Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2022.448.452

Research Article
Micropropagation Protocol for Goji Plant (*Lycium barbarum* L.)

[1,2]Rania A. Taha

[1]Department of Pomology, Laboratory of Biotechnology and Micropropagation, National Research Centre (NRC), Giza, Egypt
[2]Laboratory of Tissue Culture Technique, Central Laboratories Network, National Research Centre NRC, Giza, Egypt

Abstract

Background and Objective: Goji could be a promising plant in Egypt due to the suitable climate. To spread the plantation of goji in Egypt, propagation of this plant must be done. Micropropagation is a unique technique that could be used for this purpose. This investigation aimed to establish an *in vitro* culturing protocol for goji fruit in Egypt. **Materials and Methods:** Benzylaminopurine (BAP) at 0.00, 0.25, 0.50, 1.0, 2.0 and 4.0 mg L^{-1} were studied for multiplication. For the rooting stage, Indole Butyric Acid (IBA), Indole Acetic Acid (IAA) or Naphthalene Acetic Acid (NAA) as auxins with concentrations of 0.25 or 0.5 mg L^{-1}, with or without activated charcoal were studied. **Results:** Results showed that BAP, at all concentrations used, enhanced multiplication rate compared with the control (0.0 mg L^{-1} BAP). The highest average shoot number was obtained with BAP at 1.0 mg L^{-1} as well as the number of leaves and chlorophyll score. Meanwhile, the highest shoot length was achieved with 0.3 mg L^{-1} BAP+1.0 mg L^{-1} GA_3. Full MS strength was superior for the average number of shoots, length of shoots and the moderate number of leaves. IBA at 0.5 mg L^{-1} without AC gave the highest rooting (50%) and root number per rooted shoots (5.67). **Conclusion:** A complete *in vitro* protocol was determined for the goji berry plant.

Key words: Goji, *in vitro* culture, GA_3, BAP, auxin, medium strength, *Lycium barbarum*

Citation: Taha, R.A., 2022. Micropropagation protocol for goji plant (*Lycium barbarum* L.). Asian J. Plant Sci., 21: 448-452.

Corresponding Author: Rania A. Taha, Department of Pomology, Laboratory of Biotechnology and Micropropagation, National Research Centre (NRC), Giza, Egypt

Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Goji (*Lycium barbarum* L.) belongs to the family of *Solanaceae* is used as a food and medicinal plant in China. This plant is commonly called goji in China but has other names like a boxthorn, wolfberry and Chinese wolfberry. It is generally consumed in soups, with rice or added to a meat and vegetable dish. Polysaccharides, carotenoids and flavonoids are the major metabolites in this fruit[1]. Various health effects of *L. barbarum* polysaccharides were confirmed.

The fruit has an oblong shape. Its length ranges from 6-20 mm and diameter 3-10 mm. Its colour ranged from orange to dark red and it has shrunken skin. The pulp is fleshy and soft with a bitter and sweet taste. The goji fruit is usually consumed fresh, dry or as a juice. The fruit is also processed to make tinctures, powders and tablets. In addition to using as a food, it also benefits as a medicinal plant in East Asia[2].

The leaves, fruits and the root bark of *Lycium barbarum* contain abundant polysaccharides, carotenoids, flavonoids, alkaloids, amides, peptides, anthraquinones, coumarins, lignanoids, terpenoids, sterols, steroids, organic acids, anthocyanins, essential oils and glycolipids. So that, the goji plant can treat various diseases, including blurry vision, abdominal pain, infertility, dry cough, fatigue, dizziness and headache. Otherwise, *L. barbarum* has long been used in oriental medicine as a potent anti-ageing agent[3]. For instance, it is effective for counteracting premature greying of hair[4,5].

Goji is an unknown plant in Egypt despite its suitable climate. To spread the plantation of goji in Egypt, propagation for the few existing plants must be done.

Micropropagation is a unique technique that could be used for this purpose. This technique may be used for both commercial (i.e., mass production) and scientific studies (i.e., germplasm preservation, *in vitro* conservation[6], plant breeding, physiological[7,8] and genetics) also allow opportunities to improve plants against abiotic stress factors using *in vitro* selection methods[9].

This investigation aimed to establish an *in vitro* culturing protocol for the goji berry plant in Egypt.

MATERIALS AND METHODS

Study area: The present study was conducted in 2018-2020 at the Laboratory of Tissue Culture Technique, Central Laboratories Network, National Research Centre, Dokki, Giza, Egypt. Vegetative shoots of goji plants grown in the Department of Pomology greenhouse were collected and brought to the lab.

Establishment stage: Explants were prepared, leaves were detached and shoots were divided into small explants included one node for each, washed with tap water for 20 min and sterilized into the laminar flow hood.

Sterilization treatments: Quick immersion (1 or 2 sec) in mercuric chloride at 1.0 g L^{-1} or soaking in 30% Clorox (sodium hypochlorite 5%) was used for 10 or 15 min for sterilization. After that, explants were cultured individually on MS medium[10] as a basal medium. MS medium was supplemented with 0.5 mg L^{-1} 6- Benzylaminopurine (BAP), 30 g L^{-1} sucrose and 6.0 g L^{-1} agar. The pH of the medium was adjusted to 5.7 and autoclaved at 121°C and 15 lb/in^2 for 20 min. The cultured explants were incubated under 16 hrs of artificial light (fluorescent light at 30 μM sec^{-1}) and 8 hrs of darkness at an average temperature of 23±2°C. Response of cultures was determined as survival percentage.

Multiplication stage: Subculturing was done regularly at 4 weeks intervals in the multiplication stage. Thus, the following experiments were carried out:

- **Effect of 6-benzyl amino purine (BAP) concentrations on *in vitro* culture of goji plant:** *In vitro* explants collected from establishing stage were cultured on a pre-prepared MS medium. Different concentrations of BAP (0.00, 0.25, 0.50, 1.0, 2.0 and 4.0 mg L^{-1}) were investigated to evaluate the most suitable concentration inducing the highest proliferation
- **Effect of medium strength on *in vitro* culture of goji plant:** *In vitro* explants collected from the multiplication stage were cultured into full, one and a half or double strength of MS medium[10] to select the best medium-strength inducing growth vigour and proliferation
- **Enlargement stage:** The proliferated shoots were used as explants and cultured on 3/4 strength of MS medium supplemented with combinations of BAP and GA_3, 0.1, 0.2 and 0.3 mg L^{-1} with 0.5 and 1.0 mg L^{-1}, respectively. Percentage of enlarged plants, average shoots length and growth vigour were estimated
- **Rooting stage:** Effect of type and concentrations of auxins on *in vitro* rooting of goji plant, with or without Activated Charcoal (AC) were studied. The enlarged shoots were used as explants and cultured on 3/4 strength of MS medium supplemented with Indole Butyric Acid (IBA), Indole Acetic Acid (IAA) or Naphthalene

Acetic Acid (NAA) as auxins with concentrations of 0.25 or 0.5 mg L^{-1}, with or without Activated Charcoal (AC). Rooting percentage, the average number of roots per rooted explant and average root length per root were evaluated

- **Acclimatization stage:** Rooted shoots of goji were transplanted to greenhouse and cultured in a mixture of peat and sand at 1:1 (v/v), covered with plastic bags and irrigated with Hoagland solution day after day. Plastic bags were removed gradually. The survived plants were transplanted to bigger pots

Statistical analysis: Data were analyzed as a one-way completely randomized factorial design and mean separation was carried out using Duncan's multiple range test at a 5% level of significance. Data analysis was performed using CoSATAT version 7.7 beta (2015).

RESULTS AND DISCUSSION

Table 1 revealed that immersing goji explants in $HgCl_2$ at 1.0 g L^{-1} for 1 sec was optimum for sterilizing and survival percent. Meanwhile, Clorox at 30% for 10 min gave the lowest survival rate. Similarly, Neliyati *et al.*[11] found that 0.1% $HgCl_2$ was optimum for sterilization of oil palm explants.

Table 2 showed that BAP, at all concentrations used, enhanced multiplication rate compared with the control (0.0 mg L^{-1} BAP). The highest average shoot number was obtained with BAP at 1.0 mg L^{-1}. Similarly, this concentration enhanced the number of leaves and chlorophyll score. Meanwhile, the highest shoot length was achieved with the control and BAP at 0.25 mg L^{-1} (Fig. 1a). Similarly, Hassan *et al.*[12] found that BAP encouraged shoot multiplication of plum Cv. 'Santa Rosa'. In addition, Taha *et al.*[13] assured that BAP included in the medium is surpassed other treatments for data palm multiplication rate.

Table 3 showed that medium supplemented with full MS was superior for an average number of shoots, length of shoots and the moderate number of leaves while the double MS supplementary gave the highest number of leaves. Similarly, Rezali *et al.*[14] revealed that full MS strength increased shoot number of *Typhonium flagelliforme* compared with other srengths investigated.

Table 4 assured that medium supplemented with 0.3 mg L^{-1} BAP+1.0 mg L^{-1} GA_3 gave the highest percent of enlarged plantlets, plantlet length and growth vigour score (Fig. 1b). Similarly, Geng *et al.*[15] assured that GA_3 increased shoot elongation when included with BAP in 2 dwarfing apple rootstock cultures.

Table 5 indicated that the type of auxin, concentration and AC affected the rooting of goji shootlets, significantly. The highest significant percent of rooting was observed with IAA or IBA at 0.5 mg L^{-1}, without AC (50.0%). IBA at 0.5 mg L^{-1} gave the highest root number per rooted shoots followed by IAA at 0.5 mg L^{-1} (Fig. 1c-d). Interestingly, the rooting medium included activated charcoal did not enhance rooting parameters. In addition, rooting medium included NAA without AC gave the lowest results. Hassan *et al.*[12] found that the highest root percentage was observed with IBA at 0.25 mg L^{-1}. In addition, Rashotte *et al.*[16] stated that IBA was better than IAA for *Arabidopsis*, it is might be due to it is transported at greater levels than is IAA.

Table 1: Effect of time of exposure and type of detergents of goji explants

Treatments	Survival rate (%)	Treatments	Survival rate (%)
Clorox at 30% for 10 min	16.67	$HgCl_2$ at 1.0 g L^{-1} immersion for 1 sec	58.33
Clorox at 30% for 15 min	50.0	$HgCl_2$ at 1.0 g L^{-1} immersion for 2 sec	50.00

Table 2: Effect of BAP concentrations on multiplication of goji explants

BAP (mg L^{-1})	Shoot numbers	Shoot length	Leaf numbers	Chlorophyll scores
0.0	2.00^{f}	1.70^{a}	6.60^{d}	2.50^{c}
0.25	3.43^{e}	1.70^{a}	9.13^{a}	3.00^{b}
0.5	4.29^{c}	1.38^{b}	7.90^{c}	3.14^{ab}
1.0	6.25^{a}	1.40^{b}	8.44^{b}	3.25^{a}
2.0	4.14^{d}	1.29^{c}	5.86^{e}	2.57^{c}
4.0	4.67^{b}	0.57^{d}	5.39^{f}	2.00^{d}

Means followed by the same letter within each column do not significantly differ from each other at a 1.0% level

Table 3: Effect of MS strength on multiplication and growth of goji *in vitro* shoots

MS strength	Shoot numbers	Shoot length (cm)	Leaf numbers
Full MS	12.00^{a}	1.37^{a}	6.10^{b}
1.5 MS	7.50^{b}	1.19^{a}	4.92^{c}
Double MS	5.00^{c}	1.29^{a}	10.05^{a}

Means followed by the same letter within each column do not significantly differ from each other at a 1.0% level

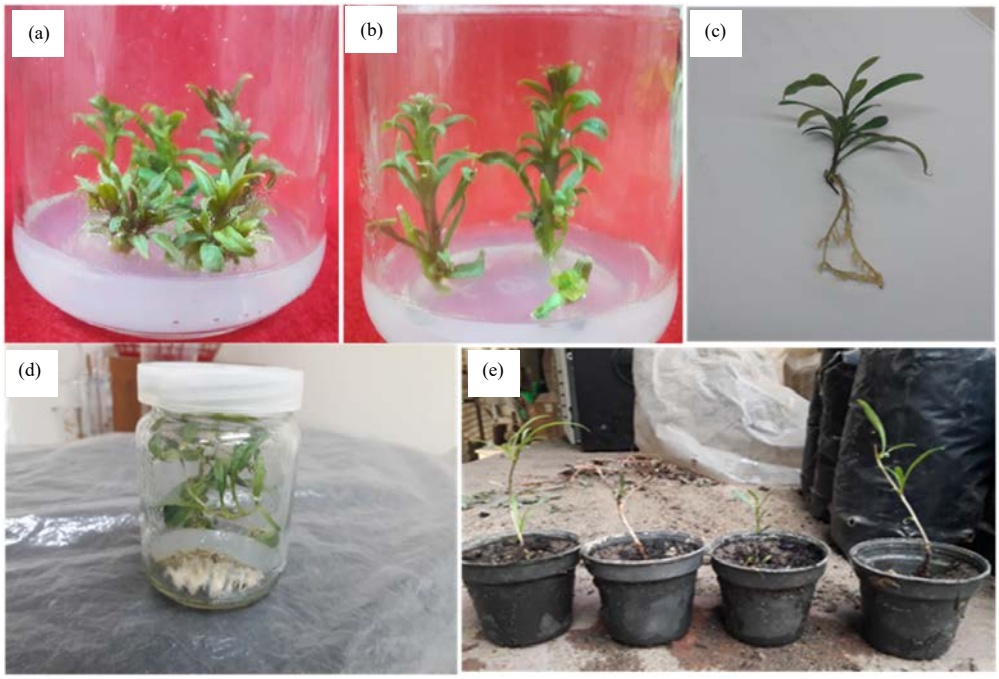

Fig. 1(a-e): Complete stips of goji micropropagation, (a) Multiplication, (b) Elongation, (c) Rooting, (d) Excessive rooting and (e) Successive acclimatization

Table 4: Effect of GA_3 on the enlargement of goji *in vitro* shoots

Combination treatments (mg L^{-1})	Enlarged plantlets (%)	Plantlet length (cm)	Growth vigor (score)
0.1 BAP+0.5 GA_3	30.00	2.0^{d}	2.0^{c}
0.2 BAP+0.5 GA_3	23.33	3.0^{c}	2.0^{c}
0.3 BAP+0.5 GA_3	26.67	3.0^{c}	2.0^{c}
0.1 BAP+1.0 GA_3	35.33	4.0^{b}	3.0^{b}
0.2 BAP+1.0 GA_3	38.67	4.0^{b}	3.0^{b}
0.3 BAP+1.0 GA_3	85.33	6.0^{a}	5.0^{a}

Means followed by the same letter within each column do not significantly differ from each other at a 1.0% level

Table 5: Effect of type of auxin and concentrations with or without AC on rooting of goji *in vitro* shoots

Auxin type and concentration (mg L^{-1})	Root (%)	Average number of roots/rooted explant (number)	Average root length (cm)/root
0.25 NAA	12.5	1.00^{c}	1.00^{ef}
0.50 NAA	0.00	0.00^{d}	0.00^{f}
0.25 IBA	33.33	1.50^{b}	9.33^{b}
0.50 IBA	50.00	5.67^{a}	4.35^{d}
0.25 IAA	33.33	1.00^{c}	4.25^{d}
0.50 IAA	50.00	1.50^{b}	6.00^{c}
0.25 NAA+AC	16.67	1.00^{c}	2.00^{e}
0.50 NAA+AC	16.67	1.00^{c}	11.00^{a}
0.25 IBA+AC	0.00	0.00^{d}	0.00^{f}
0.50 IBA+AC	16.67	1.00^{c}	5.00^{cd}
0.25 IAA+AC	16.67	1.00^{c}	10.00^{ab}
0.50 IAA+AC	0.00	0.00^{d}	0.00^{f}

Means followed by the same letter within each column do not significantly differ from each other at a 1.0% level

Thus, it is recommended to use BAP for goji multiplication and IBA for rooting. It is worth mentioning that rooted shoots of goji plantlets were transferred to the greenhouse, cultured in a mixture of peat and sand at 1:1 (v/v), covered with plastic bags and irrigated with Hoagland solution day after day. Plastic bags were removed gradually. The survived plants (Fig.1e) were transplanted to bigger pots.

CONCLUSION

A complete *in vitro* protocol was determined for the goji plant. Results showed that BAP, at all concentrations used, enhanced the multiplication rate. The highest average shoot number, number of leaves and chlorophyll score were obtained with BAP at 1.0 mg L^{-1}. Full MS strength was more suitable for goji multiplication. The addition of 1.0 mg L^{-1} GA_3 was crucial for achieving the highest shoot length. IBA at 0.5 mg L^{-1} without AC gave the highest rooting percentage and root number per rooted shoots (5.67).

SIGNIFICANCE STATEMENT

Goji (*Lycium barbarum* L.) is a new coming plant to Egypt that need scientific investigations about propagation, suitable planting area and weather. To process these investigations, a huge number of these plants is needed. The tissue culture technique is a suitable tool for getting large numbers and true to type plants. Results assured that full MS strength medium with BAP at 1.0 mg L^{-1} was suitable for healthy multiplication. Enlargement of *in vitro* shoots can be obtained by the addition of GA_3 to the culture medium. For obtaining roots, IBA at 0.5 mg L^{-1} was the suitable auxin concentration used. Acclimatization was successfully processed. This study discovers the *in vitro* protocol for goji plant propagation that can be beneficial for scientists who need to do researches about breeding, gene transportation and gene expression, etc.

REFERENCES

1. Cheung, B., M. Kwan, R. Chan, M. Sea and J. Woo, 2016. Potential of Asian Natural Products for Health in Aging. In: Molecular Basis of Nutrition and Aging, Malavolta, M. and E. Mocchegiani (Eds.), Elsevier, Amsterdam, Netherlands, ISBN: 978-0-12-801816-3, Pages: 18.
2. Potterat, O., 2010. Goji (*Lycium barbarum* and *L. Chinense*): Phytochemistry, pharmacology and safety in the perspective of traditional uses and recent popularity. Planta Med., 76: 7-19.
3. Gao, Y., Y. Wei, Y. Wang, F. Gao and Z. Chen, 2017. *Lycium barbarum*: A traditional Chinese herb and a promising anti-aging agent. Aging Dis., 8: 778-791.
4. Toyoda-Ono, Y., M. Maeda, M. Nakao, M. Yoshimura, N. Sugiura-Tomimori and H. Fukami, 2004. 2-O-(β-D-Glucopyranosyl) ascorbic acid, a novel ascorbic acid analogue isolated from *Lycium* fruit. J. Agric. Food Chem., 52: 2092-2096.
5. Luo, Q., Y. Cai, J. Yan, M. Sun and H. Corke, 2004. Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from *Lycium barbarum*. Life Sci., 76: 137-149.
6. Hassan, M.M., R.A. Taha and I.A. Ibrahim, 2016. *In vitro* conservation of date palm embryos under slow-growth conditions with osmotic agent and abscisic acid. Int. J. PharmTech Res., 9: 173-183.
7. Taha, R.A., M.M. Hassan, E.A. Ibrahim, N.H.A. Baker and E.A. Shaaban, 2016. Carbon nanotubes impact on date palm *in vitro* cultures. Plant Cell Tissue Organ Cult., 127: 525-534.
8. Gaafar, A.A., R.A. Taha, N.H. Abou-Baker, E.A. Shaaban and Z.A. Salama, 2018. Evaluation of regeneration, active ingredients and antioxidant activities in jojoba tissue cultures as affected by carbon nanotubes. Biosci. Res., 15: 2383-2392.
9. Taha, R.A. and M.M. Hassan, 2014. Using low levels of seawater to enhance growth and development of date palm embryogenic cultures. Asian J. Agric. Sci., 6: 69-74.
10. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.
11. Neliyati, N., L. Lizawati and Z. Zulkarnain, 2019. The evaluation of sterilization protocol for sprout explants in oil palm (*Elaeis guineensis* Jacq.) tissue culture. J. Phys.: Conf. Ser., Vol. 1402. 10.1088/1742-6596/1402/3/033024.
12. Hassan, S.A.M., R.A. Taha, N.S. Mustafa and N.S.M. Zaied, 2021. Relation between culture medium, hormones combination and shoots proliferation of plum Cv. "santa rosa". Asian J. Plant Sci., 20: 157-162.
13. Taha, R.A., M.A. Allam, S.A.M. Hassan, B.M.M. Bakr and M.M. Hassan, 2021. Thidiazuron-induced direct organogenesis from immature inflorescence of three date palm cultivars. J. Genet. Eng. Biotechnol., Vol. 19. 10.1186/s43141-021-00115-4.
14. Rezali, N.I., N.J. Sidik, A. Saleh, N.I. Osman and N.A.M. Adam, 2016. The effects of different strength of MS media in solid and liquid media on *in vitro* growth of *Typhonium flagelliforme*. Asian Pac. J. Trop. Biomed., 7: 151-156.
15. Geng, F., R. Moran, M. Day, W. Halteman and D. Zhang, 2016. Increasing *in vitro* shoot elongation and proliferation of 'G.30' and 'G.41' apple by chilling explants and plant growth regulators. HortSci., 51: 899-904.
16. Rashotte, A.M., J. Poupart, C.S. Waddell and G.K. Muday, 2003. Transport of the two natural auxins, Indole-3-butyric acid and Indole-3-acetic acid, in Arabidopsis. Plant Physiol., 133: 761-772.