Effect of AgNps and different sources of Zinc on rooting and acclimation of strawberry (*Fragaria ananassa* Duch) cv. Rubygem *in vitro*

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Abstract

This study was conducted to examine the effect of adding different concentrations of nano silver nitrate (0, 5, 10 and 15 mg L^{-1}) and zinc source {traditional(0.86mg L^{-1} as $ZnSO_4.7H_2O$) and nano zinc 0.086 mg L^{-1} as Zinc oxide) to the specifies medium of rooting strawberry (*Fragaria X ananassa* Duch) cv. Rubygem, in addition to testing the efficiency of nano silver nitrate and zinc source on successful percentage of cultures adapting stage. Results revealed that highest rooting percentage, root numbers, roots length, leaves content of total chlorophyll and macro nutrients (N, P and K) were achieved the highest average at 10mg L^{-1} + nano zinc treatment. The source of cultures resulting from rooting stage affected the plant survival at acclimatization stage. The treatment 10 and 15mg L^{-1} of nano silver nitrate + nano zinc achieved 100% success percentage.

Keywords: strawberry micropropagation, nano silver nitrate, zinc source, rooting stage, acclimatization, Nano Zn oxide**.**

Introduction

Strawberry (*Fragaria X ananassa* Duch.) belongs to Rosaceae family is small fruits plant spread widely worldwide. This genus includes more than 45 wild and cultivated species. It is believed that the origin of this plant is the Alps mountain regions and the Massif Central region in France, from which it spread to the rest of Europe and North Asia (10). Strawberry is commercially cultivated for their nutritional and medicinal value which either consume fresh or after processing (6, 25). The commercial production of strawberry has started in Iraq in recent years, where a suitable environment has been found for this crop in some areas (15).

Strawberry is propagated by two main methods: sexually by seeds and asexually by runners. The sexual method produces new genetic combinations; thus, it is not desirable by farmers. Instead, they rely on asexual propagation by runners (stolons), where the plants are genetically identical to the original plant, and they also reach maturity faster. The main challenge for plants cultivated by vegetative methods is the transmission of plant virus diseases to the seedlings. Various attempts have been made to reproduce strawberry cultivars that are free of plant diseases using tissue culture in a short period of time without being limited by the breeding cycle (29). Nevertheless, *in vitro* cultures growth is affected by several factors, including mineral nutrients, which play an important role in metabolic processes, reduce contamination, and the production of secondary compounds (32). In the recent years, attention has turned to the use of modern technologies in agriculture, including agro-nanotechnology as it increased nutrient efficiency (19). Several Nano composites are unique due to their high surface area and small particles, which contribute to increased vital processes and physiological activities (24). Zinc, one of the essential micronutrients for plant growth, is involved in the content of nutrient media as well as stimulation of metabolic processes in plants such as photosynthesis, protein synthesis, pollen formation, and nitrogen synthesis (17). Moreover, zinc is essential for producing chlorophyll and starch by effects on the enzymes of their formation (30). Many studies have shown that zinc can significantly improve plant growth *in vitro*, including *Brassica nigra* (33), *Phoenix dactylifera* (7), *Coffea arabica* (13) and *Musa acuminata* (20). Moreover, Alharby *et al*., (1) found that 15mg.L^{-1} of nano zinc increased tomato callus growth and multiplication. In contrast, Javed *et al*, (22) found that the highest percentage of shoots formation, the average shoot length, and the average fresh weight were achieved with *Stevia rebaudiana* when nano zinc oxide was added at 1mgL^{-1}. Studies published by Awad *et al*., (8) and Al-Mayahi (3) reported that nano zinc oxide had a significant effect on the growth of palm trees cultures. The aim of the current study was therefore to examine the effect of adding different concentrations of nano silver nitrate and zinc sources (traditional: 0.86mg L^{-1} as $ZnSO_4.7H_2O$) and nano zinc as 0.086mg L^{-1} as Zinc oxide) on strawberry multiplication and rooting. Additionally, the study examined the effectiveness of nano silver nitrate and zinc source on successful percentages of plantlets during the weaning stage.

Materials and Methods

The Present study was conduct in tissue culture laboratory - Directorate of Agriculture , Al-Najaf Governorate during the period 1 October 2021 to 1 April 2022, in order to observe and evaluate the rooting response of strawberry shoots, four concentrations of nano silver nitrate (0, 5, 10 15 mg L^{-1}) were use and two zinc sources {traditional(0.86mg L^{-1} as $ZnSO_4.7H_2O$) and nano zinc 0.086 mg L^{-1} as Zinc oxide) was employed.

Rooting medium

Solutions of MS and MS modification medium were prepared with {traditional (0.86mg L^{-1} as $ZnSO_4.7H_2O$) and nano zinc 0.086mg L^{-1} as Zinc oxide) respectively. 1mg L^{-1} of IBA was added with 7g L^{-1} of agar to the nutrient medium. Shoots of strawberry were planted in jars with 30ml of nutrient medium (50 replicates) then cultures were incubated in growth room at 25^0C and luminous intensity for 16h followed by 8h of dark daily for a period of 6 weeks then root percentage, number of roots, and length (cm) were calculated at the end of the rooting stage.

Chemical properties of plant total vegetative

The total chlorophyll was determined in leaves based on the approach proposed by Goodwin (16). Leaves content of nitrogen: Nitrogen was estimated using microKjeldahl as outlined by Al-Sahaf (5).

Leaves content of phosphorous: It was estimated using ascorbic acid–ammonium hepta molybdate then measured by UV-Visible Spectrophotometer at 420nm as described by Al-Sahaf (5).

Leaves content of potassium: Potassium content in leaves was estimated using the Flame photometer device, followed Horneck and Hanson (21).

Acclimatization of plants

Rooted plantlets came from various treatments were washed carefully with tap water to remove traces of adhering medium. Plantlet then dipped with fungicide (Benlate 1.0ml L-1) for 1 minute. After that, plantlet was planting in 7 cm diameter pots filled with autoclaved substrate (peat moss, sand and perlite in 3:1:1 ratio). Plantlets were initially covered with transparent polyethylene cover to prevent transpiration and to maintain relative humidity at 95% for 4 weeks and kept in the culture room at 25±1ºC under light and irrigated with MS nutrient solution (28) every 10 days. One month later, the survive percentage of plantlet was calculated according to equation below:

Number of successful acclimatized plants =

$$\frac{\text{The percentage of successful acclimatized}}{\text{Total number of acclimatized plants}} \times 100$$

Statistical analysis

A factorial experiment was arranged using complete randomize design (CRD) with 10 replicates. Data were analyses using GenStat program. The differences between means were tested using Duncan multiple range test at 5% level of significance ($P_{>0.05}$) (4).

Results and Discussion

The results showed that due to the difference in the concentration of nano-silver nitrate, there were significant differences in rooting rate. The best rooting percentage was achieved by 10 mg

L^{-1} nano-silver nitrate (75%), while the control treatment recorded the lowest percentage of 32.5% (Table 1).

As a result of the interaction between nano silver and zinc source, rooting percentage was significantly increased when 10mg L^{-1} + nano zinc treatment was applied over other treatments. where, the highest percentage amounted to 90.0% and showed no difference from (15mg L^{-1} + nano zinc treatment). Meanwhile, treatment 0mg L^{-1} + traditional zinc produced a rooting percentage of 30%.

Table 1. The effect of nano silver nitrate zinc source in MS medium and their interaction on the percentage of rooting after 6 weeks of planting

Concentration of nano silver nitrate (mg L^{-1})	Rooting percentage%		The average of nano silver concentration
	Traditional zinc (0.86(mg L^{-1} $ZnSO_4.7H_2O$)	Nano zinc (0.086mg L^{-1} as nano Zinc oxide)	
0	30.0	35.0	32.5
5	40.0	45.0	42.5
10	60.0	90.0	75.0
15	50.0	80.0	65.0
The average of zinc source	45.0	62.5	
L.S.D 0.05	Nano Silver	Zinc source	Nano Silver X Zinc source
	15.80	11.17	22.35

The recorded results in (Table -2) indicated that there were significant differences in the root numbers rate according to the differences in nano-silver nitrate concentrations. However, the highest rate of root numbers (7.50) was recorded in the silver nitrate treatment (10mg L-1). A significant difference was seen in the average root number after adding zinc and nano- silver nitrate to the culture medium. Most effective result was observed with Nano-Silver Nitrate + Nano-Zinc (10 mg) (Table-2).

Table 2. The effect of nano silver nitrate zinc source in MS medium and their interaction on the average of roots number after 6 weeks of planting

Concentration of nano silver nitrate	roots number		The average of nano silver concentration
	Traditional zinc	Nano zinc	

(mg L^{-1})	(0.86(mg L^{-1} $ZnSO_4.7H_2O$)	(0.086mg L^{-1} as nano Zinc oxide)	
0	3.67	3.33	3.50
5	4.00	4.33	4.17
10	6.00	9.00	7.50
15	5.00	8.33	6.67
The average of zinc source	4.67	6.25	
L.S.D 0.05	Nano Silver	Zinc source	Nano Silver X Zinc source
	1.580	1.117	2.235

Results in (Table -2) indicated that there were significant differences in the root length according to the differences in nano-silver nitrate concentrations. However, the highest root length average (10.83cm) was recorded compare to 5.17cm for 0mg L^{-1} treatment (Table 3). A significant difference was seen in root length after adding nano zinc alone or Inco bination with nano- silver nitrate to the culture medium. Most effective result was observed with 10 mg Nano-Silver Nitrate + Nano-Zinc (Table-3).

Table 3. The effect of nano silver nitrate zinc source in MS medium and their interaction on the average of roots length(cm) after 6 weeks of planting

Concentration of nano silver nitrate (mg L^{-1})	Root length(cm)		
	Traditional zinc (0.86(mg L^{-1} $ZnSO_4.7H_2O$)	Nano zinc (0.086mg L^{-1} as nano Zinc oxide)	The average of nano silver concentration
0	3.67	6.67	5.17
5	6.33	5.67	6.00
10	10.33	11.33	10.83
15	10.67	9.67	10.17
The average of zinc source	6.75	8.33	
L.S.D 0.05	Nano Silver	Zinc source	Nano Silver X Zinc source
	4.832	3.416	6.833

One of the most critical components of photosynthetic pigments is chlorophyll, which is susceptible to damage under various circumstances, including the nutrient medium composition. It is obvious from the results that the concentration of nano-silver nitrates increases the chlorophyll content of the plant. Based on the recorded results, nano-silver nitrate concentration significantly increased chlorophyll content. the highest total chlorophyll content was recorded with 10 mg-1 of nano-silver nitrate treatment. According to the study, the chlorophyll content of strawberry leaves increased significantly with an increase in nano-silver nitrate concentration. Where the highest value of total chlorophyll content was recorded with 10mg l-1 nano-silver nitrate treatment (57.10mg 100g-1 fresh weight). There were no significant differences between treatments using 5 and 15 mg L-1 of nano silver nitrate. Study also found that chlorophyll content was also affected by zinc sources that were added to the culture medium. With respect to total chlorophyll content, nano-zinc proved to be the most effective treatment, yielding high chlorophyll levels (57.82mg 100g-1 fresh weight). In contrast, traditional zinc sources yielded the lowest chlorophyll content (44,30 mg 100g-1 fresh weight) (Table-4). The interaction between nano silver nitrate and zinc source had significant effect on shoot dry weight when 10mg L^{-1} nano silver nitrate + nano zinc treatment was achieved the highest content amounted 66.10mg $100g^{-1}$ fresh weight, while, the lowest content was recorded in the control treatment (0mg L^{-1} of nano silver nitrate + traditional zinc) reached 35.60 mg 100 g^{-1} fresh weight.

Table 4. The effect of nano silver nitrate zinc source in MS medium and their interaction on the total leaves content of chlorophyll (100g-1 fresh weight) at rooting stage

Concentration of nano silver nitrate (mg L^{-1})	Total chlorophyll (100g-1 fresh weight)		The average of nano silver concentration
	Traditional zinc (0.86(mg L^{-1} $ZnSO_4.7H_2O$)	Nano zinc (0.086mg L^{-1} as nano Zinc oxide)	
0	35.60	41.40	38.50
5	48.30	64.10	56.20
10	48.10	66.10	57.10
15	45.20	59.70	52.45
The average of zinc source	44.30	57.82	
L.S.D 0.05	Nano Silver	Zinc source	Nano Silver X Zinc source
	8.91	6.32	12.10

Table 5 showed that there were significant differences in the concentration of nitrogen content in shoots based on the nano silver nitrate concentration used in the nutrient medium. However, a high nitrogen content (2.04%) was observed in shoots grown on medium content (15mg L^{-1}) Ag nano-particals. Whereas, shoots grown on control medium had a lower nitrogen content (1.51%). Conversely, adding zinc to the culture medium resulted in a significant increase in nitrogen content in the leaves. Where, nano zinc treatment resulted in the highest content of nitrogen (2.05%), while the traditional zinc treatment reduced the nitrogen content level to 1.51%. Also, high nitrogen content (2.17%) was obtained in shoots grown on medium supplemented with 10 mg nano silver along with nano-zinc. Results from 15 mg L^{-1} of nano silver nitrate, however, showed no significant differences. In contrast, the control treatment (0mg L^{-1} of nano silver nitrate + traditional zinc) exhibited the lowest nitrogen content (1.13%).

Table 5. The effect of nano silver nitrate zinc source in MS medium and their interaction on the leaves content of nitrogen (%) at rooting stage

Concentration of nano silver nitrate (mg L^{-1})	N% Traditional zinc (0.86(mg L^{-1} $ZnSO_4.7H_2O$)	Nano zinc (0.086mg L^{-1} as nano Zinc oxide)	The average of nano silver concentration
0	1.13	1.88	1.51
5	1.94	1.99	1.97
10	1.85	2.17	2.01
15	1.92	2.16	2.04
The average of zinc source	1.51	2.05	
L.S.D 0.05	Nano Silver	Zinc source	Nano Silver X Zinc source
	0.400	0.283	0.566

The phosphorus content analysis study for shoots found that it level was increased to 0.45% on medium enriched with 10 mg L^{-1} nano-silver nitrate. The same results were obtained with shoots grown in medium supplemented with nano zinc, where phosphorus content increased to 0.47 % (Table-6). The interaction treatments differed significantly according to the concentration of nano silver nitrate and nano zinc. The interaction of 10mg L^{-1} of nano silver + nano zinc treatment was superior in recording the highest rate for P% in leaves.

Table 6. The effect of nano silver nitrate zinc source in MS medium and their interaction on the leaves content of phosphorous (%) at rooting stage

Concentration of nano silver nitrate (mg L^{-1})	P% Traditional zinc (0.86(mg L^{-1} $ZnSO_4.7H_2O$)	Nano zinc (0.086mg L^{-1} as nano Zinc oxide)	The average of nano silver concentration
0	0.31	0.43	0.37

5	0.32	0.42	0.37
10	0.32	0.52	0.42
15	0.30	0.52	0.41
The average of zinc source	0.31	0.47	
L.S.D 0.05	Nano Silver	Zinc source	Nano Silver X Zinc source
	0.024	0.017	0.034

Results in Table (7) cleared that leaf content of K significantly increased by adding nano silver nitrate to culture medium compared with the control. Also, it could be observed that, cultured which grow in media supplemented with nano zinc achieved significant increasing in shoots content of potassium. The interaction treatments showed that there were significant effect of nano silver nitrate nano zinc on K%, when treatment 15mg L-1 of nano silver + nano zinc was exceeded control treatment(0mg L^{-1} of nano silver nitrate + traditional zinc) and recorded the highest K% in leaf amounted 1.73%,, while the lowest average 0.466%.was recorded in the control treatment.

Table 7. The effect of nano silver nitrate zinc source in MS medium and their interaction on the shoots content of potassium (%) at rooting stage

Concentration of nano silver nitrate (mg L^{-1})	K% Traditional zinc (0.86(mg L^{-1} $ZnSO_4.7H_2O$)	K% Nano zinc (0.086mg L^{-1} as nano Zinc oxide)	The average of nano silver concentration
0	0.57	0.64	0.60
5	0.66	0.72	0.69
10	1.43	1.66	1.54
15	1.52	1.73	1.62
The average of zinc source	1.04	1.18	
L.S.D 0.05	Nano Silver	Zinc source	Nano Silver X Zinc source
	0.077	0.054	0.109

Zinc is a micro nutrients (17) which has important functions in the biosynthesis pathway of IAA from tryptophan (12). Nano $AgNo_3$ and nano zinc have different physical and chemical properties than bulk metals. Properties such as lower melting points, higher specific surface areas, specific optical properties, mechanical strength and specific magnetizations make nanoparticles attractive in various agriculture industrial applications like plant tissue culture (24). The positive effect of silver nitrate and zinc oxide that added to nutrient medium for the formation of adventitious roots for strawberry as they affected rooting percentage, roots number and roots length. The reason may be attributed to their role in endogenous auxin biosynthesis which can be synthesized naturally in plants, and so the action of auxin,was increases the number of meristem sites at the base of shoots, which increases the process of dedifferentiation of specialized tissues and transform them into meristematic cells by the process which form root initial, which continues to grow and develop into the root primordium, which grows outside the stem tissues, forming the adventitious root(18). These results are coincided with Helaly *et al.* (20) and El-Mahdy *et al.,* (14) in banana, Karakeçili *et al.* (23) in *Pyrus* spp. and Alizadeh and Dumanoglu (2) in apple in increasing of rooting percentage and roots number when adding nano silver and nano zinc to the nutrient medium. Increasing total chlorophyll content in leaves of strawberry when adding nano zinc may be due to its entering to cultured shoot tissues in a large amounts more than the traditional zinc and for its characterized by ease of diffusion, which makes it more available to plants due to the smallness of the nanoparticles, which facilitates the penetration of the pores of the cell walls easily to the vascular bundles and from there to the tissues of the leaf(26), so it activated the reactions of chlorophyll synthesis process(31). Moreover, it participates in the vital reactions necessary for the chlorophyll and carbohydrates synthesis and participates as a cofactor for enzyme carbonic anhydrase, which has a pH-regulating effect inside the chloroplasts and thus keeps proteins from losing their vital nature (11). Also the increasing of leaves content of nutrients as a result of zinc may attributed to the fact that nano fertilizers are provide a big surface area for the metabolic reactions which increases photosynthesis, as a result, the need of nutrients that the plantlets required to uptake from the medium encourages to continuous its growth(27). so, the increase in the root growth (Tables 1,2and 3) increased the activity of the plantlets in absorbing water and nutrients, which was reflected in the increase in the availability of these nutrients, and leads to an increase in their uptake from the medium and then an increase in their concentration in the leaves. Our findings coincide with Al-Mayahi(3)who found that nZnO and was effective on total chlorophyll in fronds of *in vitro* date palm plantlets

Results in (Table 8) showed the percentage of successful transferred plantlets from rooting stage after 30 days to pots as according to its source. Plantlets that transferred from shoots produced in medium supplied by 10 and 15mg L^{-1} of nano silver nitrate + nano zinc showed high successful acclimatization percentage (100%) compare to 85% in 0mg L^{-1} of nano silver nitrate + traditional zinc (control treatment). The reason for that may be due to the effect of nano zinc and nano silver on rooting percentage and improvement of rooting system of plantlets and this is in consistent with Barbosa *et al,* (9).

Table 8. The effect of cultures source (treatments of nano silver and nano zinc interaction at rooting stage) on the percentage of successful plant acclimatization

Cultures source at rooting stage	Plantlets successful acclimatization percentage %

0mg L^{-1} of nano silver nitrate + 0.86mg L^{-1} $ZnSO_4.7H_2O$	85.00
5mg L^{-1} of nano silver nitrate + 0.86mg L^{-1} $ZnSO_4.7H_2O$	90.00
10mg L^{-1} of nano silver nitrate + 0.86mg L^{-1} $ZnSO_4.7H_2O$	95.00
15mg L^{-1} of nano silver nitrate + 0.86mg L^{-1} $ZnSO_4.7H_2O$	100.00
0mg L^{-1} of nano silver nitrate + 0.086mg L^{-1} as Nano Zinc oxide	90.00
5mg L^{-1} of nano silver nitrate + 0.086mg L^{-1} as Nano Zinc oxide	80.00
10mg L^{-1} of nano silver nitrate + 0.086mg L^{-1} as Nano Zinc oxide	100.00
15mg L^{-1} of nano silver nitrate + 0.086mg L^{-1} as Nano Zinc oxide	100.00
L.S.Dp> 0.05	12.74

Conflict of interest

The authors have no conflict of interest.

References

1- **Alharby H.F.; E.M.R. Metwali; M.P. Fuller and Aldhebiani Y.A. 2016**. Impact of application of zinc oxide nanoparticles on callus induction, plant regeneration, element content and antioxidant enzyme activity in tomato (*Solanum lycopersicum* Mill.) under salt stress. Arch. Biol. Sci., 68:723–735.

2- **Alizadeh, S. and H. Dumanoglu. 2022**. The effects of zinc oxide nanoparticles loaded with IAA and IBA on in vitro rooting of apple microcuttings. Turkish Journal of Agriculture and Forestry, 46(3), 306-317.

3- **Al-Mayahi A.M.W. 2021**.The effect of humic acid (HA) and zinc oxide nanoparticles (ZnO-NPS) on *in vitro* regeneration of date palm (*Phoenix dactylifera* L.) cv. Quntar. Plant Cell Tiss. Organ Cult., 145: 445–456. https://doi.org/10.1007/s11240-021-02020-7.

4- **Al- Rawi, K. M. and A. Khalaf-Alla.2000**.Design and Analysis of Agricultural Experiments. College of Agriculture and Forestry. University of Mousel. Iraq. pp.487.

5- **Al-Sahaf, FH.1989**.Applied Plant Nutrition. Dar al-Hikma Press. University Baghdad. Ministry of Higher Education and Scientific Research. Iraq. pp.258.

6- **Amatori S.; L. Mazzoni; J. Alvarez-Suarez; M. Gasparrini; T. Y. Forbes-Hernandez; S. Afrin; A. E. Provenzano; G. Persico; B. Mezzetti; A. Amici; M. Fanelli and Battino, M .2016**. Polyphenol-rich strawberry extract (PRSE) shows *in vitro* and *in* *vivo* biological activity against invasive breast cancer cells. Sci. Rep., 6: 30917. https://doi.org/10.1038/srep30917.

7- **Amiri H.; M. Mousavi and Torahi M.A. 2016**.Improving date palm (*Phoenix dactylifera* L.CV. Estamaran) calogenesis by the use ofzinc oxide nanoparticles. Journal of Experimental Biology and Agricultural Sciences, 4(5): 557-563. DOI: 10.18006/2016.4(5).557.563.

8- **Awad K.M.; A.M.W. Al-Mayahi; M. A. Mahdi; A. S. M. Al-Asadi and Abass M. H. 2020**.*In vitro* assessment of ZnO nanoparticles on *Phoenix dactylifera* L. Micropropagation. Sci. J. King Fai. Univ. (Basic and Applied Sciences), 21(1):149–161. https://doi.org/10.37575/b/agr/2000.

9- Barbosa W.M., Otoni W.C., Carnelossi M., Silva E., Azevedo A.A. and Vieira G. 2001. Rhizogenesis in Vitro Shoot Cultures of Passion Fruit (*Passifloraedulisf. flavicarpa* Deg.) Is Affect by Ethylene Precursor and by Inhibitors. International Journal of Horticultural Science, 7, 47-51.

10- **Darnell R.L.2003.** Strawberry Growth and Development. The Strawberry: A Book for Growers, Others. Gainesville, FL: Dr. Norman F. Childers Publications. Taylor and Francis Group. London. England. pp.3-10.

11- **Das S and A. Green. 2016**. Zinc in crops and human health. In Biofortification of Food Crops. Springer, New Delhi. India. pp.31-40.

12- **Davies P. J. 2010**.Plant Hormones: Biosynthesis, Signal Transduction. Action. Springer. Netherlands.

13- **Devasia J.; B. Muniswamy and Mishra M. K.2020**.Investigation of ZnO Nanoparticles on *In Vitro* Cultures of Coffee (*Coffea Arabica* L.). International Journal of Nanoscience and Nanotechnology, 16(4): 271-277.

14- **El-Mahdy M. T.K.; A. A. Radi and Shaaban M. M.2019**.Impacts of exposure of banana to silver nanoparticles and sliver ions *in vitro*. Middle East J. Appl. Sci., 9(3):727-740.

15- **Franco S., 2009**. The Production of Strawberries in Iraq. U.S. Agency for International development (USAID). USA, pp.1-10.

16- **Goodwin T.W. 1976**.Chemistry and Biochemistry of Plant Pigment. 2nd ed. Academic Press, N.Y., Sanfrancisco, USA. pp.373.

17- **Hacisalihoglu G. 2020**. Zinc (Zn): The Last Nutrient in the Alphabet and Shedding Light on Zn Efficiency for the Future of Crop Production under Suboptimal Zn. Plants, 9: 1471. DOI: 10.3390/plants9111471.

18- **Hartmann, H. J.; D. E. Kester; R. L. Geneve and Davies, J. F. T.2014**.Plant Propagation: Principles and Practices. (8thedn.) Pearson Education Limited. USA.

19- **Hayat S.; J. Pichtel; M. Faizan and Fariduddin Q. 2020**.Nanotechnology for Plant Growth and Development. Sustainable Agriculture Reviews 41. Springer. Northlands.

20- **Helaly, M. N.; M. A. El-Metwally; H. El-Hoseiny; S. A. Omar and El-Sheery N. I .2014.** Effect of nanoparticles on biological contamination of *in vitro* cultures and organ genic regeneration of banana. Australian Journal of Crop Science 8(4):612-624

21- **Horneck D. A. and D. Hanson 1998**. Determination of Potassium and Sodium by Flame Emission Spectrophotometry. pp. 153-155. (In: Kalra, Y. P., (ed). Handbook of Reference Methods for Plant Analysis.Soil and Plant Analysis Council, Inc., CRC Press. FL., USA. PP. 287.).

22- **Javed R.; M. Usman; B. Yücesan; M. Zia and Gürel E.2017.**Effect of zinc oxide (ZnO) nanoparticles on physiology and steviol glycosides production in micropropagated shoots of *Stevia rebaudiana* Bertoni. Plant Physiol. Biochem.,110:94–99.doi: 10.1016/j.plaphy.2016.05.032.

23- **Karakeçili A; S. Korpayev; H. Dumanoğlu and Alizadeh, S.2019**.Synthesis of indole-3-acetic acid and indole-3-butyric acid loaded zinc oxide nanoparticles: Effects on rhizogenesis. Journal of Biotechnology, 303: 8-15. doi: 10.1016/j.jbiotec.2019.07.004.

24- **Kim D.H.; J. Gopal and Sivanesan I.2017**.Nanomaterials in plant tissue culture: the disclosed and undisclosed. RSC Adv., 7:36492– 36505. https://doi.org/10.1039/C7RA0 7025J.

25- **Liu L.; M. L. Ji; M. Chen; M. Sun; X. L. Fu; L. Li; D. S. Gao and Zhu C. Y. 2016.** The flavor and nutritional characteristic of four strawberry varieties cultured in soilless system. Food Sciencis and Nutrition, 6(4): 858-68. doi: 10.1002/fsn3.346.

26- **Ma X.; J. Geiser-Lee; Y. Deng and Kolmakov A.2010**.Interactions between engineered nanoparticles (ENPs) and plants: phytotoxicity, uptake and accumulation. Science of the Total Environment, 408(16):3053-3061. doi: 10.1016/j.scitotenv.2010.03.031.

27- **Malakooti S. H.; M. Majidian; S. M. Ehteshami and Rabiee M.2017**.Evaluation of iron and zinc foliar and soil application on quantitative and qualitative characteristics of two soybean cultivars. IIOAB Journal, 8(3):1-7.

28- **Murashige T. and F. Skoog.1962**. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15:473-497.

29- **Palei S.; A. Kumar Das and Rout G. R. 2015.** *In vitro* Studies of Strawberry - An Important Fruit Crop: A Review. Jour. Pl. Sci. Res., 31 (2) 115-131.

30- **Rudani K. and V. P. K. Prajapati.2018**. The importance of zinc in plant growth: A review. International Research Journal of Natural and Applied Sciences, 5(2):38-48.

31- **Ruttkay-Nedecky B.; O. Krystofova; L. Nejdl and Adam J.2017**. Nanoparticles based on essential metals and their phytotoxicity. Journal of Nanobiotechnology, 15:33.

32- **Tariq A.; S. Ilyas and Naz S.2020**.Nanotechnology and Plant Tissue Culture. (In: Javad, S. (eds) Nanoagronomy. Springer, Cham. https://doi.org/10.1007/978-3-030-41275-3_2.).

33- **Zafar H.; A. Ali; J. S. Ali; I. U. Haq and Zia M. 2016**. Effect of ZnO nanoparticles on *Brassica nigra* seedlings and stem explants: growth dynamics and

antioxidative response. Front. Plant Sci., 7:535.
https://doi.org/10.3389/fpls.2016.00535.