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A Comparative Study on Different Cytokinin Types and Carbon Source Concentrations on In Vitro Proliferation of Jojoba (*Simmondsia chinesis* Link (Schneider))

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Abstract : Nodal explants of jojoba (*Simmondsia chinesis* Link (Schneider)) were cultured individually on MS medium supplemented with various concentrations of 6-benzyl aminopurine (BAP) for establishment stage. Furthermore, different cytokinin type; BAP and thaidiazoron (TDZ) with different concentrations (0.25, 0.50, 1.00 and 2.00 mg/L) were tested on proliferation. Sucrose and stevia solution alone or in combination were also tested. Data indicated that BAP at 0.5 mg/L gave the best sprouting percent. BAP was better than TDZ in improving shoot and leaf numbers as well as shoot length while, TDZ stimulated callus production more than BAP. Moreover, all partial replacement of sucrose with stevia solution enhanced proliferation compared with other treatments. The highest average of shoot numbers and shoot length were obtained at 10 mg/L sucrose plus 13 mL/L stevia solution.
Key words: Carbon source, stevia, TDZ, BAP, *in vitro*, jojoba.

Introduction

Jojoba (*Simmondsia Chinesis*) is a potential shrub bearing an oil seed which can be commercially cultivated in arid and semi-arid areas having low rainfall, extreme cold and hot temperature. Jojoba can be propagated through seeds, cutting and tissue culture. It tolerates drought and salinity [1,2].

Micropropagation is a technique which provides a large scale mass production of planting materials within short possible time[3-9]. Meanwhile, the process of *in vitro* multiplication is affected by many factors[10-13]. With the success of rising in tissue culture plants, the micropropagation has reached a commercial level in many plant species in recent years[14].

Shoot proliferation from nodal segments of female jojoba was greatest on MS medium supplemented with 1.0 to 5.0 mg/L BAP[15,16]. Agrawal et al.[17] found that average shoot length of jojoba was maximum on 20 µM 2ip. Only 19% of the explants induced multiple shoot at 50 µM 2ip.

Sugars as a carbon source is a very important component in tissue culture medium and its addition is essential for plant growth due to the limited photosynthesis due to the weak development of the leaves, the limited gas exchange and the high relative humidity *in vitro*[18]. Sugars enter the metabolism pathway and transformation of energy which are required for growth of cell[19].

Sucrose is the most commonly used sugar to serve as a carbon source and osmotic stabilizer in tissue culture technique. It is universally used as the principal energy source although in certain cases glucose and fructose may be substituted, but most other sugars are poor carbohydrate sources for the plant[20]. However, there are many available carbon sources; glucose, fructose, maltose, mannose, etc. play an important role in some *in vitro* plants; in Fig[21, 22], in asparagus[23] and in cucumber [24].

Stevia rebaudiana (Bertoni) is a medicinal plant belongs to Astaraceae family. Leaves of stevia contain sweetening compounds; stevioside, steviolbioside, rebausiosides A-E, and dulcoside A[25]. Stevioside is a glycoside which is non-fermentable, non-discoloring and heat stable at 1000°C and comprising 6-18% of the stevia leaf [26]. Cardello, et al.[27] stated that the active compounds of stevia are steviol glycosides which have up to 150 times the sweetness of sucrose.

This study determined the effect of cytokinins as well as carbon sources concentration on *in vitro* establishment and proliferation of jojoba plantlets.

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[28] of jojoba distinguished seedling grown in greenhouse of Pomology Dept., National Research Centre, Egypt were excised and brought to the Lab. 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[29] as a basal medium[30] 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°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°C.

Preparation of stevia solution

Stevia leaves were dried and subjected (as 10 g/L) to a water extraction process with heating lower than 60°C. The solution was filtered to remove any remains and added to the medium before autoclaving. The following experiments were carried out:

1- Effect of cytokinins on starting and multiplication stages

Sterilized jojoba explants were cultured in 3/4 MS medium supplemented with BAP at 0.0, 0.25, 0.50, 1.00 and 2.00 mg/L, sucrose at 30 g/L and Difco Bacto Agar at 6.0 g/L. The percentage of sprouted explants (sprouting percent) was measured in establishment stage. Furthermore, clusters of jojoba shoots derived from the establishment stage were cultured on 3/4 MS medium supplemented with BAP or TDZ as a cytokinin. BAP and TDZ were tested at different levels (0.0, 0.25, 0.50, 1.00 and 2.00 mg/L) to find out the most effective cytokinin with suitable concentration for multiplication stage.

2- Effect of carbon source and its concentration

Sterilized jojoba explants were cultured in MS medium supplemented with 0.5 mg/L BAP. Sucrose and stevia solution were added at different concentration alone or in combinations to study their effect on sprouting and multiplication stages (30 g/L sucrose, 20 mL/L stevia solution, 20 g/L sucrose+ 7.0 mL/L stevia solution and 10 g/L sucrose+13 mL/L stevia solution) . Callus production was determined as scores: negative response =1, below average =2, average =3, above average =4 and high response =5, according to Pottino[31]. Meanwhile, number of shoots, number of leaves and shoot length (cm) were recorded after four subcultures with four weeks interval.

Statistical design:

Treatments were arranged in complete randomized design, each treatment was replicated three times, each replicate included three jars, and each contained three explants or clusters developed *in vitro*. Means were compared according to the method described by Snedecor and Cochran[32].

Result and Discussion

Starting stage:

1-1 Effect of BAP concentration

It is clear from Fig. (1) that 0.5 mg/L BAP gave the highest sprouting percent (100%). Higher concentrations of BAP reduced the percentage but still be better than the control.

1-2 Effect of carbon source and its concentration

It is clear from Fig. (2) that sucrose alone and its combinations with stevia solution gave 100% sprouting percent. Meanwhile, stevia solution alone gave 75%. It is clear that sucrose must be presented to the established media to obtain the best sprouting percent.

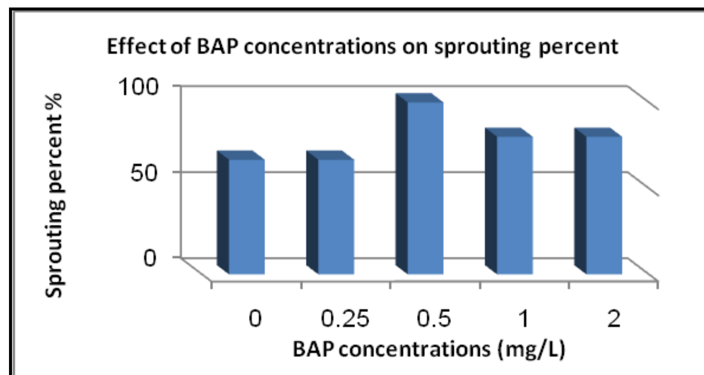

Fig.(1): Effect of BAP and its concentrations on starting stage of jojoba explants

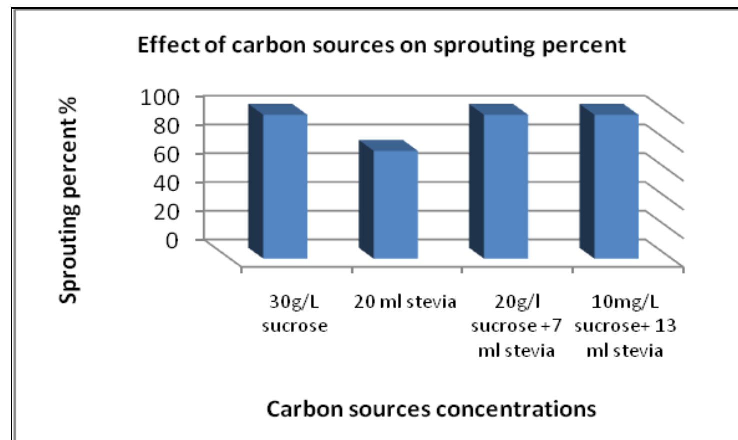

Fig. (2): Effect of type of carbon source and combination on starting stage of jojoba explants

Multiplication stage

2-1 Effect of cytokinin type and concentration

It is clear from Table (1a) that callus production was significantly decreased while shoot number, number of leaves and shoot length increased when BAP was used more than TDZ. Meanwhile, Table (1-b) reflects that the lower concentration (0.25 mg/L) enhanced shoot length and reduced both shoot number and callus production, significantly. However, using 0.50 mg/L enhanced the highest shoot number in relation to the other concentration. In addition, callus production was significantly increased by using 2 mg/L. Furthermore, higher concentrations (1.0 & 2.0 mg/L) encouraged a significant increase in number of leaves as compared to lower concentrations. Moreover, Table (1-c) explains that BAP at the rates of 0.5 mg/L induced the highest significant shoot number in relation to all other interactions. However, addition of 1.0 & 2.0 mg/L BAP were significant in increasing number of leaves in relation to all other interactions, also, using 0.25 mg/L BAP

increased shoot length with high significance. On the other hand, using 2.0 mg/L either BAP or TDZ enhanced significantly callus production as compared with the others. The previous results indicate that BAP surpassed TDZ in improving shoot and leaf number and shoot length while TDZ stimulated callus production than BAP. These results are in general agreement with the findings of Hassan[15] and Bashir et al.[16]. They reported that best shoot proliferation from nodal segments of female jojoba occurred by BAP.

Table (1): Effect of different concentration of BAP and TDZ on multiplication stage

Table (1-a): Effect of Cytokinin type

Growth parameter / Cytokinin type	Shoot number (No.)	Leaf number (No.)	Shoot length (cm)	Callus (score)
BAP	10.55a	4.43a	1.91a	1.75b
TDZ	5.61b	2.91b	1.42b	2.19a

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

Table (1-b): Effect of Concentration

Growth parameter / Concentration (mg/L)	Shoot number (No.)	Leaf number (No.)	Shoot length (cm)	Callus (score)
0.00	5.00e	2.67d	1.13d	1.00e
0.25	5.44c	3.43c	2.09a	1.50d
0.50	10.68a	3.32c	1.83b	2.34b
1.00	10.17b	4.53a	1.40c	2.01c
2.00	9.11c	4.40b	1.88b	3.00a

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

Table (1-c): Effect the interaction between cytokinin type and Concentration

Growth parameter	Shoot number (No.)		Leaf number (No.)		Shoot length (cm)		Callus (score)	
Cytokinin type / Concentration	**BAP**	**TDZ**	**BAP**	**TDZ**	**BAP**	**TDZ**	**BAP**	**TDZ**
0.00	5.00g	5.00g	2.67f	2.67f	1.13e	1.13e	1.00d	1.00d
0.25	6.22e	4.67h	3.93c	2.93ef	2.64a	1.54c	1.00d	2.00b
0.50	16.33a	5.03g	4.44b	2.20g	2.34b	1.33de	1.67c	3.00a
1.00	14.67b	5.67f	5.45a	3.61d	1.48d	1.32de	2.06b	1.97b
2.00	10.55c	7.67d	5.67a	3.13e	1.98	1.78	3.00a	3.00a

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

2-2- Effect of carbon source and its concentration

Table (2) and photo (1) show the effect of stevia solution and sucrose on proliferation and growth parameters of jojoba plantlets after four subcultures. It is clear that all combination treatments raised shoot and leaf numbers and shoot length as compared with the other treatments. Supplementation of the culture medium with sucrose at 10g/L plus stevia at 13 mL/L maximized shoot number and shoot length in comparison to the other treatments under study. While, the highest leaf number was achieved by supplementing the culture medium with sucrose at 20 g/L plus stevia at 7 m/L level.

Photo (1): Effect of stevia solution and sucrose on multiplication of jojoba shoots, from right to left: 30 g/L sucrose, 20 g/L sucrose+ 7mL/L stevia solution, 10 g/L sucrose+13 mL/L stevia solution and 20 mL /L stevia solution.

Generally, the above results can recommended that media supplemented with combinations of sucrose with stevia solution enhanced shoot and leaf numbers as well as shoot length compared with other treatments. Stevia contains steviol glycosides which consist mainly of stevioside and rebaudioside. Glycosides are molecules that contain glucose and other non-sugar substances called aglycones[27]. This probably indicates that glucose has a promoting impact on jojoba proliferation and surpassed sucrose as a carbon source. Similar results were indicated by Taha[30]. It was found that glucose enhanced shoot length and leaf number of jojoba *in vitro* shoots of two clones (69 and 51). Similar results were obtained with Fayek et al.[33]. In addition, Duong et al.[34] tested the effect of different sugars on growth of Hymalaya Yew cells in shake liquid culture condition; they found that fructose exhibited a better growth of cell when compared with sucrose or glucose. Also, the best proliferation of cells was obtained at the combination of 30 g\L glucose and 30 g\L fructose. However, stevia solution alone enhanced multiplication and growth significantly compared with sucrose alone. Plant extracts have been used as a carbon source in many researches. Maximum shoot length and higher number of multiple shoots of *Pogostemon cablin* Benth were observed on MS media supplemented with 20% sugarcane juice compared with sucrose, glucose, fructose and table sugar[35].

Combination of sucrose with stevia solution surpassed stevia alone in shoot and leaf number as well as shoot length and this may be clarify that two sources of carbon may be more benefit for jojoba *in vitro* multiplication. Taha and Hassan[36] recommended that other types of sugar should be considered in date palm *in vitro* culture.

Meanwhile, sucrose at 30 g/L gave significantly the highest callus production followed by sucrose at 20g/L plus stevia at 7 ml/L as compared with the other treatments.

Table (2): Effect of stevia solution and sucrose on proliferation and growth parameters of jojoba shoots

Growth parameter / Carbon source	Shoot number (No.)	Leaf number (No.)	Shoot length (cm)	Callus (score)
30g/L sucrose	3.33d	3.20c	0.95d	2.67a
20 ml stevia	4.67c	2.57d	1.54c	1.00c
20g/l sucrose +7 ml stevia	10.33b	5.55a	1.89b	1.67b
10mg/L sucrose+13ml stevia	16.00a	4.83b	2.14a	0.80c

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

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References

1. Fayek, M.A.; Shabaan E.A.; Zayed, N.S.; El-Obeidy, A.A., and Taha, R.A.: Effect of salt stress on chemical and physiological contents of jojoba (*Simmondsia chinensis* (Link) Schneider) using *in vitro* culture. World J. Agric. Sci., 6: 446-450. (2010).
2. Taha, R.A.: Effect of growth regulators and salinity levels on *in vitro* cultures of jojoba plants. World App. Sci. J., 31 (5): 751-758. (2014).
3. Tiwari, P.: Effect of different growth hormones on *in vitro* response of a leguminous medicinal herb. Int. J. ChemTech Res., 5 (2): 894-898. (2013).
4. Hashish, Kh.I.; Taha, L.S. and Ibrahim, S.M.M.: Micropropagation potentiality and pigments content of *Hibiscus rosasinensis* L. as affected by gamma radiation. International Journal of ChemTech Research., 8 (9):131-136. (2015).
5. Mandour, H.M.; Soliman, S.S.A.; Abd El-Hady, M.S.; Mahmoud, A.A. and El-Naggar, H.M.H..: In vitro Selection for Drought Tolerance in Wheat (*Triticum aestivum* L.). Int. J. ChemTech Res., 8 (9): 318-333. (2015).
6. Bredy, S.; Najla, S. and Albiski, F.: In vitro evaluation of six tomato genotypes for water stress. Int. J. ChemTech Res., 8 (11): 257-270. (2015).
7. Abd allatif, A.M.; Hassan, S.A.M. and El-Sharony, T.F.: In vitro germination and development of Arbequina and Coratina olive cultivars. Int. J. ChemTech Res., 8, (12):471-476. (2015).
8. El-Minisy, A.M.; Abbas, M.S.; Aly, U.I. and El-Shabrawi, H.M.: In vitro selection and characterization of salt tolerant cell lines in cassava plant (*Manihot esculenta* Crantz). Int. J. ChemTech Res., 9 (5): 215-227. (2016)
9. Khater, M.A. and Elashtokhy, M.M.A.: Effect of growth regulators on in vitro production of Hyoscyamus aureus L. and tropane alkaloids. Int. J. ChemTech Res., 8 (11): 113-119. (2015).
10. Anwar, H.M.; Hossain, M.T.; Raihanali, M. and Rahman S.M.M.: Effect of different carbon sources on in vitro regeneration of Indian penny wort Centellaa statistical. Pak. J. Boil. Sci., 8 (7): 963-965. (2005).
11. Bansal, S.; Bharati, A.J.; Bansal Y.K.: In vitro Callogenesis and Phytochemical Screening of Harsingar (*Nyctanthes arbortristis*) a Multipotent Medicinal Tree. Int. J. PharmTech Res., 5 (4): 1786-1793. (2013).
12. Mustafa, N.S.; Hassan, S.A.M.; Taha, R.A. and Zayed, N.S.: Studies on the behavior of proliferated shoots and roots of two fig cultivars *in vitro*. Int. J. ChemTech Res., 9 (7): 01-07 (2016).
13. Abd El-Motaleb, M.; Abd El-Hameid, A.R.; Elnaggar, H.M.H. and Abdel-Hady. M.S.: Callus induction and regeneration of *Stevia rebaudiana* Bertoni. Int. J. ChemTech Res., 8 (6): 868-877. (2015).
14. Chandra, R. and Mishra, M.: Comprehensive micropropagation of Horticultural Crops. International Book distributing Co. Lucknow. O.P. (India). (2003)
15. Hassan, N.S.: In vitro propagation of jojoba (*Simmondsia Chinesis*) through alginate-encapsulated shoot apical and axillary buds. Int. J. Agric. Biol., 5: 513-516. (2003).
16. Bashir, M.A.; Rashid, H. and Anjum, M.A.: In vitro shoot multiplication of six promising strains of jojoba (*Simmondsia chinensis*). Biotechnol, 6: 309-315. (2007).
17. Agrawal, V.; Prakash, S. and Gupta, S.C.: Effective protocol for invitro shoot production through nodal explants of *Simmondsia Chinesis*. Biol. Plant., 45: 449-453. (2002)
18. Kozai, T.: Micropropagation under photo autotrophic conditions. Kluwer Academic Publishers. Pp. 447-469. (1991).
19. Gauchan, D.P.: Effect of different sugars on shoot regeneration of maize (*Zea mays* L). Kathmandu University. J. Sci. Eng. Technol., 8 (1): 119-124. (2012).
20. Petersen, K.K.; Hansen, J.; bouvet A.; Gavayrac, R. and Pqaues, M.: Significance of different carbon sources and sterilization methods on callus indication and plant regeneration of Miscanthus X ogiformis Honda Giganteus. Plant Cell Tiss. Org. Cult., 58: 189-197. (2001).
21. Taha, R.A.; Mustafa, N.S. and Hassan, S.A.M.: Protocol for Micropropagation of Two *Ficus carica* Cultivars. World J. Agri. Sci., 9 (5): 383-388. (2013).

22. Mustafa, N.S.; Taha, R.A.; Hassan, S.A.M. and Zaied, N.S.: Effect of medium strength and carbon source on *in vitro* shoot multiplication of two (*Ficus carcial*) cultivars, Journal of Appl. Sci. Res., 9 (4): 3068-3074. (2013).
23. Mamiya, K. and Sakamoto, Y.: Effects of sugar concentration and strenght of basal medium on conversion of somatic embryos in *Asparagus officinalis* L. Scientia Horticulturae., 84:15-26. (2000).
24. Lou, H.; and Sako, S.: Role of high sugar concentration in inducing somatic embryogenesis from cucumber cotyledons. Scientia Horticulturae, 64:11-20. (1995).
25. Farooqi, A.A. and Sreemu, B.S.: Cultivation of medicinal and Aromatic crops. University Press (India) Ltd. Hyderabad. Indiain coffea conephora. Plant Cell Tiss. Org. Cult, 60; 5-13. (2001).
26. Gujaral, R.: Zero% calorie, 100% sweet, 100% nature science tech, Enterpreneur, pp: 10-12. (2004).
27. Cardello, H.M.A.B.; Da Silva, M.A.P.A. and Damasio, M.H.: Measurement of the relative sweetness of stevia extract, aspartame and cyclamate/saccharin blend as compared to sucrose at different concentrations. Plant Foods for Human Nutrition, 54 (2): 119–129. Doi: 10.1023/A: 1008134420339. (1999).
28. Taha, R.A.; Taha, L.S. and Metwally, S.A.: In vitro cultures of jojoba (*Simmondsia chinensis* L.) affecting by laser irradiation. J Chemi Biol Phys Sci, Sec B., 5 (4): 3906-3913. (2015).
29. Murashige, T. and Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-497. (1962).
30. Taha, R.A.: In Vitro Propagation of Mango (*Mangifera indica* L.) and Jojoba (*Simmondsia chinensis* (Link) Schneider) and Tolerance to Salt Stress". Ph.D. thesis, Cairo University, Faculty of Agriculture, Pomology Dep. (2009).
31. Pottino, B.G.: Methods in plant tissue culture. Dept. of Botany. Agric. College. Maryland Univ., College Park. Maryland, USA, pp. 8-29. (1981).
32. Snedecor, W.B. and Cochran, G.W.: Statistical Methods 8th Ed. Lowa State Univ. Press. Ames, Low, U.S.A. (1989).
33. Fayek, M.A.; Shaaban, E.A.; El-Obeidy, A.A. and Taha, R.A.: In vitro propagation of three female jojoba clones (*Simmondsia chinensis* (Link) Schneider). Egypt J App Sci., 22 (6B): 652-665. (2007).
34. Duong, T.N.; Nguyen, T.T. and Nguyen T.D.: Effect of sucrose, glucose and fructose in proliferation of Hymalaya Yew (*Taxus wallichiana* ZUCC.) cell suspension cultures-a potential source for taxol production. Proceeding of International Workshop on Biotechnology in Agriculture. Nong Lam University HoChi Minh City, pp. 20-21. (2006).
35. Kumara Swamy, M.; Sudipta, K.M.; Balasubramanya, S. and Anuradha, M.: Effect of different carbon sources on in vitro morphogenetic response of patchouli (*Pogostemon cablin* benth.) J Phytology, 2 (8): 11–17. (2010).
36. Hassan, M.M. and Taha, R.A.: Callogenesis, somatic embryogenesis and regeneration of date palm Phoenix dactylifera L. cultivars affected by carbohydrate sources. International Journal of Agricultural Research, 7 (5): 231-242. (2012).

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