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Studies on silver nitrate impact on jojoba *in vitro* culture

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Abstract : Many problems have been faced in micropropagation of jojoba. Slow growth, low multiplication rate, hyperhidresity, difficulty of rooting and low survival rate in acclimatization. Many reports have shown the positive effect of $AgNO_3$ on plant tissue culture. This investigation aimed to study the effect of $AgNO_3$ on multiplication and rooting of jojoba *in vitro* shootlets. Results indicated that silver nitrate (SN) has an elevating and boosting effect on jojoba multiplication. It raised average multiplication rate from 3 to 14 shoots with the same benzylaminopurine (BAP) concentration. The best multiplication rate was achieved with SN and BAP at 7.0 and 6.0 mg/L, respectively. The combination of SN and BAP at 7.0 and 2.0 mg/L, respectively showed the highest shoot length and leaf number. Moreover, SN enhanced root induction, root number and root length of jojoba shootlets when combined with Indole Butyric acid (IBA). The highest rooting percentage appeared with silver nitrate and IBA at 3 and 7 mg/L, respectively. Meanwhile, silver nitrate and IBA at 5.0 and 7.0 mg/L, respectively gave the highest number of roots per shoot and satisfying root length. As we know, it is the first time to study the effect of silver nitrate on shooting and rooting of jojoba *in vitro* culture.
Keywords: silver nitrate, jojoba.

Introduction

Jojoba (*Simmondsia Chinesis*) is a distinguished dioccious shrub. It tolerates drought and salinity. It is suitable for arid and semiarid areas and could be the suitable plant for invading the desert. It is native to Sonoran desert, north-west Mexico and Baja California[1].

Jojoba is propagated by sexual and vegetative methods. Propagation by sexual method makes it difficult to determine sex type in early stages of growth and shows variability in growth and yield[2]. In addition, planting jojoba at the field needs a 5: 1 female to male ratio[3]. Nowadays, jojoba stem cutting is only the vegetative propagation method used commercially, but it needs a long procedure[4,1]. Therefore, tissue culture techniques can be the alternative method for commercial production of female distinguished clones if a jojoba *in vitro* protocol is determined[5].

Jojoba *in vitro* culture has attracted a massive interest in this decade[6-9]. Moreover, many advantages have been achieved with tissue culture technique; producing free virus plants, large number in short time, valuable tools for breeding programs, good tool to overcome problems of traditional propagation methods and research studies[10-16]. Otherwise, tissue culture protocols are affected by many factors like plant hormone type and concentration, carbon source, type of media, etc [17-20].

Silver nitrate is known to promote multiple shoot formation in different plants. *In vitro* shoot formation was improved by incorporating silver nitrate in the culture medium. Ganesh and Sreenath [21] reported that the addition of N6-benzyladenine with $AgNO_3$ greatly enhanced the rate of sprouting and improved growth of the

proliferated shoots in *Coffea canephora* as well as in *C. Arabica*[22-24]. Addition of putrescine and silver nitrate to the medium influenced morphogenesis in chicory (*Chichorium intybus*) shoot cultures[25]. Silver nitrate was found to be beneficial in the regeneration and clonally propagation of several economically important plants such as buffalo grass[26] and Pomegranate[27].

This investigation was conducted to improve growth, health, multiplication and rooting of jojoba micropropagation using silver nitrate.

Materials and Methods

This study was carried out at the Biotechnology and micropropagation Lab, Pomology Dept., National Research Centre, during the period from 2014 to 2016.

Preparation of plant material

Nodal segments of jojoba distinguished seedling grown in greenhouse of Pomology Dept., National Research Centre, Egypt were excised and brought to the laboratory in plastic bag. Explants were exposed to sterilization process including treatment with commercial bleach (5% sodium hypochlorite) at 30% and mercuric chloride at 0.1 %. Sterilized explants were cultured into 3/4 MS medium (Murashigue and Skoog, 1962) as a basal medium[5] supplemented with 0.5 mg/L 6-benzylaminopurine (BAP), unless noted otherwise, 30 g/L sucrose and 6.0 g/L Difco Bacto Agar. The pH of the media was adjusted to 5.7 and media were autoclaved at 121^{o}C and 15 Ib/in^{2} for 15 minutes. The cultured explants were incubated under 16 hours of artificial light (Fluorescent light at 30 µM/sec) and 8 hours of darkness at average temperature of 25+2^{o}C.

Multiplication stage

Clusters with two shoots of jojoba derived from the establishment stage were cultured on 3/4 MS medium supplemented with various combinations of BAP and silver nitrate for multiplication as presented in Table (1).

Rooting stage

Individual shoots (2-3 cm, in length) derived from the multiplication stage were cultured on 3/4 MS medium supplemented with various combinations of Indole Butyric acid (IBA) and silver nitrate (Table 2) for rooting.

Table (1): Silver nitrate and Benzylaminopurine (BAP) concentrations supplemented to multiplication medium

Treatments	BAP (mg/l)	Silver nitrate (mg/l)
M 1	2.0	0.0
M 2	2.0	3.0
M 3	2.0	5.0
M 4	2.0	7.0
M 5	4.0	0.0
M 6	4.0	3.0
M 7	4.0	5.0
M 8	4.0	7.0
M 9	6.0	0.0
M 10	6.0	3.0
M 11	6.0	5.0
M 12	6.0	7.0

Table (2): Silver nitrate and Indole Butyric acid (IBA) concentrations added to rooting medium

Treatments	IBA (mg/l)	Silver nitrate (mg/l)
R 1	5.0	0.0
R 2	5.0	3.0
R 3	5.0	5.0
R 4	5.0	7.0
R 5	7.0	0.0
R 6	7.0	3.0
R 7	7.0	5.0
R 8	7.0	7.0
R 9	9.0	0.0
R 10	9.0	3.0
R 11	9.0	5.0
R 12	9.0	7.0

Statistical design:

Treatments were arranged in complete randomized design. Each treatment was replicated three times, each replicate included three jars, and each contained three clusters developed *in vitro*. In rooting stage ten shootlets were presented to each treatment. Each experiment was repeated twice. Data were statistically analyzed according to Duncan's multiple range test at 5% level of probability [28].

Results

Multiplication stage:

Data in Table (3) and Fig. (1) show that the combination of silver nitrate and BAP significantly affected *in vitro* multiplication of jojoba shoots. Silver nitrate and BAP at 6.0 and 7.0 mg/L, respectively (M12) gave the highest number of adventitious shoots followed by treatment (M11). It is clear from data that silver nitrate enhanced shoot multiplication especially with higher concentrations of BAP. It was observed that the highest concentration of BAP used in this investigation depressed shoot multiplication when used alone while, silver nitrate when added neglected this depression.

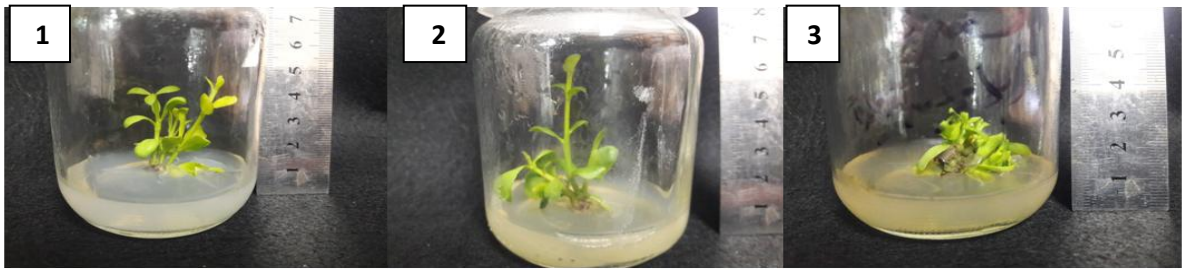

Fig. (1): Effect of SN and BAP on jojoba multiplication: treatment M3 showed low number of shoots and moderate length (1), M4 showed low number of shoots and higher length (2) and M12 showed higher number of shoots and lower length (3).

With respect to the effect of these combinations on shoot length and leaf number, it is clear that M4 treatment gave the highest results. It was observed that silver nitrate treatments affected hyperhydricity (data not included) as it highly decreased this phenomenon in jojoba shoots either in multiplication or rooting stage.

Table (3): Effect of silver nitrate and Benzylaminopurine combinations on multiplication stage of jojoba shoots

Treatments	Shoot No.	Shoot Length	Leaf No.
M 1	4.30 i	1.63 d	1.67 e
M 2	5.00 h	2.17 c	3.00 b
M 3	4.30 i	2.5 b	4.75 a
M 4	4.00 i	3.25 a	5.00 a
M 5	6.00 g	1.00 g	1.17 f
M 6	7.03 f	1.02 g	1.75 de
M 7	8.37 e	1.19 f	2.50 c
M 8	9.00 d	1.47 e	2.60 c
M 9	3.00 j	0.68 i	1.00 f
M 10	11.00 c	0.85 h	1.22 f
M 11	12.50 b	1.00 g	2.00 d
M 12	14.00 a	1.17 f	2.75 bc

Means followed by the same letter(s) within each parameter are not statistically different at 5% level.

Rooting stage:

It is obvious from data in Table (4) and Fig. (2) that the combinations of silver nitrate and IBA significantly affected *in vitro* rooting of jojoba shoots. Silver nitrate and IBA combination at 3 and 7 mg/L, respectively (R6) gave the highest rooting percentage compared with other combinations, followed by R7, R8 and R12. In addition, silver nitrate and IBA at 5.0 and 7.0 mg/L, respectively (R7) gave the highest number of roots per shoot followed insignificantly by R12 which also gave the highest root length.

Fig. (2): Effect of SN and IBA on jojoba *in vitro* rooting: rooted shootlets at treatment R6 (3 and 7 mg/L, respectively)

Table (4): Effect of silver nitrate and Indole Butyric acid concentrations on jojoba *in vitro* rooting

Treatments (mg/L)	% Rooted cuttings	Root No.	Root Length
R 1	0.0	1.00 e	1.00 e
R 2	22.22	1.75 bc	1.33 cd
R 3	11.11	1.14 de	1.14 de
R 4	11.11	1.14 de	1.24 de
R 5	11.11	1.14 de	1.07 de
R 6	44.44	1.42 cde	1.35 cd
R 7	33.33	2.64 a	1.93 b
R 8	33.33	1.52 bcde	1.58 c
R 9	11.11	1.14 de	1.14 de
R 10	22.22	1.66 bcd	1.59 c
R 11	22.22	1.47 cde	2.12 ab
R 12	33.33	2.08 ab	2.31 a

Means followed by the same letter(s) within each parameter are not statistically different at 5% level.

Discussion

Silver nitrate is widely used in plant tissue culture. Many reports have shown the positive effect of $AgNO_3$ on plants such as apple[29] and date palm[30]. In our research, it induced multiplication, shoot length and leaf number of jojoba shootlets compared with treatments free of SN. Similarly, it enhanced multiplication of *Vanilla planifolia*[31].

Some properties of SN such as easy availability, solubility in water, specificity and stability make it very useful for various applications in enhancing plant growth regulation and morphogenesis *in vivo* and *in vitro*. Silver ion mediated responses seem to be involved in polyamines, ethylene and calcium- mediated pathways, and play a crucial role in regulating physiological process including morphogenesis[32].

Our results assured that SN could be the solution of slow multiplication and difficult to root problems. These results are in harmony with Petrova *et al*.[33] as silver nitrate combined with IBA (at 1 mg/l for each) enhanced the development and growth of *G. lutea* shoots and increased the percentage of rooted plants. Similarly, *in vitro* root formation of *Decalepis hamiltonii* was achieved by addition of 40 µM $AgNO_3$ [25,34]. Similar results were conducted in *Vanilla planifolia* as SN not only induced shoot multiplication but also influenced rooting[31].

Silver nitrate has proved to be a very potent inhibitor of ethylene action. Some reports suggested beneficial effects of silver nitrate on *in vitro* development of axillary buds due to ethylene inhibition. When ethylene action and biosynthesis were inhibited, a significant stimulated regeneration of shoots from cotyledon explants of *Helianthus annus* was observed[35].

Conclusion

Our results indicated that silver nitrate could be the solution of slow multiplication and difficult to root problems in jojoba *in vitro* cultures.

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