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Improving Shoot Multiplication Of Strawberry (Fragaria Ananassa L. Cv. Roby Gem) In Vitro By Using Agnps And Iron Nanoparticles

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ABSTRACT

The current study was carried out to study the effect of adding different concentrations of nano silver nitrate (0, 5, 10 and 15 mg L^{-1}) to the cultures in initiation stage of strawberry (Fragaria ananassa cv. Ruby Gem) and adding the same concentrations of iron (traditional and nano iron) on shoot multiplication stage. Results showed that there were significant differences between nano silver nitrate concentrations in initiation stage as it increased the response percentage, number and length of shoots and reduced the percentage of contamination of cultures at 10 and 15 mg L^{-1} concentrations compare to the control treatment (0 mg L^{-1}). The outcome of shoot multiplication experiment indicated that silver nitrate concentration and the source of iron separately had significant positive effect on studied traits as well as their interaction, when the 10 mg L^{-1} of nano silver nitrate and nano iron treatment gave the highest average of shoots number and its dry and fresh weight as it reached 11.00 shoot, 2.23g and 0.26g respectively in comparison with lowest average recorded 5.33 shoot, 1.28g and 0.16g respectively in control treatment with traditional iron.

Key words: strawberry, nano silver nitrate, traditional iron, initiation stage, shoot multiplication.

INTRODUCTION

The cultivation of strawberry (Fragaria ananassa L. Duch) is spreading in more than 64 countries and its productivity amounted 9.22 million ton globally (FAO 2017, and many arab countries are cultivated it widely particularly Egypt, Syria, Palestine and Lebanon. However, the cultivation of this vegetable crop is limited in some areas, scientific experiment stations and gardens, with low consuming rate and most strawberry are imported from Syria, Iran and Turkey. Strawberry is reproduced by two main methods, sexual method when the seeds are used to produce new cultivars but this method is not preferred due to different varieties that produced from original plant and low germination percentage of its seeds. Therefore, many growers use the asexual method (vegetative method) widely by dividing plants that produce few rhizoid as this method is considered the most dominant to produce strawberry commercially (Mohan et al., 2005; Neamah et al., 2020). One of the main challenges that facing plants cultivated by vegetative method is the infection with one or more plant pathogens especially viruses which transferred from infested to healthy plants by aphid insects and lead to reduce the growth and yield. Many attempts has been done to reproduce the important

cultivars of strawberry by free of plant diseases using tissue culture in short period without being restricted to the breeding date (Palei et al 2015; Kadhim and Abdulhussein 2021). The growth of cultures outside of living body is affected by several factors including mineral nutrients in the medium which play significant role and contributed in metabolic processes. In recent years, attention has turned to the use of modern technologies in the field of plant nutrition such as nanotechnology as it increased the efficiency of nutrients (Ghorbanpour et al 2017; Saleh and Hameed, 2019). Nanoparticles were used in tissue culture, improve seed germination in vitro, genetic modification, reduce microbial activity and production of secondary compounds (Ruttkay-Nedecky et al 2017). Silver nitrate and iron oxides are among the nanocomposites that have been tested for their efficiency in improving the growth of tissue cultures of different plants as they reduce fungal and bacterial contamination (Arab et al 2014; Shokri et al 2014). In addition, nano silver nitrate and iron oxides that added to tissue culture media has a positive effect on improving the growth of plant parts (Alvarez et al 2019), for instance, banana cultures (Helay et al 2014), palm trees (Amiri et al 2016), apple (Avestan et al 2018) and potato (Al-Jibouri et al 2017).

Thus, the aim of current study was conducted to examine the effect of adding different concentrations of nano silver nitrate to the cultures in initiation stage of strawberry and adding the same concentrations of iron (traditional and nano iron) on shoot multiplication stage.

MATERIALS AND METHODS

The study was carried out in tissue culture laboratory - Directorate of Agriculture of Al-Najaf Governorate from 1/4/2020 to 1/5/2021. Strawberry plant parts were obtained from rhizoids growing in greenhouses.

Preparing the culture medium

MS medium was used (Murashige and Skoog 1962) with vitamins, inositol, 30g of sucrose, growth regulators (Table 1) and nano compounds (Table 2) based on multiplication stage then the size was completed to 1L by distilled water and pH maintained to 5.7 ± 0.1 by HCl or 0.1 NaOH. 7g/L of Agar-Agar was added and the medium was put on hot plate magnetic stirrer to mix the medium then poured in culture tubes (10ml/tube) and autoclaved at 121^0C, 1.04kg/cm^2 for 20 minutes, after that, tubes left to cool down at room temperature.

Table 1. Growth regulators added to the medium based on multiplication stage.

Multiplication stage	Combination of growth regulators
Cultures initiation stage	0.5mg L^{-1} of BA + 0.1mg L^{-1} of IBA
Shoots multiplication	1mg L^{-1} of BA + 0.1mg L^{-1} of IBA

Table 2. Characteristics of nanocomposites used in experiments.

Nano compound	Particle size (nano meter)	Purity	Suppling company
$AgNO_3$	50 nm	99.99	Hangwu International Group LTD.China
Fe_2O_3	20 nm	99%	Skysping Nanomaterials,Inc, USA

The effect of nano silver nitrate on cultures initiation

Runner tips of strawberry (Ruby Gem cultivar) were collected, leaves were removed and the top of Runner were cut into 1cm then washed under tap water with liquid soap for 30 minutes and put in antioxidant solution consist of 100mg L^{-1} ascorbic acid + 150mg L^{-1} citric acid for 15 minutes to get rid of phenolic secretions of the tissue used in culture and transferred to laminar air flow cabinet for surface sterile. Runner tips were sterile by submerging it in Clorox (6% of sodium hypochlorite) for 15 minutes then washed three times to get rid of Clorox residues, plant parts then transferred to Petri plates and the apex was excised.

Excised apex was put in culture tubes by needle and grow on MS medium with adding nano silver nitrate (0, 5, 10 and 15 mg L^{-1}) to the medium and making 10 replicates for each treatment (1 plant part for each replicate). Cultures were incubated in growth room for 21 days at 25^0C±2 and 1000 lox luminous intensity for 16h followed by 8h of dark daily for a period of 4 weeks then the following indicators were recorded:-

The percentage of contamination after 15 days: It was calculated according to the following equation:

$$\text{Contamination \%} = \frac{\text{The number of contaminated tubes}}{\text{Total number of tubes}} \times 100$$

The percentage of response (percentage of open buds): It was calculated using the following equation:

$$\text{Percentage of open buds \%} = \frac{\text{The number of contaminated tubes}}{\text{Total number of tubes}} \times 100$$

The length of shoot: The length of the shoot formed in each of the cultivation tubes was calculated using a sterile ruler after extracting plants at the end of initiation stage to grow it on the medium that prepared for multiplication stage.

The number of shoots: The number of the shoot formed in each of the cultivation tubes was calculated at the end of initiation stage for each replicate and divided on shoots number to get the average of shoot length in one replicate.

The effect of nano silver nitrate and iron oxide on shoots multiplication stage

The shoots that produced at initiation stage were cultured in 250ml containers contain 30ml of culture medium and incubated for 30 days. When enough cultures were obtained, an experiment was conducted to examine the effect of nano silver nitrate (0, 5, 10 and 15 mg L^{-1}) and adding the same concentrations of iron (traditional and nano iron) on shoot multiplication stage for a period of 4 weeks then the following indicators were calculated:

The average of shoots formed (plant^{-1}): The number of the shoot formed in each container was calculated at multiplication stage and the shoots of each treatment were divided on the number of replicates.

The average of leaves formed: Leaves of each plant was calculated then the average of leaves for each treatment was obtained.

Fresh weight of total vegetative of multiplication shoots (mg): It was calculated after the end of incubation stage of multiplication using sensitive scale.

Dry weight of total vegetative of multiplication shoots (mg): It was calculated after the end of incubation stage by taking 10 replicates and put it in electric oven at 48^{0}C until the weight is stable.

Statistical analysis

A factorial experiment was arranged using randomize complete block design (RCBD) with 10 replicates. The differences between means were tested using least significant difference test (L.S.D.) at 5% level of significance (P>0.05) (AL–Rawi and Khalf Allah 2000). Data were analysed using GenStat International 12.1 VSN (2009).

RESULTS AND DISCUSSION

Results of Table 3 showed that the percentage of plant parts grew on the culture medium that supplied with nano silver nitrate in culture tubes after surface sterile was higher compare to control treatment (without adding nano silver nitrate), as this percentage decreased when the concentration of silver nitrate increased. Treating plant parts with 10 and 15 mg/L concentration of nano silver nitrate was gave the lowest percentage of contamination reached 0% and was not significantly differ between both concentrations.

Adding nano silver nitrate was significantly affected the percentage of open buds as the results indicated the superiority of plant part grown in the nutritional medium MS prepared with 10 and 15 mg/L of nano silver nitrate in its response to ex vivo cultivation and reached 90 and 100% respectively compared to control treatment by 50%. There were significant differences in the average of shoots number as a result of adding different concentrations of nano silver nitrate when the 10 mg/L was exceeded other concentrations and achieved the highest average 2.76 shoot with no significant difference from 15 mg/L concentration in comparison with the lowest average 1.33shoot in control. Results also showed that there was no significant effect of adding nano silver nitrate on shoot length.

Table 3. The effect of nano silver nitrate on the percentage of contamination, the percentage of open buds, shoots number and the length of shoot after 3 weeks of culturing.

Concentration of nano silver nitrate (mg L^{-1})	The percentage of contamination %	The percentage of open buds %	Shoots number	The length of shoot (cm)
0	20.00	50.00	1.33	3.57

5	10.00	80.00	1.36	4.10
10	0.00	90.00	2.76	4.17
15	0.00	100.00	2.06	4.37
L.S.D 0.05	10.00	30.75	0.64	1.76

The increasing of response percentage and the decreasing of contamination was occurred as a result of nano silver nitrate which is very effective as anti-fungal or bacterial (Kim et al 2017), in addition, silver is one of the inhibitors of ethylene, which is produced and gathered in tissue culture vessels, whether it is produced by cultivated plant tissues or generated by the flame used to sterilize the nozzles of culture vessels, which considered one of the factors that negatively affecting cultures growth and their continued development later, as it works to curb the multiplication of shoots and the production of buds (Kumar et al 1998). High concentrations of ethylene are increase the effectiveness of cellulase and pectinase enzymes or both which damages cells and thus fails the development process (Salman 1988). According to Saltveit et al (1977), the reason for increasing of response percentage and shoots growth is the presence of silver nitrate as it works to inhibit the action ethylene by binding to the active part of cytolytic enzymes which inhibits their work and stimulates growth. Results of current study are in agreement with the findings of Shokri et al (2014) on shrub roses, Huong et al (2021) on banana and Sreelekshmi (2021) on clove, in terms of the positive effect on the response of cultures and reducing contamination.

Results showed that there were significant differences between treatments in the average of shoots multiplication as a result of adding nano silver nitrate to the culture medium when the 10mg L^{-1} treatment was exceeded other treatment except the 15mg L^{-1} treatment and achieved the highest average 10.00 shoot. While, the 5mg L^{-1} treatment gave the lowest average 6.00 shoots which was not significantly differ from control and 15mg L^{-1} treatments(Fig.1).

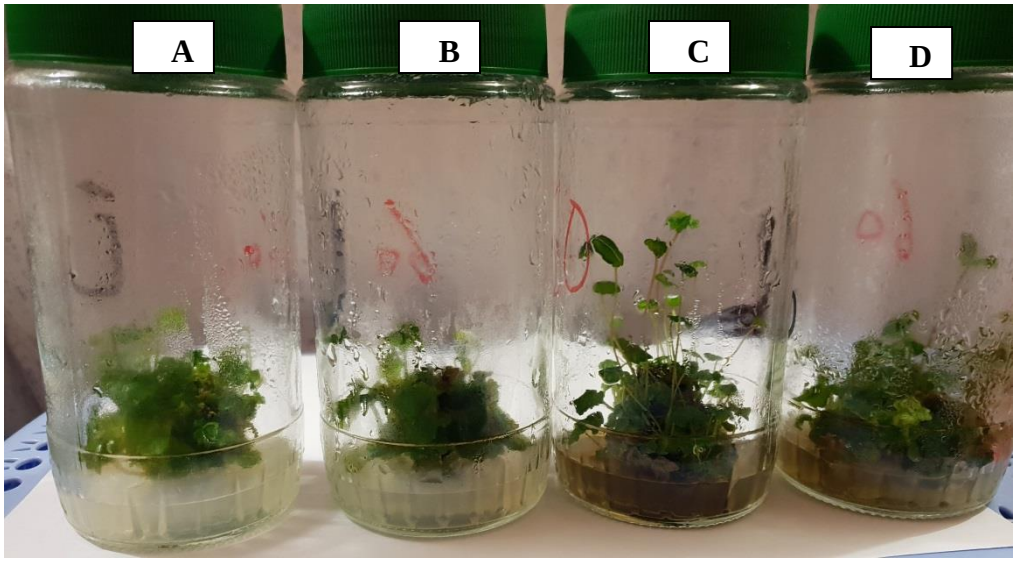

Fig.1. Strawberry shoots multiple after 4 weeks of culturing on MS medium supplemented with 0mg.L^{-1} (A), 5mg.L^{-1}(B) 10mg.L^{-1}(C) and 15mg.L^{-1}(D) of silver nanoparticles.

Table 4 results indicated that the iron source was significantly affected the shoots multiplication as the nano iron treatment was significantly exceeded control treatment (traditional iron). The interaction between nano silver nitrate and iron source was significantly increased shoots number

when 10mg L^{-1} + nano iron treatment was exceeded other treatments (except 15mg L^{-1} + nano iron treatment) and recorded the highest average 11.00 shoot, while, the 0mg L^{-1} + traditional iron treatment gave the lowest average 5.33 shoot(Fig.2).

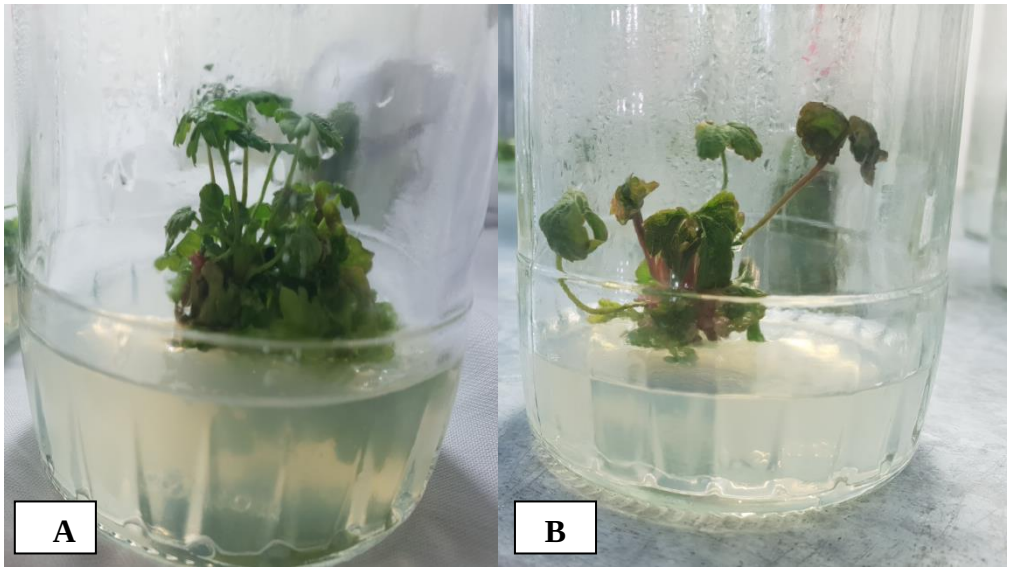

Fig.2. Strawberry shoots multiple after 4 weeks of culturing on MS medium supplemented with: (A) 10mg.L^{-1} Silver nanoparticles + Nano Iron. (D).without Nano particles.

Table 4. The effect of nano silver nitrate and iron source in MS medium and their interaction on the average of shoots number after 4 weeks of planting.

Concentration of nano silver nitrate (mg L^{-1})	Iron source in MS medium		The average of nano silver concentration effect
	Traditional iron	Nano iron	
0	5.33	6.67	6.00
5	8.00	8.67	8.33
10	9.00	11.00	10.00
15	8.00	9.67	8.83
The average of iron source effect	7.58	9.00	
L.S.D 0.05	Silver concentration = 1.95 Iron source = 1.38 Interaction = 2.76		

Results of Table 5 indicated that there were significant differences in leaves number on the vegetative shoot when adding different concentrations of nano silver nitrate to the culture medium during initiation stage. The 10mg L^{-1} concentration was increased leaves number significantly and reached 23.83 leaf compare to 17.33 leaf in 0mg L^{-1} treatment. The adding of iron to the culture medium was significantly affecting leaves number when nano iron treatment was exceeded traditional iron treatment and gave the highest leaves number.

The interaction between nano silver nitrate and iron source was significantly increased leaves number when 15mg L^{-1} + nano iron treatment was exceeded other treatments and recorded the highest average 27.33 leaf, while, the 5mg L^{-1} + traditional iron treatment gave the lowest average 12.67 leaf.

Table 5. The effect of nano silver nitrate and iron source in MS medium and their interaction on the average of leaves number after 4 weeks of planting.

Concentration of nano silver nitrate (mg L^{-1})	Iron source in MS medium		The average of nano silver concentration effect
	Traditional iron	Nano iron	
0	13.00	21.67	17.33
5	12.67	23.00	17.83
10	20.67	27.00	23.83
15	15.00	27.33	21.17
The average of iron source effect	15.33	24.75	
L.S.D 0.05	Silver concentration = 4.483 Iron source = 3.170 Interaction = 6.340		

There were significant differences in the average of total vegetative fresh weight between treatments of adding nano silver nitrate when the 10mg L^{-1} treatment was exceeded and recorded the highest average 1.92g compare to 1.49g in the 0mg L^{-1} treatment (Table 6). Iron source also made significant differences between treatments as nano iron treatment gave the highest average of shoot fresh weight amounted 1.97g in comparison with the lowest average 1.53g in control (0mg L^{-1}). The interaction between nano silver nitrate and iron source made significant effect on shoot fresh weight when 10mg L^{-1} nano silver nitrate + nano iron treatment was significantly exceeded other treatments followed by 15mg L^{-1} nano silver nitrate + nano iron as it reached 2.23g and 2.11g respectively. While, the lowest average was recorded in the control treatment (0mg L^{-1} of nano silver nitrate + traditional iron) reached 0.53g.

Table 6. The effect of nano silver nitrate and iron source in MS medium and their interaction on shoots fresh weight after 4 weeks of planting.

Concentration of nano silver nitrate (mg L^{-1})	Iron source in MS medium		The average of nano silver concentration effect
	Traditional iron	Nano iron	
0	1.28	1.73	1.49
5	1.53	1.84	1.68
10	1.61	2.23	1.92
15	1.72	2.11	1.91
The average of iron source effect	1.53	1.97	
L.S.D 0.05	Silver concentration = 0.485 Iron source = 0.343 Interaction = 0.678		

Adding nano silver nitrate to the culture medium leaded to increase dry weight of shoots when the highest average of shoot weight was achieved at 10mg L^{-1} concentration reached 0.22g, while, the lowest average 0.18g recorded at 0mg L^{-1}. Table 7 showed that there were significant differences of iron source as the nano iron treatment was exceeded and recorded the highest average 0.23g compare to the lowest average 0.18g recorded in control.

The interaction between nano silver nitrate and iron source had significant effect on shoot dry weight when 10mg L^{-1} nano silver nitrate + nano iron treatment was gave the highest average amounted 0.26g. While, the lowest average was recorded in the control treatment (0mg L^{-1} of nano silver nitrate + traditional iron) reached 0.16g and not significantly differ from other silver concentrations with traditional iron.

Table 7. The effect of nano silver nitrate and iron source in MS medium and their interaction on shoots dry weight after 4 weeks of planting.

Concentration of nano silver nitrate (mg L^{-1})	Iron source in MS medium		The average of nano silver concentration effect
	Traditional iron	Nano iron	
0	0.16	0.21	0.18
5	0.18	0.23	0.20
10	0.19	0.26	0.22
15	0.19	0.22	0.20
The average of iron source effect	0.18	0.23	
L.S.D 0.05	Silver concentration = 0.014 Iron source = 0.023 Interaction = 0.031		

The effect of nono silver nitrate and nano iron on shoots multiplication indicated the role of silver on shoots number, fresh and dry weight of shoots and these results are in agreement with Aghdaei et al (2012), Saha and Gupta (2018) who reported an increasing of the multiplication of Swertia chirata and Tecomella undulate plants when nano silver was added to the culture medium. Since this kind of increase requires the presence of BA in culture medium to make the multiplication and increase its level, which promotes the use of nanocatalysts in applied tissue culture.

There was significant effect of iron source with silver nitrate as it showed an increasing in studied traits. This may be due to the fact that Nano composites possess unique properties due to their high surface area and small particles, which leads to an increase in their entry into the tissues of the cultivated plant part and thus affects their ability to spread and availability to plants compared to traditional particles that exist in micron size (Tariq et al 2020). Iron is one of the important elements that play a role in many enzymatic systems involved in the processes of oxidation and reduction; it is also involved in the production of NADP, the reduction of nitrates and sulphates and the representation of nitrogen (Briat and Gaymard 2015). In addition, iron is included in the composition of lipids, nucleoles walls, chloroplasts, mitochondria as well as cytochromes which transfer electrons in the process of photophosphorylation (Mohebi et al 2010). All of these roles lead to stimulating the growth of shoots and increasing their length and dry weight, when nanoparticles are added in low concentrations to the nutrient medium (Phogat et al 2016).

CONCLUSION

Adding of AgNPs to initiation and multiplication medium was leaded to reduce the the fungal and bacterial contamination of cultures. In addition, adding nano silver and iron was leaded to improve the average of shoots multiplication. Enhancing the MS medium with 10mg L-1 concentration of nano silver nitrate in initiation stage of cultures was gave the best shoots number of strawberry.

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