Effects of $AgNO_3$ in combination with some plant growth regulators on micropropagation of strawberry (*Fragaria ananassa* Duch)

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Abstract

Strawberry *(Fragaria ananassa* Duch*),* belong to Rosaceae family, is widely appreciated, mainly for its fruit characteristic aroma, bright red color, juicy texture, It is traditionally asexually propagated by rooted runners. To improve the strawberry varieties this method was not suitable due to incidence of many serious diseases infection resulting in the gradual degeneration of cultivars performance, so micropropagated strawberry plant has been introduced to prevent most of the plant and soil transmissible diseases. Micropropagated strawberry has several advantages, such as its ability to multiply virus free stock rapidly and, the improved capacity of these plants to produce runners for planting in the field. In this study, it was aimed to examine the interaction of BA, IBA and $AgNO_3$ combinations at different micropropagation stages**.** Results showed the addition of $AgNO_3$ to MS medium led to increase shoot number, shoot length, fresh and dry weight at multiplication shoots stage, shoots rooting % and root number at rooting stage. Survival plants %, plant height, leaves number and dry weight at acclimatization stage**.**

Keywords: Strawberry, $AgNO_3$, Micropropagation, *In vitro*

Introduction

Strawberry plant (*Fragaria ananassa* Duch) is belong to Rosaceae and it was able to adapts to a wide range of temperatures and spread worldwide. It grows as wild and cultivated plant and carry small fruits with height value of nutrition and cure benefits. Strawberry plant propagation in two methods, the first by sexual propagation by using seeds to produce seedlings which plant direct in soil or to produce new cultivars, the second by vegetative propagation by divided the crown plant or by runners. Results of previous studies proven there is a capability to propagate strawberry plant by using different tissue culture techniques such as using runners tip culture but this method will face trouble of browning in shoot initiation stage, which in most case led to the death of explant (15).Therefore, most of specialists try to propagate strawberry plant in tissue culture by apical meristem it considered more confidential because it saves genetic stability of cultivar and their ability to constantly renew themselves (17).Sterilization conditions are one of the basic requirements for tissue culture protocol success. Contaminants, despite their different types (fungal or bacterial pollutants), are a major source of damage to the micropropagation protocol during its various stages (initiation, multiplication or even rooting). Microbial contamination can stop or completely damage the micropropagation process, even in its early stages. Usually the plant parts are collected from the field or the greenhouse and then transported to the laboratory for superficial sterilization and then tissue culture. However, in many cases, bacterial contamination appears in the advanced stages of shoots multiplication, which exposes the culture to damage and consequently a loss of time and effort with an economic loss (13). Silver nitrate has proved to be a very potent inhibitor of ethylene action and is widely started to use in plant tissue culture experiments. It is also having easy availability, solubility in water, specifity and stability traits make it very useful for various applications in exploiting plant growth regulation and *in vitro* and *in vivo* morphogenesis(21).According to several reports $AgNO_3$ influence *in vitro* somatic embryogenesis, enhancing shoot regeneration, shoots multiplication, root formation and reducing the ethylene hormone negative effects in several crops cultures such as potato(1),*Anthurium andraeanum*(3),*Moringa oleifera* (5), *Dillenia indica*(9),*Citrus australasica*(10) and banana(22),because of the increasing markets demand of the strawberry crop in Iraq day after day, also the lack of studies on the effect of $AgNO_3$ in combination with some plant growth regulators in the field of plant propagation by tissue culture, this study was carried to test the effects of $AgNO_3$ in different concentration on improving the characteristics of plants resulting from tissue culture.

Materials and Methods

The study was carried out in tissue culture laboratories of Horticulture and Landscape Department - Faculty of Agriculture, University of Kufa.

Culture initiation stage:

Runner tips of RubyGem cultivar were excised with the help of a sharp blade and washed 2-3 times with sterile distilled water. Surface sterilization was carried out by dipping the shoot tips into 3% Clorox

(6% Sodium hypochlorate) solution with shaking for 15 minutes followed by 3-5 times washing with sterile distilled water. Surface sterilized runner tips were carefully cultured on MS medium(11) supplemented with 30gl^{-1}sucrose ,100 mgl^{-1} myo-inositol with 0.5 mgl^{-1} BAP + 0.1 mgl^{-1} IBA in test tube, after solidified with 0.7% agar, in the presence of $AgNO_3$ at 0, 5 and 10 $mg.l^{-1}$. The medium pH was adjusted to 5.8 prior to autoclaving at 121 ◦C and 108 Kpa for 20 min. The culture were incubated under 16/8 h light/ dark cycle at 25 ± 1°C in a growth chamber at an intensity of 1000 lux for 3 weeks. Data about bud response (%), shoots number and length, and cultures contamination percentage was calculated at the end of the incubation period.

Shoots multiplication:

Suitable multiply shoot were transferred to new media for further multiple shoot induction. For the testing response of culture to different concentration of $AgNO_3$(0, 5 and 10 $mg.l^{-1}$), shoots were cultured on MS medium supplemented with 1 $mg.l^{-1}$ BAP + 0.1 $mg.l^{-1}$ IBA for 4 weeks and data on the number and length of shoots/culture were recorded.

Rooting of Regenerated Shoots:

For testing response of regenerated Shoots to roots formation under different $AgNO_3$ concentrations, regenerated shoots were separated and cultured on half- strength MS salts medium(2)supplemented with 0.3 mgl^{-1} IBA with (0, 5 and 10 mgl^{-1}) $AgNO_3$for 4 weeks incubation period and data on rooting percentage, number of roots and length of roots were recorded.

Acclimation of micro-propagated plantlets:

Randomly *in vitro*-rooted shoots (Plantlets) of strawberry cv. RubyGem were selected for acclimatization. Plantlets with good vegetative and root groups, washed with clean water several times to remove the residue of the medium growth from the roots, which may be a source of contamination in the future, and treated with fungal pesticide Benliet (1.0 $ml.l^{-1}$) for 1 minute. Then each plantlet was planted in 7 cm diameter pots filled with a mixture of Peat moss, sand and perlite(1: 3 :1 v:v:v) and then irrigated with (1/4MS) solution nutrient media and the potted-plantlets were covered with transparent plastic film to prevent moisture loss and incubated. After 10 days, gradually remove the cover. After 30 days, at the end of the period of acclimation, plant survival(%)was recorded.

Experimental design and statistical analysis:

All experiments was carried out according to Completely Randomized Design(CRD) with one factor (three concentration of $AgNO_3$) with 10 replicates and collected data statistically analysis in one-way ANOVA followed by Duncan's multiple range tests, at the P(0.05), using Genstate (12th Edt.) statistics program.

Results and Discussion

Results in Table 1 indicate that the percentage of contamination of explants grown in MS media with silver nitrate in the culture tubes after surface sterilization was high in the control treatment (without adding silver nitrate), as this percentage decreased with the increase of silver nitrate concentration. The treatment with concentrations of 5 and 10 $mg.l^{-1}$ achieved the lowest percentages of contamination

amounted to 0%, which did not differ significantly among them. Also, the supply of the nutrient medium with silver nitrate had a significant effect on the percentage of growth buds (response rate). The same table indicated the preference of cultures grown in the MS nutrient medium supplemented with 5 and 10 $mg.l^{-1}$ silver nitrate its response to *in vitro* culture, in which, the response rate of shoots to growth was 90 and 100%, respectively, compared to the lowest percentage(50%)recorded in control**.** Also, there are significant differences in shoots number as a result of adding different concentrations of silver nitrate, as 5 $mg.l^{-1}$ concentration was superior in achieving the highest rate of 2.86 shoots without a significant difference from 10 $mg.l^{-1}$ compared with the control treatment, which achieved the lowest rate of 1.33 shoots**.** The results of the same table showed that adding silver nitrate had no significant effect on shoot length

Table 1: Effect of $AgNO_3$ on culture contamination, response and shoots initiation

$AgNO_3$ ($mg.l^{-1}$)	Contamination (%)	Response rate (%)	Shoot number	Shoot length (cm)
0	20.00a	50.00b	1.13a	3.17a
5	00.00b	90.00a	2.86ab	4.11a
10	00.00b	100.00a	2.09a	4.27a

Results in Table 2 and Figures 3 indicate that there were significant differences in the number of shoots, their length, the number of leaves and shoots dry weight as a result of theaddition ofdifferent $AgNO_3$, as the concentration $10mg.l^{-1}$ is significantly superior by achieving the highest rates compared to the lowest in cultures grow in MS free of $AgNO_3$.

Table 2: *In vitro* strawberry shoot multiplication as affected by silver nitrate addedin combination with 1 $mg.l^{-1}$BAP and 0.1 $mg.l^{-1}$IBA

$AgNO_3$ ($mg.l^{-1}$)	shoots number	Plant height(cm)	Leaves number	Shoots dry weight (g)
0	4.22 b	2.02 b	11.1 b	6.50 b
5	7.72 ab	2.62 b	13.0 b	8.53 a
10	12.30a	3.06 a	16.2 a	8.89a

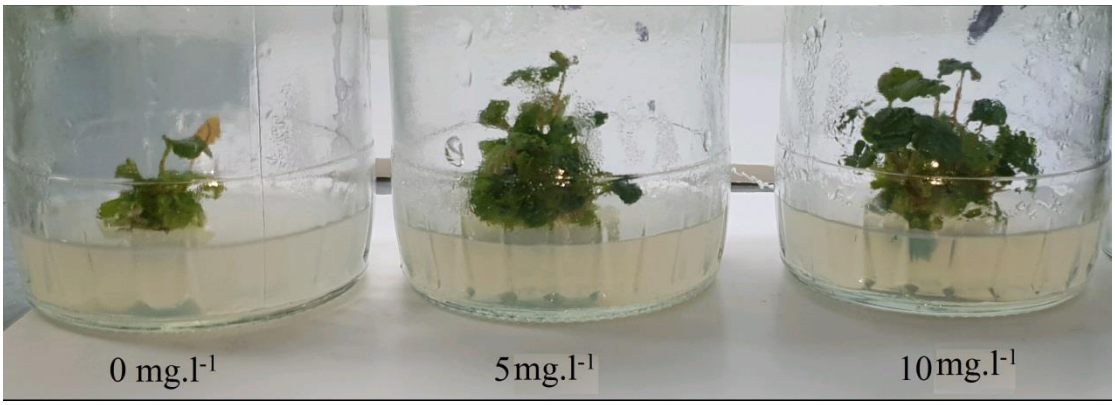

Fig.1. Strawberry shoot multiplication cultured on MS medium with different concentrationsof AgNO3 (0, 5, and 10 mg.l^{-1})

There were significant differences in the percentage of rooted shoots and root number according to the concentration of $AgNO_3$ used, as the shoots grown in MS with $AgNO_3$s outperformed in that, while 5 and 10mg $^{l-1}$ did not differ among them in the average length of roots, as shown in Table3.

Table 3: rooting, root number, and length as affected by differentconcentrations of$AgNO_3$ in MS medium

$AgNO_3$ (mg.l^{-1})	Shoots Rooting (%)	Root number/shoots	Root length (cm)
0	55.00b	11.67b	2.88a
5	65.50ab	13.33ab	3.25a
10	75.00a	16.33 a	3.38a

Data in Table 4 showed that the survival (%) for plantlets produced from cultured grown in MS supplemented with 10mg was superior over all $AgNO_3$ concentration used in the experiment, itsshows 100% success compared to 84% of control treatment, in addition, same$AgNO_3$ achieved superior in vegetative growth characteristics of Strawberry acclimated plantlets as Plantlets height, number of leaves / plant and dry weight of plantlets.

Table 4: Survival and vegetative growth characteristics of strawberry plantlets under different $AgNO_3$ after one month of acclimation

$AgNO_3$ (mg.l^{-1})	Plant survival (%)	Plantlets height (cm)	Leaves number / plantlets	Dry weight (g)
0	80b	6.50b	8.33b	0.088b
5 mg	85ab	7.50ab	11.33a	0.294 b
10 mg	100a	8.83a	13.11a	0.710 a

Results of this study showed that MS medium supplemented with 10 $mg.l^{-1}$ AgNO3, not only increased shoot proliferation of *in vitro* shoot tips cultured strawberry but also reduced cultured contamination and improved shoot elongation, leaf formation, root formation and elongation as well as plant survival and their good characterestics (tables 1, 2,3 and 4).Increasing the response rate and decreasing contamination due to the addition of silver nitrate, being highly effective as antifungal and bacterial agents(7), in addition to silver being one of the ethylene inhibitors, which is produced and collected in tissue culture vessels, whether caused by cultivated plant tissues or generated by the flame used to sterilize the nozzle of planting vessels is one of the factors that negatively affect the growth of cultures and their continued development later, which works to curb the multiplication of shoots(8**).** Also, high concentrations of ethylene increase the activity of the enzyme cellulase and pectinase or both, which leds to cell damage and thus failure of the subsequent development process (18). According to Saltveit *et al.* (19), the response and growth of shoots can be attributed to the presence of silver in silver nitrate, which inhibits the action of ethylene by binding to the active part of the catalytic enzymes (cellulase and pectinase), which inhibits their work and stimulates growth. The results of the experiment agree with the results of several researchers who attributed the beneficial effect of AgNO3 to its ability to ameliorate the detrimental effect(s) of ethylene on cultured plant tissues rather than inhibiting its production by the cultured plant tissues (6, 20). The results of study are similar to those of Qin, *et al.* (16) whoenhanced shoot regeneration efficiency and adventitious bud formation instrawberry by the inclusion of AgNO3 in the culture medium. AgNO3 hasbeen widely, and in most cases, successfully used to overcome the inhibitoryeffects of ethylene on *in vitro* cultured plant tissues. In this study, supplementationof the culture medium with 10 mg/l AgNO3 enhanced growth and morphologicaldevelopment in strawberry shoot. Similar results were reported in anumber of agricultural plants such as,date palm (4), *Dillenia indica*(9), citrus (10), pomegranate(12),pistachio(14) and*Lantana camara*(23) in terms of the positive effect on the response of their *in vitr*o culture and reducing contamination.

Conclusion

There were avariable effects among$AgNO_3$ concentration on shoots multiplication, rooting and acclimated plantlets growth with superiore by using $AgNO_3$ at $10mg.l^{-1}$, so we recommended using $AgNO_3$ in plant tissue culture laboratory for strawberry micropropagation

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