European Journal of Biological Sciences 14 (1): 14-19, 2022
ISSN 2079-2085

DOI: 10.5829/idosi.ejbs.2022.14.19

Effect of Hydrogel as an Alternative Gelling Agent and Stress on *In vitro* Protocol for Goji Berry (*Lycium barbarum* L.)

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Abstract: Goji (*Lycium barbarum* L.) plant is considered from the richest nutrient plant on earth. It has been used for centuries in traditional Chinese medicine. The aim of this investigation was to study the effect of type and strength of gelling agents; gerlite and hydrogel on micropropagation of goji shoots derived from *In vitro* cultures. The highest shoot proliferation was obtained on MS medium with 0.5 g/l Gerlite + 6 g/l Hydrogel + 1.0 mg/l benzyl adenine. At this concentration, hydrogel as a gerlite alternative was more effective in increasing shoot number, shoot length, leaf number and chlorophyll degree. While, leaf N, P and K concentration was significantly higher at 2.0 g/l Gerlite + 16 g/l Hydrogel + 1.0 mg/l BA than the other treatments. Use of 2.0 g/l Gerlite + 4.0 g/l Hydrogel + 0.5 mg/l IBA was superior in rooted plantlets due to significant increase in number of leaf, root length and chlorophyll (SPAD value). Plantlets were successfully transferred to small pots filled with mixture of sand and peatmoss.

Key words: Hydrogel · Gelrite · Proliferation · *In vitro* · Goji

INTRODUCTION

Goji (*Lycium barbarum* L.) or wolfberry is a fruit bearing woody shrub. This fruit has gained early attention for its important nutritional and medicinal properties. Interestingly, goji berry contains bioactive polysaccharides [1, 2]. It has a neuro-protective role for nerve cells [3], an anticancer effect [4] and a protective effect against oxidative stress and inflammation [5, 6]. *Lycium* root bark contains alkaloid (kukoamine) and coumarin (scopoletin) that has hypotensive and some medicinal properties [7, 8]. Micropropagation is an important technique for safe conservation of valuable genotypes [9]. Few researches investigated *In vitro* culture of *L. barbarum*. Some of them had reported plantlet regeneration by adventitious organogenesis as well as somatic embryogenesis using leaf explants [10, 11, 12]. Meanwhile, Taha [13] propagated goji by *In vitro* cultures using nodal explants.

Gelling agent is a crucial for tissue culture possessing. Agar and gelrite are the famous solidifying materials usually used in tissue culture labs but still considered the major cost. Otherwise, the suitability of starch as a gelling agent was studied previously in many scientific researches due to its accessibility and low cost and proven effectiveness [14, 15]. Tapioca starch proved to give a good result for banana micropropagation [16]. In addition, a mixture of corn starch and gelrite as gelling agent enhanced growth and proliferation of *In vitro* culture of some apple and raspberry cultivars, as compared to agar medium [17, 18]. However, these materials haven't the transparency which is needed for culturing and checking up contamination processes.

Hydrogel has a transparency property and could be used as a gelling agent. Hydrogel treatments increased all growth parameters compared with the control treatment which had 100% of agar. However, the highest number of leaves, root/explants and shoot length was achieved in medium with 50% agar + 50% hydrogel. On the other hand using of sucrose at 20 g/l with 50% agar + 50% hydrogel enhanced the shoot chlorophyll content, number of leaves, shoot length, number of root and root length. Moreover, we found that 0.2 g or 0.5 g improved plant performance during acclimatization stage [19].

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So that, the aim of the present research was to investigate the effect of hydrogel as a gelling agent on all stages of goji (*L. barbarum*) micropropagation.

MATERIALS AND METHODS

This study was carried out in Biotechnology and Micropropagation Lab., Pomology Department and Tissue Culture Technique Lab, Central Laboratories Network, National Research Centre, Giza, Egypt.

Plant Material and Establishment of Explants: Shoots derived from previous *In vitro* culture [13] of goji (*Lycium barbarum* L.), were cultured individually on Murashige and Skoog [20] as a basal medium supplemented with 1.0 mg/l BAP, 30 g\l sucrose and 7 g\l Difco Bacto agar for multiplication stage. The pH of the media was adjusted to 5.7 and autoclaved at 121°C and 151 b/ In^2 for 15 minutes. The culture explants were incubated under 16 h of artificial light (Fluorescent light at 30 uM/ sec) and 8 h of darkness at average temperature of 22 ± 2°C. Thus, the following experiments were carried out.

Effect of Gelling Agent Strength on Multiplication of Goji Shoots: Hydrogel is a hydropolymer (Barbary) usually used as a soil improver registered in France (9010133). Its components were hydropolymer (42%), source: acrylamide + total Nitrogen (6.5%), source: ammonium nitrate and potassium nitrate + P_2O_5 (4.8%), source: phosphoric acid + K_2O (8.2%), source: potassium nitrate.

Initial experiment had been established to determine the suitable concentration of hydrogel that could be used for *In vitro* culture at 2, 4, 8 g/l (unpublished data). It was found that there is a need for increasing these concentrations and trying to include gerlite as a gelling agent with hydrogell. So that, different gelling agent strengths (0.5, 1, 2 and 4 g/l Gerlite) and (4, 6, 8 and 16 g hydrogel) were tested to find out the best strength that encourage the multiplication and mineral content rate of goji (*Lycium barbarum* L.).

Effect of Gelling Agent Combination on Goji Root: The culture rooting medium consisted of MS basal salts, sucrose (30 g/l) + indole-3-butyric acid (IBA) (0.5 mg/l) + 2 g/l gerlite supplemented with various concentrations of Hydrogel (0, 4, 6 and 8 g/l). Medium pH was adjusted to 5.8. Cultures, consisting of 3 replicates per treatment were incubated at 25±2°C under 16 hphotoperiod. Thenumber of leaves, plant length, leaf chlorophyll content and root parameters were noted 6 weeks after culture. The effect of treatments applied on root initiation and elongation was also examined.

Chemical Leaf Characteristics

Leaf Mineral Content: Total nitrogen content was estimated by modified Kjeldahl's methods Motsara and Roy [21]. The percentages of phosphorus and potassium in the acid digested samples of goji dry shoots were determined; Phosphorus was determined calorimetrically by NH4-Metavanidate method and Potassium was flame-photometrically estimated [21].

Determination of Leaf Chlorophyll Content: Chlorophyll was measured by chlorophyll meter (Minolta- SPAD-502, Japan); the data were expressed as SPAD units [22].

Statistical Design: Treatments were arranged in complete randomized design, each treatment was replicated three times, each replicate involved three jars, and each contained three plants developed *In vitro*. Means were compared according to the method described by [23].

RESULTS

Effect of Gelling Agent Type and Strength on Multiplication Stage: Table (1) and Figure (1) shows the effect of gerlite and hydrogel on goji (*Lycium barbarum* L.), growth at multiplication stage. It is obvious that gerlite mixture with hydrogel was significantly more superior to using gerlite alone; it increased growth parameters. Using mixture of 0.5 g/l Gerlite + 6 g/l Hydrogel + 1 mg/l BA was more effective in increasing shoot number, shoot length, leaf number and chlorophyll degree followed by 1.0 g/l Gerlite + 4.0 g/l Hydrogel + 1.0 mg/l BA as compared with other treatments. Meanwhile, increasing the concentration of gerlite alone or mixture with hydrogel caused decreasing the growth parameter and increasing the callus induction as the effect of stress on explant culture.

Effect of Gelling Agent Type and Strength on Mineral Content: It is clear from data in Table 2 that leaf N, P and K concentration was significantly higher at 2.0g/lGerlite+16g/l Hydrogel+1.0 mg/l BA than the other treatments, followed by 1.0g/lGerlite+16g/l Hydrogel+1.0 mg/l BA. However, the residual effect stress of all treatments significantly produced the highest Na concentration compared with low concentration in control.

Table 1: Effect of gelling agent strength on development and growth of Goji (*Lycium barbarum* L.) *In vitro* plants

Treatment	Shoot number	Shoot length	Leaf number	Color degree	Callus induction
2g/l Gerlite+1mg/lBA (control)	31.00	2.90	8.50	5.00	1.00
0.5g/lGerlite+6g/l Hydrogel+1mg/lBA	52.50	3.75	12.50	4.25	1.00
1.0g/lGerlite+4g/l Hydrogel+1mg/lBA	51.00	3.30	10.50	3.85	1.00
4g/l Gerlite +1mg/lBA	30.50	2.25	7.50	3.25	2.00
2.0g/lGerlite+8g/l Hydrogel+1mg/lBA	19.50	1.90	6.50	2.75	3.00
1.0g/lGerlite+16g/l Hydrogel+1mg/lBA	22.33	2.00	5.34	2.17	3.27
2.0g/lGerlite+16g/l Hydrogel+1mg/lBA	19.00	1.74	5.00	2.03	3.70
LSD 5.0%	4.88	0.15	1.12	0.38	0.15

Table 2: Effect of gelling agents type and strength on mineral content of goji (*Lycium barbarum* L.), *In vitro* shoots

Treatment	N (ppm)	P (ppm)	K (ppm)	Na (ppm)
2g/l Gerlite (control)	350.00	39.00	300.00	25.00
0.5g/lGerlite+6g/l Hydrogel	310.00	33.00	280.00	30.00
1.0g/lGerlite+4g/l Hydrogel	290.00	30.00	201.00	31.00
4g/l Gerlite	390.00	42.00	305.00	42.00
2.0g/lGerlite+8g/l Hydrogel	420.00	48.00	350.00	50.00
1.0g/lGerlite+16g/l Hydrogel	510.00	53.00	390.00	45.00
2.0g/lGerlite+16g/l Hydrogel	630.00	61.00	400.00	52.00
LSD 5%	21.44	4.75	11.33	7.29

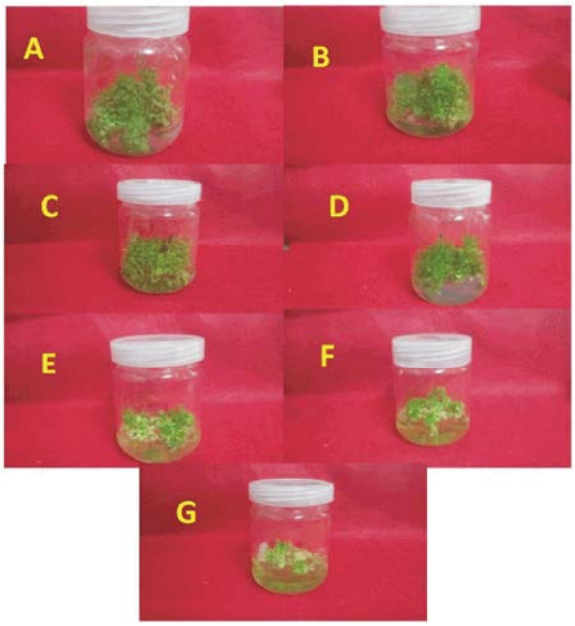

Fig. 1: Effect of gelling agent type and strength on development and growth of goji (*Lycium barbarum* L.), *In vitro* shoots
A: 2g/l Gerlite +1mg/l BA (control), B: 0.5g/lGerlite+6g/l Hydrogel+1mg/l BA, C: 1.0 g/l Gerlite+4g/l Hydrogel+1mg/l BA, D: 4g/l Gerlite +1mg/l BA, E: 2.0g/lGerlite+8g/l Hydrogel+1mg/l BA, F:1.0g/lGerlite+16g/l Hydrogel+1mg/l BA, G: 2.0g/lGerlite+16g/l Hydrogel+1mg/l BA

Effect of Gelling Agent Combination on Root: Data in Table (3) recorded that explants showed significant increase in number of leaf, root length and chlorophyll (SPAD value) with 2.0 g/l Gerlite+4.0 g/l Hydrogel +0.5mg/l IBA, followed by 2.0 g/l Gerlite+6.0 g/l Hydrogel +0.5mg/l IBA. Moreover, Table (3) explains that increase in hydrogel concentration was more stressful for all root parameters of goji (*Lycium barbarum* L.) plants.

Table 3: Effect of gelling agents type and strength on rooting stage of Goji (*Lycium barbarum* L.), plantlets

Treatment	Number of leaf	Shoot length (cm)	Root length (cm)	Chlorophyll (SPAD value)
2g/l Gerlite (control)	49.00	8.50	4.50	10.40
2g/l Gerlite +4g/l Hydrogel	55.00	9.00	5.50	13.30
2g/l Gerlite +6g/l Hydrogel	53.00	9.80	5.27	11.07
2g/l Gerlite +8g/l Hydrogel	33.00	8.00	3.00	8.30
LSD 5.0%	2.20	0.72	0.85	0.84

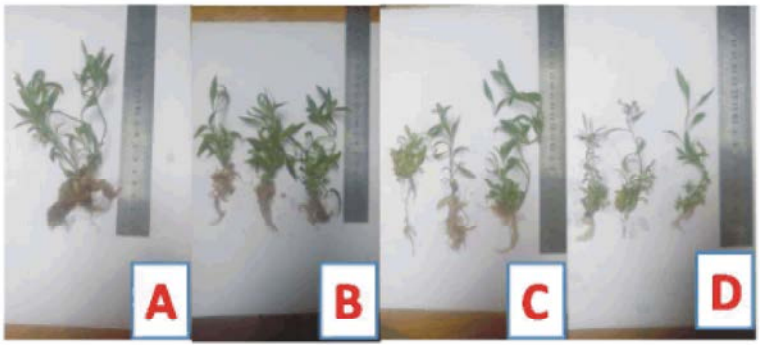

Fig. 2: Effect of gelling agents type and strength on *In vitro* rooting of Goji (*Lycium barbarum* L.) plants
A: 2g/l Gerlite +0.5mg/l IBA (control), B: 2g/l Gerlite+4g/l Hydrogel +0.5mg/l IBA, C: 2g/l Gerlite+6g/l Hydrogel +0.5mg/l IBA, D: 2g/l Gerlite+8g/l Hydrogel +0.5mg/l IBA

Fig. 3: Acclimatized plants

Acclimatization Stage: Rooted plantlets were cultured in a mixture of sand: perlite: peat moss (2:1:1). Pots were covered with plastic bags to keep moisture. (Fig. 3). After one week, gradual removal of plastic bags was applied. After a month, successful acclimatized plants were transferred to bigger pots with a mixture of sand: peat moss (1:1) and fertilized twice per week with NPK fertilizer (20:20:20) at 0.5 g/l.

DISCUSSION

In fact, many researches assured that gelling agent type can influence the growth and development of tissue cultured plants [24, 25]. Similarly, our results indicated the effect of the type of gelling agents on goji *In vitro* culture. In addition, concentration of the gelling agents affects the plant *In vitro* growth. Mbanaso *et al.* [17] stated that using 6 and 7% (w/v) cassava starch was adequate to support *Musa* shoot tip explants, also, cassava starch at 7% enhanced banana survival percentage in tissue culture and produced consistent results in terms of shoots and roots growth. Gelrite proved to be more superior compared with agar and the concentration of 3.5 g/l gave the highest shoot, leaf and root number for polyembryoid converted oil palms [25].

Interestingly, it was found that many gelling agents contain minerals that could affect plant growth [26]. Our results assured that the more concentration of gelling agent are used the more mineral content in goji shoots are determined.

CONCLUSION

Using mixture of 0.5g/lGerlite+6g/l Hydrogel+1mg/l BA had best effect on goji (*Lycium barbarum* L.) by increasing shoot number, shoot length,leaf number and chlorophyll degree. Due to N, P, K concentration in leaves, treatment of 2.0g/lGerlite+16g/l Hydrogel+1.0 mg/l BA had superiority than the other treatments but 2.0 g/l Gerlite+4.0 g/l Hydrogel +0.5mg/l IBA had significant increase in number of leaf, root length and chlorophyll (SPAD value).

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