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Influences of carbon sources and plant growth regulators on *in vitro* rooting of *Lycium chinense*

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ABSTRACT

In vitro propagation of *Lycium chinense* is very important, which contributes to its effective multiplication as well as preservation. Accordingly, this study aimed to explore the influence of different carbon sources, auxins, silver nitrate ($AgNO_3$), and putrescine on *in vitro* shoot regeneration and rooting from stem explants of *L. chinense*. Young shoots were generated on hormone-free Murashige and Skoog (MS) basal medium, and root and shoot experiments were then conducted to evaluate their effects. Among different carbon sources, sucrose (at 130 mM) and glucose significantly promoted root regeneration (highest number and length of roots). Of the auxins, the best root production occurred with indol-3-butyric acid (IBA) at 0.5 mg/L, and IAA and NAA generated root production at less than optimal levels with increased concentration. The co-application of IBA (0.5 mg/L) with $AgNO_3$ or putrescine enhanced shoot regeneration. The best rate of shoot production was observed with putrescine on both concentrations of $AgNO_3$ (5 mg/L on $AgNO_3$ and 100 mg/L on putrescine respectively) indicating the capability of these substances to enhance in vitro culture systems. These results will contribute to improving the efficiency of *L. chinense* regeneration via adventitious shoots through an easy setup of the culture conditions with an interest in propagation and other biotechnological applications.

KEYWORDS: *Lycium chinense*, Root regeneration, Shoot proliferation, Auxins, *In vitro* culture

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INTRODUCTION

Goji berry (*Lycium chinense*), traditional food and medicine in East Asia, is a shrub belonging to the Solanaceae family. This technique has been increasingly recognized in Europe and North America as well (Potterat, 2010; Nomura *et al.*, 2015). Liangzi, as a typical component of traditional Chinese herbal medicine, shows a wide range of beneficial effects, particularly immune modulation, anti-inflammatory, antidiabetic, anticancer, anti-obesity, antimicrobial, and anti-aging effects (Gan *et al.*, 2004; Luo *et al.*, 2004; Chang & So, 2008; Mao *et al.*, 2011; Oh *et al.*, 2012; Kruczek *et al.*, 2020; Jee *et al.*, 2024), on its own or by synergism with other herbs/foods. Glycerogalactolipids, phenylpropanoids, coumarins, lignans, flavonoids, amides, alkaloids, anthraquinones, organic acids, terpenoids, sterols, and betaine are among many of the chemical constituents and nutrients isolated from *L. chinense* (Shin *et al.*, 1999; Bungheza *et al.*, 2012; Youn *et al.*, 2012; Zhang *et al.*, 2013; Lee *et al.*, 2014; Qian *et al.*, 2017).

Rooting is the final culture stage before acclimatization which is the prime concept of the micropropagation system (Ismail *et al.*, 2011; Millán-Orozco *et al.*, 2011; Choi *et al.*, 2024). A strong rooting system is critical for the *in vitro*-grown plantlets to survive after their transfer to the field, as it helps in the uptake of water and nutrients from the soil (Benková & Bielach, 2010). *In vitro* rooting is affected by several factors, being natural or synthetic auxin one of the most important since it not only supports root induction but also favors plant physiology and anatomy adjustments during in vitro propagation and subsequent acclimatization (Osterc & Stampar, 2011; Martins *et al.*, 2018).

Carbon sources used for *in vitro* tissue culture are mainly sucrose, fructose, glucose, and maltose which serve as important sources

of energy for metabolism and growth points of the cells. These sugars are instant sources of energy for explants, stress healthy growth, and development. Carbon sources also play a role in such processes as osmoregulation, morphogenetic responses, biochemical synthesis, and the regulation of regeneration and development (Park *et al.*, 2023).

Silver ions (Ag^+) are powerful inhibitors of ethylene action, usually supplied as e.g. silver nitrate ($AgNO_3$) or as complexes with silver thiosulfate (STS) (Beyer Jr, 1979). Silver has been reported to improve different phases of plant regeneration *in vitro* (Kumar *et al.*, 2007; Park *et al.*, 2022) and $AgNO_3$ has also been found to be effective for root formation (Petrova *et al.*, 2011; Tamimi, 2015; Kim *et al.*, 2020). Polyamines are growth regulators involved in embryogenesis, cell division, development, and root formation (Tang & Newton, 2005; Liu *et al.*, 2006). Polyamines, mainly putrescine, have recently been highlighted as a means to enhance root formation and development in difficult-to-root species (Wu *et al.*, 2010).

It is essential for *in vitro* rooting systems to find the right concentrations of carbon source, auxin, $AgNO_3$, and putrescine. Nevertheless, little has been researched on the *in vitro* propagation of *L. chinense* considering the influences of these mentioned factors. This study is conducted to explore the effect of different carbon sources, auxins, $AgNO_3$, and putrescine on the root induction of *L. chinense*.

MATERIALS AND METHODS

Plant Materials

Young one-year-old independent plantlets of *Lycium chinense* plants were harvested and further cultured in the greenhouse at Chungnam National University, Daejeon, Korea. Young shoots were selected from the plant, their leaves removed, and the remaining shoots were trimmed to about 5 cm in length for use in establishing *in vitro* shoot cultures. First, the explants were washed three times with tap water (5-10 min) and then surface sterilized by dipping in 70% (v/v) ethanol for 30 sec and then in 1% sodium hypochlorite for 10 min. All explants were rinsed with sterile distilled water after sterilization and placed on a 50 mL hormone-free MS (Murashige & Skoog, 1962) basal medium, under controlled light. A basal medium containing mineral salts and vitamins was used, supplemented with 30 g/L sucrose and 8 g/L phytagar as a solidifying agent. The pH of the medium was set at 5.8 before the addition of phytagar and autoclaved at 121 °C for 20 min. The shoot result was then confirmed eight weeks post-inoculation by expanding longer shoots at a distal end of shoots towards the end of the period under *in vitro* controlled conditions for additional utilization.

Effect of Carbon Sources on *In Vitro* Rooting

A range of carbon sources was evaluated for their effectiveness in promoting root regeneration in *L. chinense*, including 100 mM concentrations of dextrose, fructose, galactose, glucose, maltose, mannose, sucrose, as well as varying sucrose concentrations (0, 30, 70, 100, 130, and 170 mM) in combination with SH medium (Schenk & Hildebrandt, 1972). Magenta boxes containing 50 mL of each media were used to lay out seven 2-cm-long shoot segments. The gelrite was subsequently added, and the pH of the medium was adjusted to 5.8 before autoclaving at 121 °C, 20 min under 1.1 kg/cm^2 pressure. Incubation of cultures was performed at 25±1 °C with 16 h of light per day, provided by normal cool white fluorescent tube lights. All experiments were performed in triplicate. After incubation for 4 weeks, the leaf explants were assessed for rooting response based on the rooting efficiency, number of roots per explant, and root length.

Effect of Auxin Concentrations on *In Vitro* Rooting

The ability of different concentrations (0, 0.1, 0.5, and 1.0 mg/L) of indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and 1-naphthaleneacetic acid (NAA) to promote root regeneration on SH culture medium in *L. chinense* was tested. Seven 2 cm-long shoot segments were transferred into each magenta box filled with 50 mL of the respective medium. The zones of the medium and the culture conditions were identical to those described in the first experiment.

Promoting Root Regeneration with $AgNO_3$ and Putrescine

The best auxin concentration of 0.5 mg/L IBA was selected for further tests based on previous preliminary tests of rooting capability. We tested different concentrations of silver nitrate ($AgNO_3$; 0, 1, 3, 5, 10, and 20 mg/L) and putrescine (0, 10, 30, 50, 100, and 200 mg/L) in combination with 0.5 mg/L IBA for root regeneration in *L. chinense*. Seven 2 cm-long shoot segments (taken from the above plant culture) were inserted into Magenta boxes containing 50 mL of the corresponding medium. The media were autoclaved to sterilize them, as performed for all the plant materials in previous experiments. Treatments were repeated three times and data was recorded after two weeks of culture.

Statistical Analysis

The analysis of variance (ANOVA) method in SPSS 20 (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The data obtained was analyzed as mean±standard deviation from 50 shoot explants tested. Different letters in the values signify statistically significant variations among the means using Duncan's multiple tests (ANOVA, $p<0.05$).

RESULTS

Data presented in Table 1 showed that the carbon sources tested have a significant effect on root regeneration and root growth of *L. chinense*. Moderate root formation under standard conditions was observed without the addition of any carbon source (control treatment; 1.43±0.11 roots/explant; 1.35±0.08 cm) although this was significantly lower compared to the average number of roots/per explant in other treatments (Table 1). The presence of dextrose (100 mM) improved root regeneration with 1.52±0.09 roots/explant, and an average root length of

Table 1: Influence of different carbon sources on the regeneration and growth of roots from the excised stem of *L. chinense* after four weeks of *in vitro* culture on SH medium

Carbon sources (100 mM)	No. of root/ explant	Root length (cm)
Control	1.43±0.11^{e}	1.35±0.08^{d}
Dextrose	1.52±0.09^{e}	1.93±0.14^{c}
Fructose	2.80±0.15^{b}	2.38±0.18^{b}
Galactose	2.14±0.17^{c}	1.92±0.16^{c}
Glucose	3.36±0.22^{a}	3.24±0.22^{a}
Maltose	1.73±0.13^{de}	2.07±0.13^{c}
Mannose	1.83±0.17^{d}	2.01±0.15^{c}
Sucrose	3.51±0.23^{a}	3.38±0.26^{a}

1.93±0.14 cm. The number of roots (2.80±0.15) and root length (2.38±0.18 cm) was significantly enhanced in fructose treatment compared to either, control and dextrose, whereas, galactose was just slightly stronger with 2.14±0.17 roots/explant and a root length of 1.92±0.16 cm. Glucose proved to be the maximum carbon source, giving the greatest number of roots (3.36±0.22) and also the longest roots (3.24±0.22 cm), demonstrating a significant increase in root induction and elongation. Maltose addition induced 1.73±0.13 roots/explant with a 2.07±0.13 cm root length that shows a less significant influence in root growth relative to glucose and fructose. Mannose had a positive but modest effect with 1.83±0.17 roots/explant and root length 2.01±0.15 cm. Out of the various carbon sources, sucrose produced the maximum number (3.51±0.23) and length of roots (3.38±0.26 cm) in *L. chinense*, suggesting that sucrose was the most suitable carbon source for root regeneration.

Data in Table 2 illustrates the effect of different concentrations of sucrose on the regeneration and growth of *L. chinense* roots from stem explants after 4 weeks of culture *in vitro*. They also prepared explants transected within a top 2 cm rooted segment at 0 mM (lowest level) of sucrose level revealing measly response at root formation numbers of 1.38 per explant and 1.32 cm root lengths per various lengths. Root regeneration and growth also increased with higher concentrations of sucrose, peaking at 130 mM where each of the explants was able to produce an average of 4.13 roots with a root length of 3.82 cm. However, at the highest sucrose concentrations (170 mM) the number of root plantlets decreased to (3.45) and the root length (3.52) cm, which may indicate the inhibition of platelets formation at the high concentrations of sucrose. Our results indicate that a sucrose concentration of 130 mM is optimal for root growth enhancement in *L. chinense* explants.

The data on the effect of different concentrations of auxin on root regeneration and growth in stem explants of *L. chinense* are shown in Table 3 after four weeks of *in vitro* culture. On the other hand, the control (0 mg/L) showed an average of 3.48 roots/explant with a root length of 3.38 cm. IAA increased the number of roots, and the highest number of roots was produced at the concentration of 1.0 mg/L (5.25 root/explant), however, the root length was decreased to 2.65 cm. Likewise, IBA had also given a higher number of roots (5.74 root/explant at 0.5 mg/L) along with a root length of 3.45 cm and is also an auxiliary to this type of root induction. On the contrary, in NAA treatments,

Table 2: Effect of different sucrose concentrations on the regeneration and growth of roots from the excised stem of *L. chinense* after four weeks of *in vitro* culture

Sucrose concentration (mM)	No. of root/ explant	Root length (cm)
0	1.38±0.12^{d}	1.32±0.09^{d}
30	2.86±0.26^{c}	2.59±0.23^{c}
70	3.51±0.29^{b}	3.28±0.27^{b}
100	3.54±0.32^{b}	3.34±0.24^{b}
130	4.13±0.29^{a}	3.82±0.31^{a}
170	3.45±0.24^{b}	3.52±0.21^{ab}

Table 3: Effect of different auxin concentrations on the regeneration and growth of roots from the excised stem of *L. chinense* after four weeks of *in vitro* culture

Auxin (mg/L)	No. of root/explant	Root length (cm)
Control 0	3.48±0.31^{de}	3.38±0.23^{a}
IAA 0.1	4.24±0.34^{c}	3.27±0.31^{ab}
IAA 0.5	4.91±0.38^{b}	2.91±0.19^{bc}
IAA 1.0	5.25±0.46^{ab}	2.65±0.17^{cd}
IBA 0.1	5.13±0.33^{ab}	3.31±0.27^{ab}
IBA 0.5	5.74±0.46^{a}	3.45±0.29^{a}
IBA 1.0	5.26±0.44^{ab}	3.26±0.31^{ab}
NAA 0.1	3.83±0.33^{cd}	3.09±0.24^{ab}
NAA 0.5	3.61±0.31^{cde}	2.43±0.16^{d}
NAA 1.0	3.12±0.26^{e}	1.91±0.12^{e}

a slow reduction in both root number and root length was observed with increasing concentration, but at the highest NAA concentration (1.0 mg/L) the root/explant and the root length decreased to 3.12 and 1.91 cm, respectively. Together, the most favorable results regarding the quantity and quality of root regeneration were achieved at a concentration of 0.5 mg/L IBA.

The data presented in Table 4 shows the effect of combined treatments of IBA (0.5 mg/L) and different concentrations of $AgNO_3$ on shoot regeneration and growth of *L. chinense* stem explants after four weeks of *in vitro* culture. In the control (0 mg/L $AgNO_3$) group, 5.68 shoot/explants were observed with a shoot length of 3.37 cm. The number of shoots increased in a dose-dependent manner from a minimum of only 1.30 shoots in control treatments to a maximum of 7.32 shoots at 20 mg/L $AgNO_3$ (Table 4), whereas the shoot length was significantly decreased to 2.64 cm. At 5 mg/L $AgNO_3$ produced maximum shoots (7.17 shoots/explant) with moderate shoot length (4.12 cm), showing a possible promote effect on plant growth (Table 4). This data suggests $AgNO_3$ is a favorable enhancer substance for shoot regeneration, and high proportions showed the greatest stimulation but the unusually long shoot length at the highest (5 mg/L) level would need to be assessed for practical relevance.

The effect of IBA (0.5 mg/L) along with different concentrations of putrescine on shoot regeneration and growth of different concentrations of putrescine on shoot regeneration and growth of stem explants of *L. chinense* after four weeks of *in vitro* culture is shown in Table 5. The experimental group average number of shoots/explant (5.71) in 0 mg/L putrescine with an average length of shoots (3.41 cm). With the increase of putrescine concentration, the number of shoots and shoot length were

Table 4: Combined Effect of IBA 0.5 mg/L and different concentrations of $AgNO_3$ on the regeneration and growth of roots from the excised stem of *L. chinense* after four weeks of *in vitro* culture

$AgNO_3$ (mg/L)	No. of shoots/explants	Shoot length (cm)
0	5.68±0.47^{b}	3.37±0.21^{b}
1	5.83±0.41^{b}	3.47±0.27^{b}
3	6.05±0.52^{b}	3.62±0.22^{b}
5	7.17±0.43^{a}	4.12±0.29^{a}
10	7.32±0.54^{a}	3.82±0.25^{ab}
20	4.20±0.34^{c}	2.64±0.30^{c}

Table 5: Combined effect of IBA 0.5 mg/L and different concentrations of putrescine on the regeneration and growth of roots from the excised stem of *L. chinense* after four weeks of *in vitro* culture

Putrescine (mg/L)	No. of shoots/explants	Shoot length (cm)
0	5.71±0.39^{c}	3.41±0.26^{a}
10	6.02±0.31^{bc}	3.54±0.32^{a}
30	6.10±0.33^{bc}	3.57±0.27^{a}
50	6.66±0.45^{ab}	3.68±0.29^{a}
100	6.98±0.53^{a}	3.78±0.34^{a}
200	6.70±0.47^{ab}	3.81±0.35^{a}

improved gradually. Maximum shoots (6.98) and shoot length (3.78 cm) were recorded at 100 mg/L putrescine. Although there was a slight decrease in the number of shoots (6.70) with 200 mg/L, the length of the shoots increased (3.81 cm). These findings indicate a beneficial effect of putrescine on both shoot number and shoot length and the most suitable concentration of 100 mg/L achieving an optimum ratio between these parameters. Nevertheless, shoot regeneration and growth did not improve at concentrations higher than 100 mg/L, suggesting the occurrence of a plateau effect at higher concentrations.

DISCUSSION

The present study revealed that carbon source, sucrose concentration, types, and concentrations of auxin in the regeneration medium with or without $AgNO_3$ and putrescine, significantly influenced *L. chinense* root and shoot regeneration *in vitro*. Sucrose (3.51±0.23 roots/explant, 3.38±0.26 cm root length) which was tested as one of the carbon sources, caused a positive deviation of the parameters of root regeneration from the control, and this effect was pleasant. In the same way was glucose (3.36±0.22 roots/explant, 3.24±0.22 cm root length). Our results are comparable to previous studies, such as (Martins *et al.*, 2018), it was also noted how suitable carbon sources can help in enhancing root formation from plant tissue culture. The beneficial effects of sucrose and glucose are probably related to their function as a source of energy for various cellular processes such as cell division and differentiation (Schenk & Hildebrandt, 1972).

Results showed that 130 mM sucrose concentration resulted in maximal root formation (4.13±0.29 roots/explant, 3.82±0.31 cm root length), confirming the trends previously observed by Akyüz (2025) in several other plant species. Conversely, at higher sucrose concentrations (170 mM), plant root regeneration was inhibited and the root number and length were reduced (3.45±0.24 roots/explant, 3.52±0.21 cm root length), this effect may be osmotic or due to nutrient imbalance (Martins *et al.*, 2018).

Auxins affecting root regrowth were examined and the highest number of roots (5.74±0.46 roots/explant, 3.45±0.29 cm root length) was observed at 0.5 mg/L IBA. This aligns with observations by Reddy *et al.* (2001). A similar result was obtained that IBA is very effective for rooting *Zanthoxylum beecheyanum* (El-Banna *et al.*, 2023). Conversely, NAA treatments were the least effective as indicated in the literature that NAA is less effective for rooting in some species (Kumar *et al.*, 1998).

The shoot regeneration was further improved by the addition of $AgNO_3$ and putrescine into the culture medium. Maximum shoot proliferation (7.17±0.43 shoots/explant, 4.12±0.29 cm shoot length) was achieved in 5 mg/L $AgNO_3$ confirming the stimulatory effects of $AgNO_3$ on plant growth (Beyer Jr, 1979; Kumar *et al.*, 1998). The beneficial role of $AgNO_3$ might be due to its role in blocking ethylene production which directly increases shoot regeneration (Kumar *et al.*, 1998). Likewise, the increase in shoot growth observed following treatment with putrescine (100 mg/L) (6.98±0.53 shoots/explant, 3.78±0.34 cm shoot length) (Table 1) is also very consistent with the effect observed with polyamines (Tang & Newton, 2005; Liu *et al.*, 2006).

CONCLUSION

The importance of carbon sources and plant growth regulators in producing *in vitro* regeneration of *L. chinensis*. Sucrose (130 mM) was the most successful root-inducer in the carbon source tests and both glucose and fructose also showed some potency to improve root regeneration. During auxin experiments, the best concentration of IBA for rooting was 0.5 mg/L. High concentrations of both putrescine (500 mg/L) and AgNO (10 mg/L) were inhibitory, while the combined use of $AgNO_3$ and putrescine, particularly at intermediate concentrations, markedly promoted shoot regeneration with the optimal concentrations of 5 mg/L $AgNO_3$ and 100 mg/L putrescine control of medium ingredients such as carbon sources and growth regulators can offer significant potential for enhancing *in vitro* regeneration efficiency in *L. chinense*, which has important applications for both basic research and commercial propagation of this species. More research could investigate the molecular basis of these responses and allow for a more thorough understanding of the regulatory networks involved in plant growth and development.

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