Table 2 demonstrated that, all treatments increased leaves number and leaf area as compared with control at the two successive seasons. While, the highest values

were obtained by γ radiation at 20 Gy with 2000 or 1000 ppm Zn NPs, respectively.

In this respect, gamma irradiation has a profound influence on plant growth and development by inducing genetical, cytological, biochemical, physiological and morphogenetic changes in cells and tissues depending on the levels of irradiation [41]. Gamma irradiation had significant effect on average length and width of *Cucurma longa* leaves, inducing larger sized leaves at lower doses while higher doses had inhibitory effect on leaves length [42].

3.4 Leaf Chemical Composition

3.4.1 Plant Pigments

Concerning to the leaf pigments concentrations, the data in (Table 3) revealed that, in the two successive seasons all treatments increased the concentrations of chl. a chl. b and total chlorophyll, when compared with control, with some exceptions at the first season. Also, in the two experimental seasons, the highest values of chl. a, chl. b and total chlorophyll were obtained by 20 Gy +500 ppm Zn NPs treatment.

These results are in agreement with Prasad et al. [7] who noticed that, the use of nano Zn oxide in groundnut that higher leaf chlorophyll content was manifested by early flowering and effective in increasing stem and root growth, as well as Ramesh et al. [43] who observed that, there are increase in leaf chlorophyll and protein content of *Triticum aestivum* with nano-ZnO treatment.

The growth of a plant is very much dependent upon the amount of chlorophyll content as chlorophyll is the main pigment involved in the production of organic matter, i.e carbohydrates [44].

In this respect, increased chlorophyll content in Kagzi lime was due to the application of zinc and copper resulted in enhanced conversion of phylloxanthin to chlorophyllin [45]. Nano Zn treatment showed higher chlorophyll content, yield and shoot biomass of maize as compared to $ZnSO_4$ treatment [35].

Also, irradiating seeds before sowing with low gamma doses increased leaf content of chlorophyll a and b where both components exhibited the same response [46]. Growth and pigment composition of plants can be affected by both radiation and availability of nutrients [47].

Regarding to total carotenoids concentration, the data in Table 3 revealed that, in the two experimental seasons, there are significant and non- significant increases were recorded by all treatments. The highest values were obtained by 2000 ppm nano Zn with or without Gy irradiation.

In this respect, carotenoid content also rose gradually with the increasing of irradiation intensity [48].

3.4.2 Total phenols

It is clear from data presented in Table 4 that, there are negative relationship between the concentration of Zn NPs and phenolic compounds concentration, with some exceptions in the second season. The highest value of phenolic compounds was obtained by pre-sowing sour orange seeds with gamma irradiation at 20 Gy alone without soaking in Zn NPs solutions as compared with control.

In this respect, gamma rays belong to ionizing radiation and interact with atoms or molecules can damage or produce free radicals in cells. These radicals can damage or modify important component of plants eg. dilation of thalakoid membranes, alteration in photosynthesis, modulation of the antioxidative system and accumulation of phenolic compounds [49]. Accumulation of phenolic and flavonoid compounds was enhanced in leaf cells by gamma irradiation exposure [48]. There are an increase in non-enzymatic antioxidative molecules, phenolics and flavonoids, depending on NPs concentration [33].

3.4.3 Total indoles

The data in Table 4 showed that, in the two experimental seasons all treatment significantly increased the total indoles concentrations. The highest values were obtained by 20 Gy +1000 ppm Zn NPs as well as 20 Gy +2000 ppm Zn NPs, respectively when compared with control. The effect of nano- Zn treatments could be due to, the role of Zn in synthesis of IAA.

In this respect, zinc play a role in the auxin metabolism of plants, although details of the physiological processes involved have yet to be elucidate. The synthesis of tryptophan out of indole and seriene is known to be catalyzed by zinc [50]. Nanotechnology had significant effect on the regulation of plant hormones like auxin which is responsible for best root growth and seedling organization [51].

Table 2. Effect of pre-sowing gamma irradiation and nano- Zn (Zn NPs) soaking on growth characters of sour orange seedlings

Treatments	Stem length (cm)		Stem diameter (mm)		Number of leaves		Leaf area (cm^2)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0Gy+0 ZnNPs(control)	50.13 e	51.63 e	2.36 d	2.78 cd	14.80 f	16.53 f	8.68 g	8.90 e
0 Gy+ 500 Zn NPs	52.86 de	54.93de	3.38 bc	2.92 cd	21.60 de	23.80 de	13.76 e	14.16 cd
0 Gy+ 1000 Zn NPs	55.87 cd	56.03 cd	3.53bc	3.25 bc	22.90 cd	25.60 cd	14.76 de	15.97 c
0 Gy+ 2000 Zn NPs	60.57 ab	61.23 b	2.90 cd	2.59 d	26.10 bc	27.10 cd	15.53 cd	16.28 bc
20 Gy + 0 Zn NPs	53.60 de	55.58 cd	3.75abc	3.31 bc	19.00 e	20.26 ef	11.21 f	12.00 d
20 Gy + 500 Zn NPs	56.80 bcd	59.01 bc	4.49 a	4.88 a	27.40 b	27.70 bc	15.94 c	14.55 c
20 Gy + 1000 Zn NPs	60.11 abc	61.73 b	3.89 ab	3.63b	29.20 ab	31.23 ab	17.74b	18.30 b
20 Gy + 2000 Zn NPs	63.47 a	65.78 a	3.54bc	3.10 bcd	32.10 a	32.90 a	20.02 a	22.95 a

Means in each column followed by the same letters did not differ at p<0.05

Table 3. Effect of pre-sowing gamma irradiation and nano-Zn (Zn NPs) soaking on leaf pigments concentration (mg/g.f.w.) of sour orange leaves

Treatments	Chl. a		Chl. b		Total chl.		Total carotene	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0 Gy+ 0 Zn NPs (control)	0.893 b	0.909 d	0.383 b	0.386 d	1.273 d	1.290 d	0.153 d	0.155 f
0 Gy+ 500 Zn NPs	0.986 b	1.113 c	0.446 b	0.470 c	1.430 c	1.580 c	0.154 d	0.173 e
0 Gy+ 1000 Zn NPs	0.999 b	1.280 b	0.438 b	0.560 b	1.434 c	1.857 b	0.163 d	0.209 d
0 Gy+ 2000 Zn NPs	1.307 a	1.229bc	0.600 a	0.581 b	1.907 b	1.806 b	0.246 a	0.241 a
20 Gy + 0 Zn NPs	1.010 b	1.262bc	0.460 b	0.574 b	1.467 c	1.832 b	0.159 d	0.210cd
20 Gy + 500 Zn NPs	1.438 a	1.444 a	0.671 a	0.658 a	2.104 a	2.100 a	0.227 b	0.174 e
20 Gy + 1000 Zn NPs	1.288 a	1.332ab	0.608 a	0.615 ab	1.893 b	1.943 ab	0.210 c	0.221bc
20 Gy + 2000 Zn NPs	1.340 a	1.275b	0.606 a	0.583 b	1.941 b	1.855 b	0.245 a	0.225bc

Means in each column followed by the same letters did not differ at p<0.05

Table 4. Effect of pre-sowing gamma irradiation and nano -Zn(Zn NPs) soaking on total phenols, indoles, amino acids and carbohydrates concentrations of sour orange leaves

Treatments	Total phenols (mg/g.f.w.)		Total indoles (mg/g.f.w.)		Total amino acids (mg/g.f.w.)		Total carbohydrates (%)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0 Gy+0 Zn NPs (control)	1.536 f	1.866 c	0.746 g	0.833f	0.932 b	0.837 c	1.24 d	2.06 d
0 Gy+ 500 Zn NPs	1.824 b	1.804 e	0.839 e	0.923d	1.004 ab	0.904bc	2.42 bc	2.42 bc
0 Gy+1000 Zn NPs	1.665 e	1.819 d	0.964 d	0.935cd	1.059 ab	0.928 bc	2.60abc	2.42bc
0 Gy+2000 Zn NPs	1.493 g	1.273 h	1.001 c	0.955c	1.009 ab	1.001ab	2.68 ab	2.47abc
20 Gy+0 Zn NPs	2.145 a	2.205 a	0.811d	0.883e	1.193 ab	0.893bc	2.10 c	2.35 c
20 Gy+500 Zn NPs	1.683 d	1.501 g	1.007 c	1.085b	1.087 ab	0.991 abc	2.77 ab	2.64 ab
20 Gy+1000 Zn NPs	1.817 c	1.569 f	1.115 a	1.169a	1.215a	1.101 a	2.94 a	2.72 a
20 Gy+2000 Zn NPs	1.825 b	1.890 b	1.046 b	1.148a	1.125ab	1.026 ab	2.89ab	2.60 abc

Means in each column followed by the same letters did not differ at p<0.05

Table 5. Effect of pre-sowing gamma irradiation and nano-Zn (Zn NPs) soaking on minerals concentration of sour orange leaves

Treatments	N (%)		P (%)		K (%)		Zn (ppm)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
0 Gy+ 0 Zn NPs (control)	2.35bc	2.32 cd	0.200 d	0.203 d	1.160a	1.073c	26.30 c	27.07 d
0 Gy+ 500 Zn NPs	2.64abc	2.70 abc	0.230cd	0.25 abc	1.347a	1.547a	34.33 b	36.85 c
0 Gy+ 1000 Zn NPs	2.21 c	2.16 d	0.260bc	0.243 bc	1.333a	1.413ab	39.20 b	40.07 b
0 Gy+ 2000 Zn NPs	2.94 a	2.92 a	0.267bc	0.273 ab	1.273a	1.193c	44.85 a	46.67 a
20 Gy + 0 Zn NPs	2.44abc	2.54abcd	0.210 d	0.230 cd	1.167a	1.080c	27.51 c	27.75 d
20 Gy + 500 Zn NPs	2.55abc	2.71abc	0.277ab	0.273 ab	1.420a	1.393ab	35.02 b	35.82 c
20 Gy + 1000 Zn NPs	2.70 ab	2.74 ab	0.307 a	0.280 a	1.360a	1.387ab	44.45 a	45.88 a
20 Gy + 2000 Zn NPs	2.60abc	2.35 bcd	0.263bc	0.250abc	1.327a	1.380b	46.12 a	47.95 a

Means in each column followed by the same letters did not differ at p<0.05

3.4.4 Total free amino acids

It is obvious from data in Table 4 that, there are positive relationship between Zn NPs and the concentration of total free amino acids. In the two experimental seasons, the highest values of total free amino acids were obtained by 20 Gy plus 1000ppm Zn NPs when compared with control.

In this respect, zinc is very closely involved in the nitrogen metabolism of citrus, apple and grapes. In zinc deficiencies plants, protein synthesis and protein levels are drastically reduced whereas amino acids accumulate. The accumulation of amino acids occurs because zinc plays an important role in helping different combinations of amino acids link together to form enzymes and proteins. Without adequate levels of zinc, the plant is unable to synthesis the various enzymes and proteins therefore causing a buildup of amino acids [52]. Also, wheat grains from irradiated plants were also rich in proteins and essential amino acids [53]. Gamma irradiation affects proteins by causing conformational changes, oxidation of amino acids, rupturing of covalent bonds and formation of protein free radicals [5].

3.4.5 Total carbohydrates

Concerning to total carbohydrates concentration, in the two experimental seasons the highest value was recorded by 20 Gy +1000 ppm Zn NPs treatment when compared with control.

In this respect, irradiated samples exhibited increased carbohydrates over non-irradiated samples [54]. Available carbohydrate was increased with increasing doses of gamma rays [55].

In addition, the involvement of zinc in carbohydrates metabolism can be demonstrated through its effect on photosynthesis and sugar transformation [56]. Zinc and boron are essential component of enzymes responsible for nitrogen and carbohydrates metabolism respectively, there by resulting in increased uptake of nitrogen by the plant [57].

3.4.6 Minerals

Data in Table 5 demonstrated that, in the two experimental seasons the highest values of N concentration were obtained by 0 Gy + 2000 Zn NPs followed by 20 Gy + 1000 Zn NPs respectively, when compared with control.

In this respect, zinc is involved in the metabolism of nitrogen and a zinc deficiency will lead to a reduction in the RNA produced and thus in cell division [58]. Also, N level in soybean shoots was slightly affected by gamma doses. When total N content "uptake" was taken into consideration, low gamma doses markedly increased it, and vice versa with high doses [59].

As regarding to phosphorus concentration, the data showed that, pre-sowing gamma irradiation and then soaking sour orange seeds in nano- Zn solution increased phosphorus concentration as compared with control, with some exceptions. The highest values were obtained by 20 Gy+ 1000 ppm nano-Zn followed by 20 Gy +500ppm nano-Zn, respectively at the two experimental seasons when compared with control.

In this respect, irradiating seeds of some vegetable crops before sowing with low doses up to 20 Gy increased P concentration in leaves, while dose of 40 Gy decreased it [60]. Also, treatment with all Zn ion and ZnO NPs concentrations resulted in significant decline in seedling P levels [61].

The data in Table 5 revealed that, in the second season all treatments significantly increased potassium concentration as compared with control, with some exceptions.

Similar results were obtained by Hussein *et al*. [62], who found that, the effect of irradiation on macro-nutrients in datura leaves. Doses ranged from 1 to 15 K rad had significant effect on some nutrients. Maximum value of potassium in leaves was obtained at 5 K rad as well as El-Essawy [63], who noticed that irradiating gladiolus corms with low gamma doses increased K content.

Concerning to zinc concentration, the data in Table 5 showed that, soaking sour orange seeds in nano- Zn solution increased Zn leaves concentration. There is a positive relationship between Zn concentration and the soaking in nano –Zn solution.

In this respect, there is fast dissolution of Zn ions from ZnO nanoparticles, same nominal concentrations of ZnO NPs as well as Zn ions. This will facilitate the plants to be exposed to similar concentrations of Zn ions released from ZnO NPs as well as Zn ions [61].

3.5 Roots Characters

It is clear from the data presented in Table 6 that, in the second season soaking sour orange seeds

Table 6. Effect of pre-sowing gamma irradiation and nano-Zn (Zn NPs) soaking on Horizontal, Vertical extension and dry matter of sour orange roots

Treatments	Horizontal root (cm)		Vertical roots (cm)		Dry matter (%)	
	1st	2nd	1st	2nd	1st	2nd
0 Gy+ 0 Zn NPs(control)	29.50 a	25.50 c	17.83 bc	22.83 b	45.66 b	45.83 b
0 Gy+ 500 Zn NPs	32.84 a	38.00 a	36.00 a	29.00 a	45.89 b	48.09 ab
0 Gy+ 1000 Zn NPs	31.33 a	28.75 bc	17.00 bc	22.34 b	62.76 a	57.91 a
0 Gy+ 2000 Zn NPs	31.00 a	31.00 bc	22.25 b	19.67 bc	51.42 ab	47.95 ab
20 Gy + 0 Zn NPs	30.67 a	28.50 bc	34.25 a	29.00 a	43.24 b	46.32 ab
20 Gy + 500 Zn NPs	31.92 a	29.50 bc	13.58 c	18.33 bc	50.01 b	46.00 b
20 Gy + 1000 Zn NPs	29.50 a	26.50 bc	13.00 c	15.83 c	50.61 ab	50.70 ab
20 Gy + 2000 Zn NPs	32.67 a	31.83 b	17.17 bc	20.00 bc	52.33 ab	54.81 ab

Means in each column followed by the same letters did not differ at p<0.05

in Zn NPs at 500 ppm without gamma irradiation followed by 20 Gy +2000 ppm Zn NPs treatments significantly increased horizontal root extension. While, vertical root increased significantly by 0 Gy + 500 ppm Zn NPs as well as gamma irradiation 20 Gy without Zn NPs, at the two experimental seasons as compared with control.

Regarding to roots dry matter percentage, the data showed that, in the two experimental seasons the highest values of dry matter percentage were obtained by 0 Gy + 1000 ppm Zn NPs and 20 Gy + 2000 ppm Zn NPs treatments, respectively as compared with control.

These results are in agreement with those were obtained by Pramod et al. [64] who noticed that, absorbed more of ZnO nanoparticles promoted the root length biomass of *Vigna radiate* and *Cicer arietinum* and Melki and Marouani [65] who mentioned that, the roots obtained from wheat seeds irradiated at the dose of 20 Gy are significantly improved in number and in length as compared to the non-irradiated ones.

In this respect, citrus apparently has difficulty in absorbing sufficient zinc from many soils, and anything that affects the root system adversely is likely to reduce the intake markedly. In connection with root injury, other mineral deficiencies may have a pronounced effect on zinc deficiency, if this element is present in the soil in minimum quantities [66]. ZnO nanoparticles may induce roots from explants cultured on appropriate medium that can be used for production of valuable secondary metabolites. The induction of roots can be explained in two ways; function of zinc in biochemical process, and role of reactive oxygen species (ROS) [34].

3.6 Protein Analysis

The data in Fig. 3 represents the banding pattern of protein peptides in treated and untreated sour orange seedling (*Citrus aurantium*) with zinc nanoparticles and gamma radiation. In total, 18 protein subunits were observed. Variability in intensity was observed in some bands indicated that the quantity of protein peptides cumulating at a particular molecular weight (10- 20 KD). The protein markers plotted for first principal components that revealed 12 distinct groups (Fig. 3).

Principal analysis based on SDS-PAGE revealed clear grouping pattern when investigated for treatment with zinc nanoparticles and gamma radiation. Protein group pattern intensity (10-20 KD) increased in the treated sour orange seedlings with Zn NPs (1000 and 2000 ppm) compared to control and Zn NPs (500 ppm) respectively, with some exceptions. On the other hand protein pattern around 80 KD disappeared in the treated sour orange seedlings with 1000 and 2000 ppm of zinc nanoparticles.

Protein group pattern (10-20 KD) enhanced in the treated sour orange seedlings with gamma rays 20 gray; zinc nanoparticles (500 ppm) and gamma rays 20 gray; zinc nanoparticles (1000 ppm) and gamma rays 20 gray respectively, while the protein group pattern above 60 KD disappeared.

On the other hand, protein group pattern between (10-20 KD) and above 60 KD down regulated in the treated sour orange seedlings with gamma ray 20 gray and Zn NPs (2000 ppm). The genotypes of sour orange seedling (*Citrus aurantium*) treated with Zn NPs and

gamma rays were separated clearly with clear variable differences between treated sour orange seedlings and the control.

Sour orange seedlings treated with zinc nanoparticles (500, 1000 and 2000 ppm) and 20 gray of gamma radiation showed clear separation of protein pattern desilk the control with distinctive 12 main protein bands.

In this regard, it is important to clarify that, radiation either affects the gene structure and this change becomes permanent (mutation) or that affects the behavior of the gene in terms of activity (gene transcription and protein composition), which increases the gene activity.

This is the desired change in this case. Also, the genomic changes were demonstrated by principal analysis based on SDS-PAGE (Fig. 3) as different bands that disappeared or appeared (Table 7) when comparing 0 Gy + 1000 ppm Zn NPs treatment with control. Appearance of new patterns by this treatment may be explained by changes in the gene structure due to mutation. Generally, it is possible to ensure that the change is permanent or temporary by vegetative propagation of treated plants. If the changes in vegetative traits persist, that mean the change is permanent and if the changes in vegetative traits didn't reappear, it is temporary. Although, 20 Gy + 500 ppm Zn NPs treatment exhibits the best results for stem diameter, which is the main
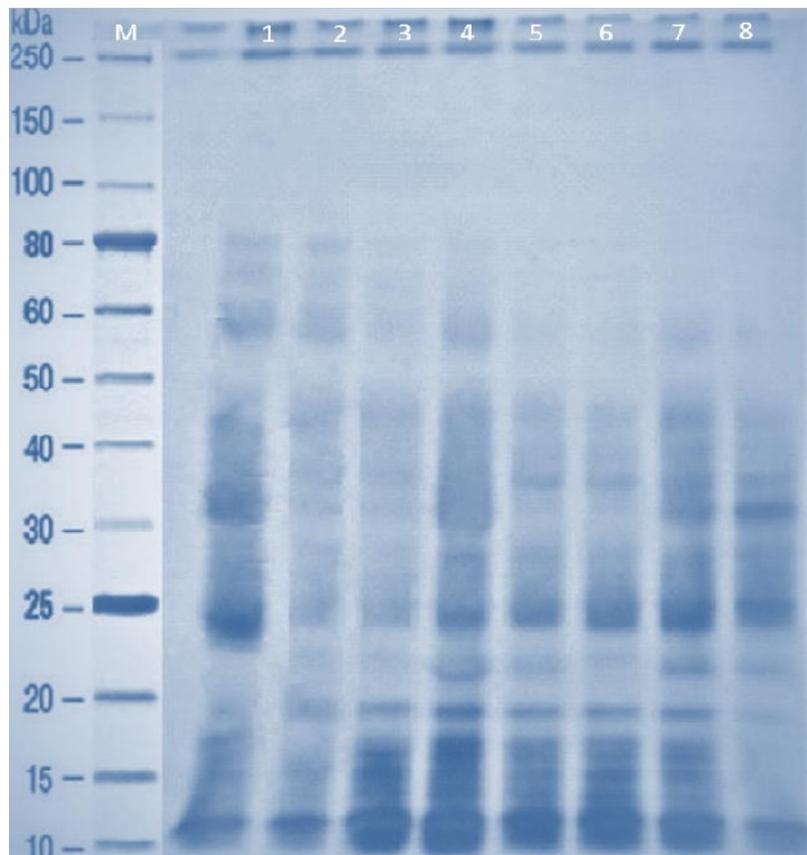
Fig. 3. SDS-PAGE separation of HMW and LMW protein subunits from sour orange seedling (*Citrus aurantium*)treated and untreated with gamma radiation and zinc nanoparticles. Lane M: Pre-stained Dual Colour Protein Molecular Weight Marker (10-250 kDa) molecular weight marker; Lane 1: untreated sour orange seedling (control); Lane 2: sour orange seedling treated with zinc nanoparticles (500 ppm); Lane 3: sour orange seedling treated with zinc nanoparticles (1000 ppm); Lane 4: sour orange seedling treated with zinc nanoparticles (2000 ppm); Lane 5: sour orange seedling treated with gamma rays 20 gray; Lane 6: sour orange seedling treated with gamma rays 20 gray and zinc nanoparticles (500 ppm); Lane 7: sour orange seedling treated with gamma rays 20 gray and zinc nanoparticles (1000 ppm); Lane 8: sour orange seedling treated with gamma rays 20 gray and zinc nanoparticles (2000 ppm)

Table 7. Effect of pre-sowing gamma irradiation and nano –Zn (Zn NPs) soaking on protein groups of orange sour seedlings

Treatments / Protein groups	Control	0 Gy +500 Zn NPs	0 Gy +1000 Zn NPs	0 Gy + 2000 Zn NPs	20Gy + 0 Zn NPs	20 Gy + 500 Zn NPs	20 Gy + 1000 Zn NPs	20 Gy + 2000 Zn NPs
250	X	X	X	X	X	X	X	X
150	X	X	X	X	X	X	X	X
100	X	X	X	X	X	X	X	X
80	√	√	X	√	X	X	X	X
60	√	√	X	√	√	X	√	X
50	X	X	X	X	X	X	√	X
40	√	√	√	√	√	√	√	√
30	√	√	√	√	√	√	√	√
25	√	√	√	√	√	√	√	√
20	X	√	√	√	√	√	√	√
15	√	√	√	√	√	√	√	X
10	√	√	√	√	√	√	√	√

characteristic for the success of grafting process, the obtained results showed changes in proteins and it possible the gene structure had been changed due to this treatment, that was so clear when compared with control. Thus, care must be taken when using nanotechnology, because it's small size and high surface reactivity which can potentially enter into the cell and interact with intracellular structures, this may be produce toxicity by different mechanisms. Adequate studies should be carried out on the safety of using nanotechnology in the field of plants because of their impact on the plant gene structure, which reflects on human health.

In this respect, in soybean seedlings evaluation the phytotoxicity of ZnO NPs at the proteome level; 16 common proteins in leaves were significantly changed, which were predominantly associated with protein degradation and photosystem [67]. NPs can potentially interact not only with DNA but also with proteins involved in DNA replication, transcription or repair [68]. Also, exposure of *Arabidopsis* to ZnO leads to distinct changes in the expression of stress genes [69]. Indirect genotoxicity resulting from interaction of nanoparticles with the nuclear proteins (proteins involve in replication, transcription and translations) or stress oxidative induced by reactive oxygen species and also by reduced DNA repair functions [70].

4. CONCLUSION

In conclusion, it could be reported that the successful formation of zinc nanoparticles inside the PVP network. Dynamic light scattering measurements indicate the nanoscale of zinc particles. These results are slightly agreed with the mentioned results obtained from TEM. Also, 20 Gy +1000 ppm Zn NPs treatment increased seed germination percentage, stem length, as well as decreased the albino and damping off percentages, while 20 Gy + 500 Zn NPs treatment increased stem diameter and plant pigments concentration. Generally, using gamma irradiation and then soaking part of radiated seeds in nano-Zn solutions had significant effects on vegetative growth and root characters as well as some chemical properties of sour orange leaves. In addition, there are variable differences in the protein pattern between treated and untreated sour orange seedlings with gamma radiation and / or zinc nanoparticles. Although, 20 Gy + 500 ppm Zn NPs treatment increased stem diameter, which is the main characteristic for the success of grafting process, the obtained results showed changes in proteins and it possible the gene structure had been changed due to this treatment. So, care must be taken when using nanomaterials and adequate studies should be carried out on the safety of using nanotechnology in the plant field.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Nawaz MA, Ahmad WD, Mumtaz MK. Role of growth regulators on preharvest fruit drop, yield and quality in kinnow

mandarin. Pak. J. Bot. 2008;40(5):1971-1981.

2. Davies FS, Albirgo LG. Citrus. CAB International, Florida, USA. 1998;254.

3. Filho FAA, Espinoza-Nunez E, Stuchi ES, Ortega EMM. Plant growth, yield, and fruit quality of 'Fallglo' and 'Sunburst' mandarin on four rootstocks. Scienti Horticulture. 2007;114(1):45-49.

4. Forner-Giner MA, Alcaide A, Primo-Millo, E, Forner JB. Performance of Navelina orange on 14 rootstocks in Northern Valencia (Spain). Sci. Hortic. 2003;98: 223–232.

5. Piri I, Babayan M, Tavassoli A, Javaheri M. The use of gamma irradiation in agriculture. African Journal of Microbiology Research. 2011;5(32):5806 5811.

6. Swietlik D. Zinc nutrition in horticultural crops. In: J. Janick (Ed.), Horticultural Reviews. John Wiley and Sons. 1999;109-118.

7. Prasad TN, Sudhakar P, Sreenivasulu Y, Latha P, Munaswamy V, Reddy KR, Pradeep T. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. Journal of Plant Nutrition. 2012;35(6):905-927.

8. Fawzy ZF, Yunsheng L, Shedeed SI, El-Bassiony AM. Nanotechnology in agriculture - current and future situation. Research & Reviews: Journal of Agriculture and Allied Sciences. 2018;7(2): 73-76.

9. Khodakovskaya MV, de Silva K, Biris AS, Dervishi E, Villagarcia H. Carbon nanotubes induce growth enhancement of tobacco cells. ACS Nano. 2012;6(3):2128–2135.

10. Nair R, Varghese SH, Nair BG, Maekawa T, Yoshida Y, Kumar DS. Nanoparticulate material delivery to plants. Plant Sci. 2010;179(3):154–163.

11. Torney F, Trewyn BG, Lin VSY, Wang K. Mesoporous silica nanoparticles deliver DNA and chemicals into plants. Nat Nanotechnol. 2007;2:295–300.

12. Kumar R, Unstedt HM. Silver ion release from antimicrobial polyamide/silver composites. Biomaterials. 2005;26:2081-2088.

13. Hartmann HT, Kaster DE. Plant propagation principles and pactices. 2nd Edition, Prentice- Hall, Inc. Cliffs, Newjersy; 1983.

14. Nornai R. Formula for determination of chlorophyllous pigments extracted with N.N Dimethyl formamide. Plant Physiol. 1982;69:1371-1381.

15. Swain T, Hillis WF. The quantitative analysis of phenolic constituent. J. Sci., Food Agric. 1959;10:63-69.

16. Larsen P. On the biogensis of some indole compounds. Physiol. Plants. 1962;15:552-565.

17. Jayaraman. Postharvest biological control. Wiely Eastern Limited. New Delhi; 1985.

18. Miller GL. Analytical Chemistry. 1959;31: 426-428.

19. Plummer DT. An introduction to practical biochem. Published by Mc Graw Hill Book Company (U.K.) Limited; 1971.

20. King EJ. Micro-analysis in medical biochemistry. 2nd Ed., Churchil, London. 1951;222.

21. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature. 1970; 227(5259):680-685.

22. Rabilloud T, Carpentier G, Tarroux P. Improvement and simplification of low-background silver staining of proteins by using sodium dithionite. Electrophoresis. 1988;9(6):288–291.

23. Snedecor GW, Cochran W. Statistical methods 7th Ed., Iawa State Univ., Press. Ames. Iawa, USA. 1982;507.

24. Duncan DB. Multiple range and multiple F. Tests Biometrics. 1955;11:1-24.

25. Abedini A, Larki F, Saion EB, Zakaria A, Hussein MZ. Radiation formation of Al–Ni bimetallic nanoparticles in aqueous system. J. Radioanal. Nucl. Chem. 2012b;292:1–6.

26. Abedini A, Saion E, Larki F. Radiation-induced reduction of mixed copper and aluminum ionic aqueous solution. J. Radioanal. Nucl. Chem. 2012a;292:983–987.

27. Eid ME, Salah MB, Soliman ME, Hegazy EA. Radiation synthesis and characterization of poly(vinyl alcohol)/poly(N-vinyl-2-pyrrolidone) based hydrogels containing silver nanoparticles. Inorg, J. Organomet. Polym. 2017;27:

1482–1490. [J. Polym. Res. 19, 9835, 2012].

28. Jawaharlal M, Sambandamoorthy S, Irulappan I. Effect of gamma ray and EMS on seed germination and seedling growth in acid lime (*Citrus aurantifolia*, Swingle). South Indian Hort. 1991;39(6):332-336.

29. Vose PB. Introduction to nuclear technique in agronomy and plant biology. First Ed. Pergman Press Ltd., Headington Hill Hall Oxford OX3 OB. V. England; 1980.

30. Kim JS, Beek, MH, Lee YK, Park YI. Stimulating effect of low dose gamma ray radiation on the growth and physiological activities of Chinese cabbage cultivars; 2004.

    Available:www.http://csiro/publish.com

31. Van Dongen JT, Ammerlaan AMH, Wouterlood M, Van Aelst ACV, Borstlap AC. Structure of the developing pea seed coat and the post phloem transport pathway of nutrients. Ann. Bot. 2003;91: 729–737.

    DOI: 10.1093/aob/mcg 066.

32. Lu CM, Zhang CY, Wen JQ, Wu GR, Tao MX. Research of the effect of nanometer materials on germination and growth enhancement of *Glycine max* and its mechanism. Soya Bean Science. 2002;21: 168-172.

33. Zafar H, Ali A, Ali JS, Ihsan UH, Ziz M. Effect of ZnO- nanoparticles on *Brassica nigra* seedlings and stem explants, growth dynamics and antioxidative response. Frotiess in Plant Sciences. 2016;7:1-8.

34. Khan N, Ali AK, Ahmad M, Nouman, M, Islam B. Evaluation and screening of sweet orange cultivars for vegetative growth and citrus canker. Sarhad Journal of Agriculture. 2016;32(2):121-126.

35. Rameshraddy SM, Geetha KN, Shankar AG. ZnO nanoparticle improves maize growth, yield and seed zinc under high soil pH condition. Int. J. Curr. Microbiol. App. Sci. 2018;7(12):1593-1601.

36. El-Otmani M, Coggins CWJ, Agusti M, Lovatt CJ. Plant growth regulators in citiculture: World current uses. Critical Reviews in Plant Sciences. 2000;19(5): 395-447.

37. Zaka R, Chenal C, Misset MT. Effects of low doses of short term gamma irradiation on growth and development through two

38. generations of *Pisum sativam*. Science of the Total Environment. 2004;320:121-129.

38. Saha A, Santra SC, Chanda S. Modulation of some quantitative characteristics in rice (*Orayza sativa*) by ionizing radiation. Radiat. Physic. Chem. 2005;74:391-394.

39. Tanaka M, Takei K, Kojima M, Sakakibara H, Mori H. Auxin controls local cytokinin biosynthesis in the nodal stem in apical dominance. Plant J. 2006;45:1028–1036.

    DOI: 10.1111/j.1365-313X.2006.02656.x

40. Nordstrom A, Tarkowski P, Tarkowska D, Norbaek R, Astot C, Dolezal K. Auxin regulation of cytokinin biosynthesis in *Arabidopsis thaliana*: A factor of potential importance for auxin-cytokinin –regulated development. Proc. Natl. Acad. Sci., U.S.A. 2004;101:8039–8044.

    DOI: 10.1073/pnas.0402504101

41. Akshatha KRC, Souframanien HMS. Effect of gamma irradiation on germination, growth, and biochemical parameters of *Terminalia arjuna* Roxb. Radiation Protection and Environment. 2015;36:39-44.

42. Ilyas S, Naz S. Effect of irradiation on morphology characteristics and isolation of cucurminoids and oleeoresoids of *Cucurma longa* L. The Journal of Animal & Plant Sciences. 2014;24(5):1396-1404. ISSN: 1018-7081.

43. Ramesh M, Palanisamy K, Babu K, Sharma NK. Effects of bulk & nano-titanium dioxide and zinc oxide on physio-morphological changes in *Triticum aestivum* Linn. J Glob Biosci. 2014;3:415-422.

44. Idrees M, Danish S, Khan MA. Effect of gamma a radiation on the chlorophyll content of brinjal. Indian J. Applied & Pure Bio. 2007;22(2):317-318.

45. Singh K, Prasad, Singh K. Effect of micro-nutrient on yield physic-chemical characteristics of Kagzi lime. Indian J. Hort. 2000;64(2):216-218.

46. Taha ATA. Effect of radiation and chemical fertilization on the productivity of Coriander (*Coriandrum sativum* L.) plant. M.Sc. Thesis, Fac. of Agric., Cairo Univ.; 2000.

47. Lavola A, Aphalo PJ, Lahti M, Julkunen-Tiitto R. Nutrient availability and the effect of increasing UV-B radiation on secondary plant compounds in Scots pine.

Environmental and Experimental Botany. 2003;49:49-60.

48. Mohajer S, Mat RMT, Ma ML, Khorasani AE, Khalili M. Stimulatory effects of gamma irradiation on phytochemical properties, mitotic behavior, and nutritional composition of sainfoin (*Onobrychis viciifolia* Scop.). Hindawi Publishing Corporation Scientific World Journal. 2014;1-9.

49. Kim JS, Lee EK, Back DH, Lee KYB. Influence of low dose γ radiation on the physiology of germinative seed of vegetable crops. Korean J. Env Agric. 2000;19:58-61.

50. Bergmann W. Nutritional disorders of plants. Gustav Fischer Verlag Jena Stuttgart, New York; 1992.

51. McLamore ES, Diggs A, Marzal CP, Shi J, Blakeslee JJ, Peer WA, Murphy AS, Porterfield DM. Noninvasive quantification of endogenous root auxin transport using an integrated flux microsensor technique. Plant J. 2010;63:1004–1016.

52. Dickinson K, Oʹ Brien J, Voet L, Edwards T. Metalosate "zinc in plant nutrition" ALBION. 2003;4(2):1-4.

53. Mashev N, Vassilev G, Ivanov K. A study of N-allyl N-2 pyridyl thiourea and gamma radiation treatment on growth and quality of peas and wheat. Bulgerian J. Plant Physiol. 1995;21(4):56–63.

54. Bamidele OP, Akanbi CT. Effect of gamma irradiation on physicochemical properties of stored pigeon pea (*Cajanus cajan*) flour. Food Science & Nutrition. 2013;1(5):377-83.

55. Devi MP, Sahoo MR, Kuna A, Deb P, Dasgupta M, Prakash N. Effect of gamma irradiation on nutritional properties and antinutrient contents of *Citrus jambhiri* Lush. fruits. Journal of Pharmacognosy and Phytochemistry. 2018;7(4):2833-2836.

56. Robson AD. Zinc in soils and plants. Soil Sci. and Plant Nutr., School of Agric. The Univ. of Western Australia Perth Western Australia. 1993;69-106.

57. Kumar NCJ, Rajngam J, Balakrishman K, Sampath PM, Kavya MV. Influence of foliar application of micronutrients on tree growth and chlorophyll statuse of mandarin orange (*Citrus reticulate Blanco*) under lower pulney Hills., Int. J. Pure App. Bioci. 2017;5(2):1100-1104.

58. Coetzee JGK. Citrus Research International (Pty) Ltd. Chapter 12: Zinc. 2007;45-46.

59. Maghraby GM. Physiological response of soybean and wheat to gamma radiation and gibbrellin. Ph.D. Thesis, Fac. of Agric., Ain Shams Univ.; 1997.

60. Orabi IOA. Effect of seed irradiation and salinity on growth, chemical composition, flowering and seed production and its quality of cowpea. Egypt. J. Appl. Sci. 1998;13(1):201-215.

61. Nair PMG, Chung IM. Regulation of morphological, molecular and nutrient status in *Arabidopsis thaliana* seedlings in response to ZnO nanoparticles and Zn ion exposure. Science of the Total Environment. 2017;575:187–198.

62. Hussein MS, EL-Sherbeny SE, Naguib NY. The effect of gamma radiation and manganese application on growth and chemical constitutes of *Datura metel* L. Egypt. J. Physiol. Sci. 1995;19(1-2):241-254.

63. El-Essawy MMA. Effect of radiation and gibberllic acid on the growth and flowering of Gladiolus corms. Ph.D. Thesis, Fac. of Agric. Ain Shams Univ.; 1995.

64. Pramod M, Dhoke SK, Khanna A. Effect of Nano-ZnO particle suspension on growth of mung (*Vigna radiata*) and gram (*Cicer arietinum*) seedling using plant agar method. J. Nanotechnology. 2011;7. Article ID: 696535.
DOI: 10.1152011/696535./696535

65. Melki M, Marouani A. Effects of gamma rays irradiation on seed germination and growth of hard wheat. Environmental Chemistry Letters. 2010;8(4):307-310.

66. Srivastava AK, Singh S. Zinc nutrition and citrus decline - A review. Agric. Rev. 2004;25(3):173–188.

67. Hossain Z, Mustafa G, Sakata K, Komatsu S. Insights into the proteomic response of soybean towards Al2O3, ZnO and Ag nanoparticles stress. J. Hazard Mater. 2016;304:291–305.

68. Magdolenova Z, Collins A, Kumar A, Dhawan A, Stone V, Dusinska M. Mechanisms of genotoxicity: A review of *in vitro* and *in vivo* studies with engineered nanoparticles. Nanotoxicology. 2014;8: 233–278.

69. Landa P, Vankova R, Andrlova J, Hodek J, Marsik P, Storchova H, White JC, Vanek

T. Nanoparticle-specific changes in *Arabidopsis thaliana* gene expression after exposure to ZnO, TiO₂, and fullerene soot. J. Hazard Mater. 2012;241–242:55–62. DOI: 10.1016/j.jhazmat.2012.08.059

70. Karami SM, De Lima R. Nanoparticles cyto and genotoxicity in plants: Mechanisms and abnormalities. Environmental Nanotechnology, Monitoring & Management. 2016;6:184-193.