Developing a protocols to micropropagate Gac fruit *(Momordica cochinchinensis* Spreng.) *in vitro*

L.K.J. Al-Amery, * M. A. Al- Shamari** , I. H. Mohammed***, H.S.A. Al-Jubori

*Horticulture department, College of Agricultural Engineering Sciences, University of Baghdad Ministry of Higher Education, Iraq.

**Research Department, Horticulture Office, Ministry of Agriculture, Iraq.

***Date palm center, Horticulture Office, Ministry of Agriculture, Iraq.

maflower2017@ gmail.com ** Lamiaa.khlifa@coagri.uobaghdad.edu.iq

Abstract

A simple and rapid protocol was established for proliferation of Gac fruit *in vitro*. In this paper, the response of Gac fruit explants for sterilization was examined. The experiments were carried to select the most appropriate methods of reproduction. Best shoots multiplication was achieved on MS medium supplemented with 2 mgL^{-1} of BA as the highest average of main and secondary brunches (3.0 and 23.2 respectively), shoots length (7.3 mm), leaves number (28.6), fresh and dry weight (0.570 and 0.054 gm respectively) was recorded. Regenerated shoots were transferred to MS media containing (0, 0.5, 1 & 1.5) mgL^{-1} Indole-3 butyric acid (IBA). The highest average of root numbers (14.10) and length (7.5 cm) was noticed on medium enriched with 1.5 mgL^{-1} of IBA. Regenerated plantlets survived and grew vigorously in the field when cultured on peat moss with survival percentage (90%). Based on the results of present paper, a good proliferation was obtained using direct pathway via organogenesis, that should be useful for the rapid propagation of this important plant and led to produce plantlets that have ability to still alive in the field.
Key words: Propagation, Organogenesis, *Momordica cochinchinensis* Spreng, *In vitro*.

Introduction:

The origin of Gac fruit (*Momordica cochinchinensis* Spreng.) is South and South-East Asia, it is a tropical vine (14), dioecious and perennial climber (43) and a species of the Cucurbitaceae family (23). In some countries such as Thailand, Cambodia, Vietnam, India, Malaysia, China and the Philippines, *Momordica cochinchinensis* Spreng is especially popular and it is also known under the name Gac. It produces rather large fruits, which until quite recently were grown only for the use in food (42). In july, M. *cochinchinensis* produces red fruits and is harvested in August. Its distinctive red color which is widely used as a natural pigment in Vietnamese cooking and natural medicine (44). Typically, the fruit is round or oblong it's about 13 cm in length and 10 cm in diameter (30). The fruit pulp contains high fatty acids, vitamin E and carotenoids. Also, the substantial part of carotenoids compounds beta-carotene (46, 24, 23, 3). In the Gac seed membrane, the concentration of lycopene was about ten-fold higher than that in known lycopene-rich fruit and vegetables. Thus, that gives indicator that Gac fruit could be a new and potentially valuable source of lycopene (9). The roots are used to cure hair falling, cough, haemorhoid, to eliminate poisonous substances, insect bite and as a contraceptive

ISSN 2072-3857

in traditional medicine system (29). It has functional effects, such as antioxidant, anticancer antimicrobial activities (41, 14) and anti-inflammatory activity as well as anti-ulcer activity (10). At present, in Europe conditions, these non-traditional plants are considered as promising sources of raw materials for the food and pharmaceutical industries. Moreover, almost all parts of plant are used: fruits, leaves, caulls and seeds (38, 4). Products like Gac powder and Gac oil are manufactured as natural colorants and medicinal supplements (14). Propagation methods need to be improved to meet the increasing demand for Gac fruit as a health product (33). Thus, this paper was conducted to increase Gac yields on a larger scale in a short time using an efficient protocol for Gac production by in *vitro* technique.

Materials and methods

Explants preparation and initiation of culture: -

Single internodes were used as explants (10-15mm) of *M. cochinchinensis* for this propagation procedure. The sterilization of explants was performed by using solution of sodium hypochloride (6% NaOCl) for 10 minutes at different concentrations (0%, 5%, 10%& 15%). (1).,Explants were cultured on the Murashige& Skoog (MS) medium without the addition of phytohormones for ten days at a temperature of 22-24º C, relative humidity not less than 70%, in the light with 16-hours photoperiod (28).

Shoots multiplication: -

In this stage, four different concentrations of BA included in MS media (0, 1, 2, &3 mgL^{-1}). All shoot tips explants (5mm in length) of Gac fruit were placed on these media which supplemented with 30 gL^{-1} of sucrose and 7gL^{-1} of agar (Sigma-Aldrich). Cultures were incubated for one month, after that, data were collected including: Main brunches number, Secondary brunches number, Shoots length, Leaves number, Fresh weight and Dry weight.

Rooting of regenerated shoots: -

Explants (10 mm in length) were used to test the effect of IBA hormone at different concentrations such as (0, 0.5, 1, 1.5 mgL^{-1}). The data including response percentage, roots number and roots length were collected after one month of culture.

Acclimatization of regenerated plantlets: -

The rooted plantlet with 3–4 fully expanded leaves and well-developed roots were taken out from the containers and washed thoroughly with tap water to remove adhering agar. Then, plantlets were placed in tubes containing tap water for one week. After that, these plantlets were dipped in Benlate herbicide for two minutes and transplanted in pots containing sterilized three types of mixture.1) soil. 2) peat moss.3) soil and peat moss (1:1) and covered with glasses jars to maintain humidity. After a week, the glasses jars were gradually removed over a period of 5 days. The potted plants were kept inside a small glasshouse and watered regularly with sprinkler for a week. Further, they were watered twice a day with the help of a water cane. A temperature of 26 ± $2C^0$ and humidity of 85 - 90% were maintained inside the glasshouse. The *ex vitro* establishment rate was assessed as the percentage of acclimatized plants that survived after four weeks of transplanting (5).

Data analysis: -

A completely randomized design with ten replicates was applied to examine the effects of treatments. One- way analysis of variance was performed. Data were analyzed using Genstat software. Test of least significant differences (LSD) at 5% level of probability was used to compare the calculated averages of traits.

Results and discussion

Explants sterilization: -

To select the most appropriate method of sterilization for explants, it was found in current study that explants are successfully sterilized in 10 or 15% percent solution of sodium hypochlorite (6% NaOCl) for 10 minutes as these treatments appeared the lowest percentage of contamination which was 0, while the highest percentage of contamination was observed using control treatment which was without addition of sodium hypochlorite (Table 1). One of the most difficult micropropagation challenges is microbial contamination (45). The effective acquisition of high-quality sterile explant material can be considered
the key to the subsequent tissue culture (11). Sodium hypochlorite is the more considered option for chemical disinfection with a broad antimicrobial spectrum, rapid bactericidal action, relative stability and solubility in water (18). Also, it has a strong oxidation (36). Therefore, it is widely applied compound in plant tissue culture (25, 45). Based on its concentration, the production of hypochlorous acid (HOCl) in diluted solution was behind the germicidal effect of NaOCl (15, 6) also to hypochlorite ion (ClO^-) oxidizing agent in concentrated form as well as to its high pH (12.5–13.5) (17). The positive effect of sterilization by NaOCL was mentioned by Benmahioul, et.al.2016 who refereed that 96% contamination−free culture can be achieved using NaOCL 2.6% (w/v) for 10 minutes to sterilize stem segments explants of *Pistacia vera* L. Also, good results were obtained when shoot tips explants of pistachio were sterilized in a 10% NaOCl but for 30 minutes (39).

Table 1. Effect of hypochlorite concentrations on level of culture contamination.

Hypochlorite concentrations	Contamination percentage%
0	100
5	70
10	0
15	0
L.S. D	3.7

Multiplication of shoots: -

In vitro, shoot propagation and multiplication are largely based on media formulations including cytokinins which considered as a major plant growth regulator (34).
The response for shooting is dependent upon concentration of cytokinin supplemented in the medium (7). According to our research results, significant effect was observed on response percentage using BA in multiplication media ($P<0.001$), especially at concentration of 2 mgL^{-1} (Fig.1). The greatest percentage of explants response (90%) was observed on this medium which differed significantly from all other concentrations used. Regeneration was observed through organogenesis only. As shown in table (2), the use of BA at 2 mgL^{-1} promoted the proliferation and elongation of shoots that produced from internodal explants as the highest average of main brunches number (3.0), secondary brunches number (23.2) and shoots length (7.3 mm) was recorded at this concentration (Fig.2). In accordance with our results, the effect of BA in inducing multiple shoots at concentration of 2 mgL^{-1} has been previously reported using internodal explants of bitter gourd *(Momordica charantia L.* cv. Faizabadi) and the best response was recorded (8). Also, a good number of shoots was shown using various concentrations of BA including 2mgL^{-1} that applied for *in vitro* propagation of

ISSN 2072-3857

Momordica charantia L. using shoot apex (27). (37) mentioned that BA alone was more favorable than TDZ in inducing shoot regeneration from leaves of cherry cultivars. Our findings in contrast with (26) who studied and reported that the best plant growth regulator for shoot multiplication in nodal segment explants of gac plant achieved by using semi-solid MS medium supplemented with 4mg/L BA that produced average of 9.2 shoots per explant and an average of 2.247 cm in length after 4 week. However, in the present paper, the highest average of leaves numbers, fresh and dry weight was also observed on medium containing 2 mgL^{-1} of BA. Cytokinins are often applied to stimulate growth and development, BA and KN being in common use (35). Based on our results, the use of BA as plant growth regulator is essential for the growth of shoot or shoot regeneration in gac plant tissue culture.

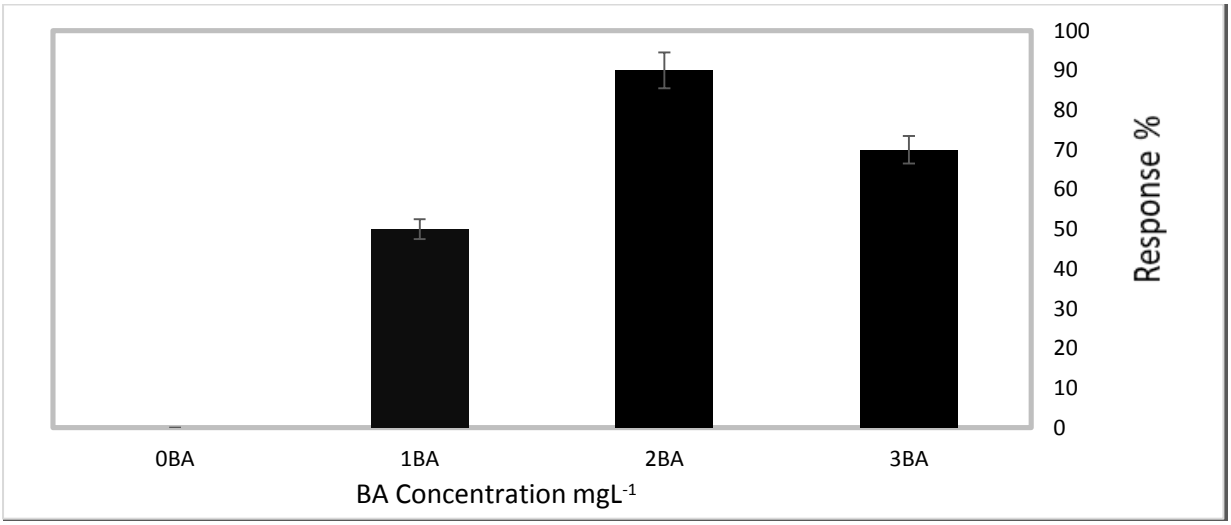

Figure 1. Effect of BA concentrations on response percentage% of explants (LSD= 7.0).

Table 2. The effect of BA concentrations on shoots formation through multiplication of Gac explants.

BA Concentration (mgL^{-1})	Main brunches number	Secondary brunches number	Shoots length mm	Leaves number	Fresh weight g	Dry weight g	Mean
0	1.0a	5.0a	4.2a	5.8a	0.178a	0.029a	2.701
1	2.6b	16.4b	4.7a	17.8b	0.176a	0.028a	6.950
2	3.0b	23.2c	7.3b	28.6c	0.570b	0.054b	10.454
3	2.2b	13.8b	2.6a	20.0bc	0.454c	0.042c	6.516
Mean	2.2	14.6	4.7	18.05	0.344	0.038	6.655
LSD	0.9	4.6	2.0	5.3	0.104	0.012	

ISSN 2072-3857

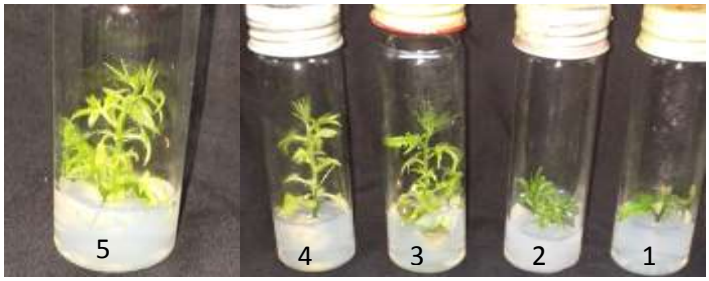

Figure 2. Effect of BA concentrations on multiplication of gac explants. 1) Explant on medium without hormone (control). 2) Explant on medium containing BA at 1 mg. L^{-1}. 3) Explant on medium containing BA at 2 mg. L^{-1}. 4) Explant on medium containing BA at 3 mg. L^{-1}. 5) Explant on medium containing BA at 2 mg. L^{-1} after one month of culture.

Rooting of explants: -

Auxins are involved in cell division, cell elongation, rhizogenesis and root formation, embryogenesis, vascular tissue differentiation and inhibition the growth of axillary shoot (13, 20, 31, and 5). At rooting stage, the plantlets are prepared for transferring from *in vitro* to *ex vitro* conditions in controlled environment rooms then in the glasshouse and later, to their ultimate location (22). In present paper, optimum induction and differentiation of root was achieved on MS media supplemented with 1.5 mgL^{-1} of IBA which showed a high percentage of response for explants reached to100% ($P<0.001$) also, the highest average for roots number and length was achieved on this concentration (Fig.3 & 4).However, in contrast to our results, (26) referred that IBA was the best PGR for root induction using nodal segment explants of gac plant that cultured on semi-solid MS medium enriched with 4mg/L IBA, the best average of 13.2 roots per explant and 4.29cm in length was achieved on this concentration. Whereas, (16) have mentioned that micro-propagated shoots of Gac plant that produced from callus tissue were subsequently transferred to half strength MS containing 0.5mg/L^{-1} (IBA) for rooting with 100% success. (39 , 40 , 2) referred that IBA was the most effective auxin for root formation in shoot regenerated using apical tips of staminate *P. vera* L. but on medium containing 2 mgL^{-1}.

ISSN 2072-3857

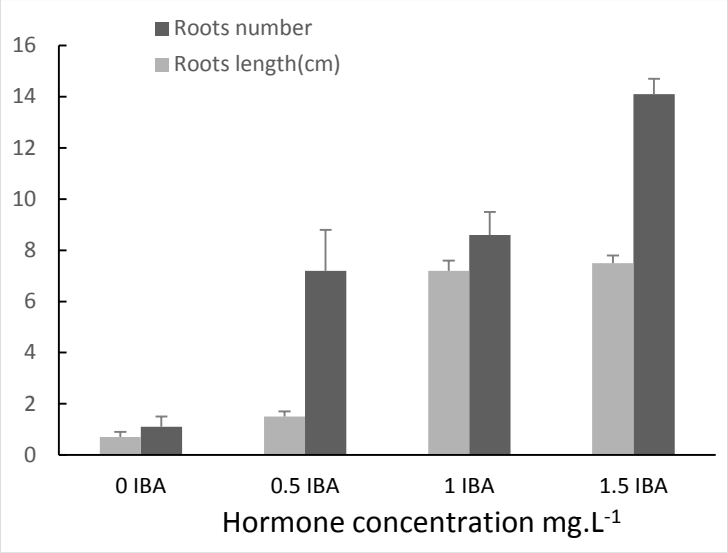

Figure 3. The effect of hormone concentrations (IBA) on roots formation from Gac fruit explants. L. S. D=3 for roots number and L.S.D=0.8 for roots length.

Figure 4. Effect of IBA at concentration of 1.5 mg.L^{-1}on roots number and length of Gac fruit explants.

Plant acclimatization:

Hardening is essential. Thus, for the establishment of plant, regenerated healthy rooted plantlets were placed in tubes containing tap water at room temperature for one week (Fig5. A), then were cultured in pots and adapted gradually to the *ex vitro* environment. (Fig5. B). after that, the plantlets that set out in green house could well-develop (Fig5. C) That indicates the ability of *M. cochinchinensis* plants for regeneration using *in vitro* culture. Survival of plantlets as much as 90% was recorded for plantlets acclimatized in peat moss, also the mixture of soil and peat moss (1:1, v/v) achieved 80% of survival percentage and the use of soil led to 50%. This result is consistent with (33), (21) who reported that gac plant has suitability for production in soilless media and also suggested that gac prefers a well-drained soil or growing medium.

ISSN 2072-3857

Figure 5. A) Plantlets via hardening stage in tap water for one week. B) Plantlets in pots covered with glasses jar to keep humidity. C) Gac plant which grown in green house after four weeks of planting.

Conclusion

It can be concluded that this approach of *in vitro* regeneration technique offers an efficient and reproducible protocol for direct multiplication from internodal explants of Gac plant. This study is particularly novel for Gac plant due to the relatively high proliferation rate and efficiency of this protocol. Hence, we expect that the method presented in this paper will be helpful for the commercial production of true-to-type plants generated *in vitro***.**

References

1. Abdul Husain, Z.M., Jawad, L.K. 2019.Effect of magnetic field on the growth, multiplication and concentration of the volatile oil of *rosemary officinalis in vitro*. Iraqi Journal of Agricultural Sciences .2019, 50(4). 982–989
2. Al-Jubori, M.T., Al-Amery, L.K.J. 2022.Effect of cytokinins BA, KIN, auxin IAA AND IBA ON propagation (*Citrus aurantifolia*) in vitro**.** International Journal of Agricultural and Statistical Sciences, 18, 2033–2040.
3. Al-Amery, L.K.J., Al-Ani, H.S.H., Al-Hasany, Z.H.A.2022. Evaluating effect of different concentrations of chemical mutagens on the vegetative growth of acclimated stevia plants and their content of rebaudioside – A and stevioside. International Journal of Agricultural and Statistical Sciences, 18. 2091–2098.
4. Al-Amery, L.K.J., ALjubori, M.T. 2021. Effect of benzyl adenine and meta-topolin on in vitro propagation of C-35 citrange rootstocks. International Journal of Agricultural and Statistical Sciences. 16, 1639–1643.
5. Al-Amery, L.K.J., Salman, B.I.H. 2016. Study the effect of cytokinins and auxins in the composition and production of In Vitro plantlets Hippeastrum Hybridum. Iraqi Journal of Agricultural Sciences (6), 1392–1403.
6. Al-Amery, L.K.J., Salman, B.I.H .2016.Influence of cytokinins and sucrose concentrations on bulblet characters of In Vitro *Hippeastrum Hybridum.* Iraqi Journal of Agricultural Sciences. 47(6). 1384–1391.
7. Agarwal, M. 2015. Tissue Culture of *Momordica charantia L.*: A Review, Journal of Plant Sciences. 3 (1): 24-32.
8. Agarwal, M. and Kamal, R. 2004. *In vitro* clonal propagation of *Momordica charantia L.* Indian Journal of Biotechnology 3: 426-430.
9. Aoki, H., Kieu, N.T., Kuze, N., Tomisaka, K., Van Chuyen N. 2002. Carotenoid pigments in GAC fruit *Momordica cochinchinensis L.*

Biotechnology and Biochemistry, 66: 2479-2482.
10. Ariffin, A. S., Shah, R. M., Ahmad,K. A. Bin Hashom, H. 2021. Addressing the sustainable agricultural agenda: Gac Fruit Benefit in Qur'an and Value Chain Perspective. Advances in Biological Sciences Research, volume 17. International Conference on Tropical Agrifood, Feed and Fuel (ICTAFF 2021).
11. Barampuram, S., Allen, G., Krasnyanski, S. 2014. Effect of Various Sterilization Procedures on the *in Vitro* Germination of Cotton Seeds. Plant Cell Tissue Organ Cult. 118, 179–185.
12. Benmahioul, B., Kaid-Harche, M, Daguin, F. 2016. *In vitro* regeneration of *Pistacia vera* L. From nodal explants. J. For Sci. 62:198–203.
13. Chawla, H. S. 2002. Introduction to plant biotechnology, 2nd ed., Science Publishers INC, New Hampshire, United States of America 528 p.
14. Chuyen, H.V.; Nguyen, M.H.; Roach, P.D.; Golding, J.B.; Parks, S.E. Gac fruit (*Momordica cochinchinensis* Spreng.) 2015. A rich source of bioactive compounds and its potential health benefits. Int. J. Food Sci. Technol. 50, 567–577.
15. Dantec, C. L., Duguet, J. P., Montiel, A., Dumoutier, N., Dubrou, S., Vincent, V. 2002 Chlorine disinfection of atypical mycobacteria isolated from a water distribution system. Appl Environ Microbiol. 68:1025–1032.
16. Debnath, B., Sinha S. and Sinha, R. K. 2013. *In vitro* differentiation and regeneration of *Momordica cochinchinensis* (Lour.) Spreng. Indian Journal of Plant Sciences, 2(2): 17-21.
17. Estrela,C., Estrela, C. R. A, Barbin, E. L., Spano, J. C., Marchesan, M. A., Pecora, J. D. 2002. Mechanism of action of sodium hypochlorite. Braz Dent J. 13(2):113–117.
18. Fukuzaki, S. 2006. Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Sci.11:147–57.
19. Gammoudi, N., Nagaz, K. & Ferchichi, A. 2022. Establishment of optimized *in vitro* disinfection protocol of *Pistacia vera* L. explants mediated a computational approach: multilayer perceptron–multi−objective genetic algorithm. *BMC Plant Biol* **22**, 324.
20. George, E. F., Hall, M. A. and Klerk, G. J. De. 2007. Plant Propagation by Tissue Culture: The Background. Exegetic, Basingstone, UK. p. 508.
21. Hamood, A.K. and Bayan.H.M. 2017. Effect of benzyl adenine and salicylic acid on growth and total alkaloids production of wethinia (*Withania somnifera* L.) *In vitro.* The Iraqi Journal of Agricultural Sciences. 48(1), 256-265
22. Iliev, I., Gajdošova, A., Libiakova, G. and Jain, S. M. 2010. Plant Micropropagation. In: Davey M.R., Anthony P. (Eds). Plant Cell Culture. John Wiley and Sons. Ltd. Publications. pp. 1–24
23. Ishida, B.K., Turner, C., Chapman, M.H., McKeon T.A. 2004. Fatty acid and carotenoid composition of gac *(Momordica cochinchinensis Spreng) fruit*. J. Agric. Food. Chem, 52 (2): 274-279.
24. Kuhnlein, H.V. 2004. Karat, Pulque, and Gac: Three shining starts in the traditional food galaxy Nutrition Reviews, 62: 439-442.
25. Lazo-Javalera, M.F., Troncoso-Rojas, R., Tiznado-Hernández, M.E., MartrtínezTellez, M. A., Varg as-Arispuro, I., Islas-Osuna, M. A., Rivera-Domínguez, M. 2016. Surface disinfection procedure and *in vitro* regeneration of grapevine (*Vitis vinifera* L.) axillary buds. Springerplus.

ISSN 2072-3857

26. Lim, C. H. 2021. Establishment of tissue culture planting materials of Gac *Momordica cochinchinensis)* plant using improved light emitting diodes (LEDS) technology. Master of Science. University Sains Malaysia.
27. Mishra, J., Tiwari, K. L. and Jadhav, S. K. 2012. Micro propagation of *Momordica charantia* L., International J. of Biological and Health Sciences, 1(1): 25-31.
28. Murashige, T., Skoog, F. 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia plantarum*, 15 (3): 473–497.
29. Nanthachit, K. and Tuchinda, P. 2008. Chemical Constituent of the Hexane Extract from Leaves of *Momordica cochinchinensis* (Lour.) Spreng. Thai Pharmaceutical and Health Science Journal, 3(2) 210-213.
30. Osman, M., Sulaiman, Z., Saleh, G., Shahril Abdul Rahman, M., Zainuddin,Z.M., Abu Sin,M. , Ana Arina, A. H. and Hamidon, A. 2017. Gac fruit, a plant genetic resource with high potential. In Conference: 12th International Genetics Congress 2017 (MiGC12) At: Bangi, Selangor, Malaysia.
31. Park, W.T., Kim, Y.K., Udin, M.R., Park, N.I.I, Kim, S.G., Young, L. and Park, S.U. 2010. Somatic embryogenesis and plant regeneration of lovage (*Levisticum officinale* Koch). Plant Omics J., 3: 159–161.
32. Parks, S., Murray, C., Gale, D., AL-Khawaldeh, B., & Spohr, L. 2013. Propagation and production of Gac (*Momordica cochinchinensis* Spreng.), a greenhouse case study. Experimental Agriculture, 49(2), 234-243.
33. Parks, S., Nguyen, M., Gale D. and Murray, C. 2013. Assessing the potential for a Gac (Cochinchin gourd) industry in Australia. Rural Industries Research and Development Corporation. Electronically published by RIRDC in July 2013 Print-on-demand by Union Offset Printing, Canberra at www.rirdc.gov.au.
34. Pati, P. K., Rath, S.P., Sharma, M., Sood, A. and Ahuja P. S. 2005. *In vitro* propagation of rose – a review. Biotechnology Advance, 94–114.
35. Pierik, R.L.M. 1987. *In vitro* Propagation of Higher Plants. Martinus Nizhoof Publisher, Boston.
36. Si, Y., Haxim, Y. & Wang, L. 2022. Optimum sterilization method for *In Vitro* cultivation of dimorphic seeds of the succulent halophyte *Suaeda aralocaspica*. Horticulturae 8(4): 289.
37. Tang, H., Ren, Z., Reustle, G., Krczal, G. 2002. Plant regeneration from leaves of sweet and sour cherry cultivars. Scientia Horticulturae 93, 235–244
38. Taylor, L. 2002. Technical data report for Bitter Melon *(Momordica charantia).* Herbal Secrets of the Rainforest. 2nd edition. Sage Press, Austin: 123- 126.
39. Tilkat E, Onay A, Yildirim H, Ayaz E. 2009. Direct plant regeneration from mature leaf explants of pistachio. *Pistacia vera* L. Sci. Hortic.121:361–5.
40. Tilkat, E., Onay, A., Yıldırım, H., Ozen Cetin, H. 2008. Micropropagation of mature male pistachio Pistacia vera L. The Journal of Horticultural Science & Biotechnology 83, 328–333
41. Tinrat, S., Akkarachaneeyakorn, S., Singhapol, C. 2014. Evaluation of antioxidant and antimicrobial activities of Momordica Cochinchinensis Spreng (Gac fruit) ethanolic extract. Int. J. Pharm. Sci. Res. 5, 3163–3169.
42. Tokhtar, V. K., Doang, Z., Tokhtar,L. A., Korotkov,O. I., Galina, I. and Safronova, G. I. 2016. *Momordica Cochinchinensis* (Lour.) Spreng. (Cucurbitaceae) In Culture *in Vitro*.

Res. Jou. Of Pharmaceutical, Biolo. & Chemi. Sci.7 (6). 3243-3246.

43. Tran, X., Parks, S., Roach, P., & Nguyen, M. 2020. Improved propagation methods for Gac (*Momordica Cochinchinensis* Spreng.). Experimental Agriculture, 56(1), 132-141.
44. Trinh, H. T. 2005. Overview of Vietnamese traditional medicine. Bull. Food Technol. 18, 91–97.
45. Tung, H.T, Bao, H.G., Cuon,g D.M., Ngan, H.T.M, Hien, V.T, Luan, V.Q. 2021. Silver nanoparticles as the sterilant in largescale micropropagation of chrysanthemum. In Vitro Cell Dev Biol – Plant. 57:897–906.
46. Vuong, L.T., King, J.C. 2003. A method of preserving and testing the acceptability of Gac fruit oil, a good source of beta-carotene and essential fatty acids. Food and Nutrition Bulletin, 24: 224-230.

ISSN 2072-3857