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Research Article

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Effect of media and gelling agents on shoot organogenesis of *Liriope platyphylla*

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ABSTRACT

Liriope platyphylla can be multiplied either by planting seeds or dividing its tuberous roots. In this study, a method for *L. platyphylla* plant shoot organogenesis from meristem explants was developed, employing medium and gelling agents. The effects of full- and half-strength B5, SH, and MS media were examined for the selection of optimal medium conditions for shoot organogenesis. Different concentrations of the gelling agents such as phytagar (6, 7, 8, and 9 g L^{-1}) and gellan gum (2, 3, 4, and 5 g L^{-1}) were examined for efficient shoot formation. The results revealed the superiority of half-strength MS basal medium in shoot organogenesis and growth of *L. platyphylla*. But the half-strength B5 media performed poorly. Compared to plant agar, gellan gum performed well in terms of shoot regeneration and shoot length. When gellan gum was used at 3 g L^{-1} the maximum number of shoots explant^{-1} (5.8) and longest shoot (45.8 mm) was observed but the lowest number of shoots explant^{-1} (3.2) and shortest shoot (21.4 mm) was registered with 5 g L^{-1}. It is proposed from our study that half-strength MS media and gellan gum gelling agent at 3 g L^{-1} could be applied in shoot organogenesis and growth of *L. platyphylla*.

KEYWORDS: Gelling agent, Shoot organogenesis, *Liriope platyphylla*, Plant agar, Gellan gum

INTRODUCTION

Liriope platyphylla Wang et Tang is a perennial herbaceous plant belonging to the Liliaceae family. It has historically been used to treat cough, sputum, asthma, and neurological illnesses. This medicinal plant is primarily found in China, Taiwan, and Korea (Kim *et al.*, 2016). It has long been used in Korea as an expectorant, an antitussive, and a stimulant (Hur *et al.*, 2004) Several biological as well as pharmacological attributes of *L. platyphylla* have been reported, which include anti-bacterial (Kim *et al.*, 2002), neuroprotective (Park *et al.*, 2015), anti-inflammatory (Kim *et al.*, 2016), and anticarcinogenic (Wang *et al.*, 2013) properties. Additionally, it is thought to postpone aging, and enhance learning and memory (Jiang *et al.*, 2007a). As the primary active components of *L. platyphylla*, multiple steroidal saponins have been discovered (Watanabe *et al.*, 1983; Jiang *et al.*, 2007b). It has been found that spicatoside A is one of the main steroidal saponins which promotes neurite outgrowth (Hur *et al.*, 2009; Park *et al.*, 2019).

There are several ways to regenerate complete plants from plant tissue that has been excised. In this research, somatic embryogenesis and shoot organogenesis, two primary methods, were taken into consideration (Phillips & Hubstenberger, 1995). When a single cell or a cluster of cells is stimulated to differentiate into shoots or roots, this process is known as organogenesis. The process of plant regeneration through organogenesis normally begins with the induction and growth of a shoot from explant tissue and then transferred to a new media, and is followed by the initiation of root and plant growth (Fleming, 2006; Boudaoud, 2010). Several plant species may successfully develop organs, according to research, if the proper medium components are chosen, an adequate explant is chosen, and the physical environment is controlled in the right way (Brown & Thorpe, 1986).

Gelling agents (GA) are polysaccharides, which are big molecules of glucose like simple sugars. As a result of their capacity to form gels, they offer semi-solid or solid surfaces on which plants can grow. A solid nutritional medium functions as soil and offers the

culture the physical support necessary to sustain contact with the air necessary for respiration. In plant tissue culture, agar, and gellan gum are the two most often used gelling agents. Red algae of the Rhodophyceae family are used to make agar. It is the most popular gelling agent because of its practical gelling characteristics, including excellent stability, clarity, resistance to metabolic processes during culture, and nontoxic behavior. Bacteria are used to make gellan gum which is marketed as "Gelrite" and "Phytagel.". There are no contaminating elements in these items. By employing just a tiny amount of this, it is feasible to create a high-strength colorless gel. As the medium is clear, contaminations are simpler to spot.

Sowing seeds or dividing the tuberous roots are the two methods used to propagate *L. platyphylla*. Due to the poor rate of reproduction and the time-consuming process of extracting roots, seed propagation is challenging. This species is reproduced traditionally by dividing the roots (Han *et al.*, 1993). According to certain investigations, *L. platyphylla* may be micro-propagated and regenerated in vitro via somatic embryogenesis and adventitious buds for repeated propagations (Kim *et al.*, 2000; Mo *et al.*, 2000). Before, with the help of *L. platyphylla* meristem cultures, a quick methodology was created successfully for effective shoot organogenesis and plant regeneration (Park *et al.*, 2011). In this study, we developed a method for choosing the best medium and gelling agent conditions for the shoot organogenesis of *L. platyphylla* plants utilizing meristem explants and media.

MATERIALS AND METHODS

Seed Sterilization and Germination

For the preparation of plant materials, *Liriope platyphylla* seeds were collected from the experimental farm at Chungnam National University (Daejeon, Korea), and kept at 4°C. Surface sterilization of seeds was done with 70% (v/v) ethanol for 30 s and 2% (v/v) sodium hypochlorite solution for 10 min. Then the seeds were washed thrice using sterilized water. Seven seeds were placed on agar-solidified culture medium in Petri dishes (100 × 15 mm). The basal medium consisted of Murashige and Skoog (MS) (Murashige & Skoog, 1962) salt and vitamin medium (Sigma, St. Louis, Mo. USA) solidified with 0.7% (w/v) agar. Before adding the agar, the pH of the MS salt and vitamin medium was adjusted to 5.8, and it was then autoclaved at 121°C for 20 min to sterilize it. After two weeks of culture, the seeds began to sprout in a growth chamber with a humidity level of 70-80%, a temperature of 25°C, at a 16-hour photoperiod with a flux rate of 35 μmol m^{-2} s^{-1}.

In vitro Plant Regeneration

Meristems of *L. platyphylla* were cut into small pieces (0.7 × 0.7 cm) in size, from the in vitro grown plants. Explants were placed on medium (approximately 25 mL) in 100 × 25 mm Petri dishes. About seven explants were cultured in each petri dish. The basal medium consisted of salts and vitamins of MS medium and solidified with 0.7% (w/v) Phytagar. The pH of the medium was adjusted to 5.8 before adding Phytagar. The media were sterilized by autoclaving at 121 °C for 20 min. For shoot regeneration, the medium was supplemented with 1 mg L^{-1} zeatin with 0.1 mg L^{-1} indole-3-acetic acid (IAA). For the selection of optimal medium conditions for shoot organogenesis, the effects of full- and half-strength B5 (Gamborg *et al.*, 1968), MS (Murashige & Skoog, 1962), and SH (Schenk & Hildebrandt, 1972) media were tested. In this study, different concentrations of the gelling agents such as phytagar (6, 7, 8, and 9 g L^{-1}) and gellan gum (2, 3, 4, and 5 g L^{-1}) were examined for efficient shoot formation. The explants were kept in a growth chamber at 25 ± 1°C, with a 16-h photoperiod, and illuminated at 35 μmol s^{-1} m^{-2} for 6 weeks. All the chemicals were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

Statistical Analysis

All the data were analyzed by using SPSS 26.0 (IBM Corp., NY, USA). The data obtained were expressed as mean ± standard deviation from 50 meristems tested.

RESULTS AND DISCUSSION

Liriope Platyphylla Micropropagation

Regenerated shoots (Figure 1a) were transferred to the culture vessels containing MS medium without any exogenous plant hormone. After 5 weeks, the roots emerged from the regenerated shoots (Figure 1b). The plants that emerged with roots were transferred to pots containing sterile vermiculite. To maintain the high humidity the pots were covered with polyethylene bags for 7 days. The regenerated plants were hardened and transferred to soil in a greenhouse. The result showed that 90% of the plants grew normally.

Effect of Media

To identify the best basal medium for shoot organogenesis and shoot growth, a comparison of MS, B5, and SH media was done.

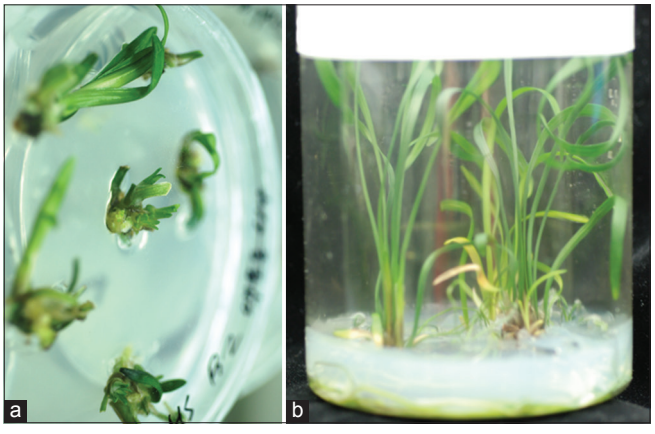

Figure 1: In vitro shoot organogenesis and plant regeneration of *L. platyphylla*. (a) Shoot emerging from a meristem explant of *L. platyphylla* 6 weeks after cultivation on MS solid media supplemented with 1 mg L^{-1} zeatin with 0.1 mg L^{-1} indole-3-acetic acid (1×). (b) The rooted plants are in a culture vessel. (0.7×)

This media's impact at full and half strength was evaluated. The development and regeneration of *L. platyphylla* meristem shoots were significantly impacted by various mediums. After 6 weeks of culture, the maximum number of shoots explant^{-1} (4.5) was found in half-strength MS media followed by full-strength MS media (4.0), full-strength SH media (3.9), half-strength SH media (3.8), full strength B5 media (3.4) and half strength B5 media (3.2). Similarly, shoot length was varied due to the usage of different media. Shoot length ranges from 19.3 mm to 29.1 mm. Maximum shoot length was attained in half-strength MS media followed by full-strength SH media, full-strength MS media, half-strength SH media, full-strength B5 media, and half-strength B5 media (Figure 2).

Different nutritional levels, such as those of micronutrients, vitamins, and amino acids, are largely responsible for the varying response of various basal media treatments. In our study, a half-strength MS medium was found superior as compared to the other media evaluated. Half-strength MS had the highest shoot organogenesis, while full-strength was next. The high ammonia concentration of MS medium may be responsible for the enhanced synthesis of nucleic acids and proteins that resulted in the expression of genes necessary for the best possible regeneration. In addition to the ammonia content, the vitamins thiamine, pyridoxine, and nicotinic acid must have made a considerable contribution to the enhancement of organogenesis (Prust *et al.*, 2022). Results made it abundantly evident that MS basal medium was superior for *L. platyphylla* shoot organogenesis and growth.

Effect of Gelling Agent

By the addition of gelling agents in various concentrations, shoot regeneration and shoot length in explants are improved while cultured in a medium. Gelling agents are one of the most crucial elements due to their involvement in regulating medium nutrient solubility and the explants' ability to absorb those nutrients (Bhatia & Ashwath, 2005).

In this investigation, plant agar and gellan gum, two different kinds of gelling agents, were employed. Gelling agents had a noticeable impact on the development and regeneration of shoots from *L. platyphylla* meristem cultures. It was noticed that plant agar at 6 g L^{-1} produced the maximum number of shoots explant^{-1} (4.8). The number of shoots explant^{-1} gradually

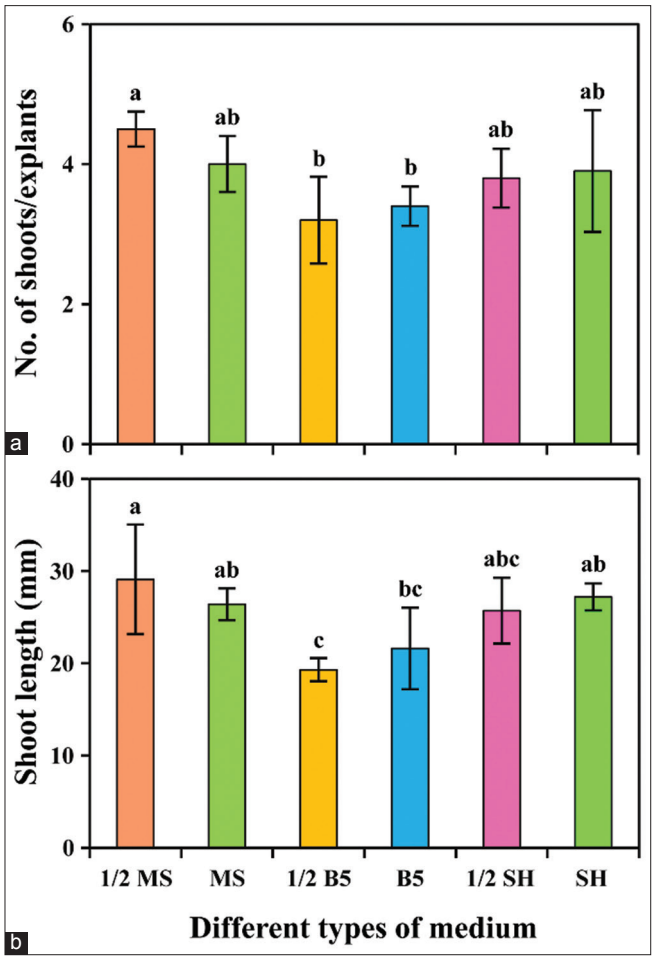

Figure 2: (a and b) Effect of different media on shoot regeneration and growth from meristem cultures of *L. platyphylla* after 6 weeks of culture

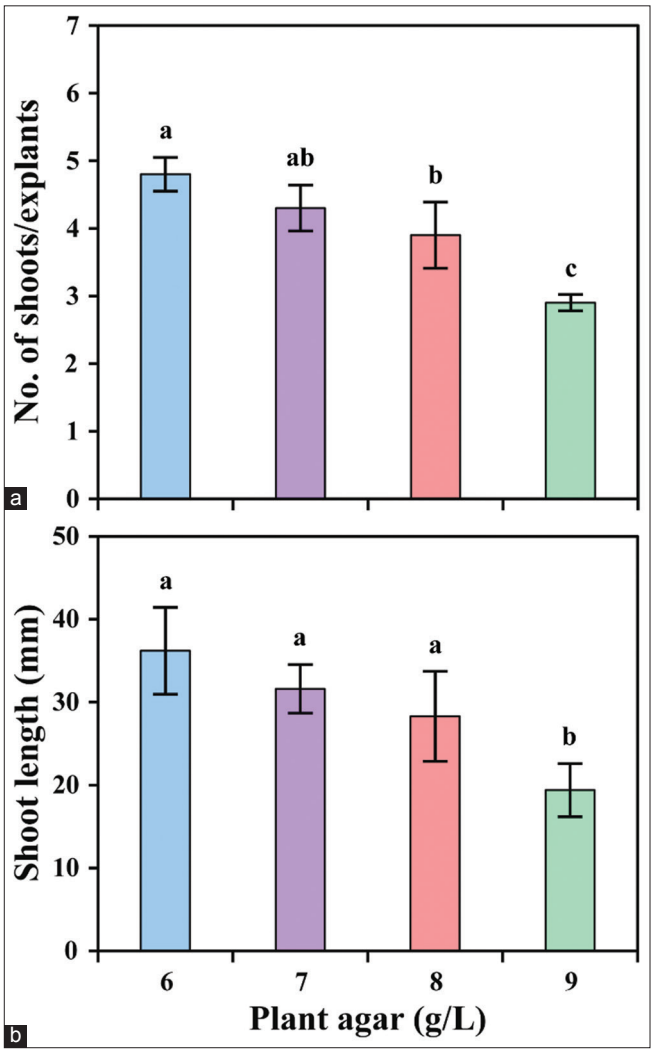

Figure 3: (a and b) The effect of plant agar on shoot regeneration and growth from meristem cultures of *L. platyphylla* after 6 weeks of culture

decreased as plant agar concentration increased, reaching its lowest point (2.9) at 9 g L^{-1}. Similarly, the longest shoot (36.2 mm) was observed at 6 g L^{-1} plant agar gelling agent, while the shortest (19.4 mm) was noted at 9 g L^{-1} plant agar (Figure 3). Gellan gum was used at 2, 3, 4, and 5 g $L^{-1,}$ and different concentrations of this gelling agent showed variation in the number of shoots $explant^{-1}$ and shoot length. When gellan gum was used at 3 g L^{-1} the maximum number of shoots $explant^{-1}$ (5.8) and longest shoot (45.8 mm) was recorded. Whilst the lowest number of shoots $explant^{-1}$ (3.2) and shortest shoot (21.4 mm) was registered with 5 g L^{-1} (Figure 4).

Variations in how shoots react to various galling agents are caused by variations in the water potential of the medium, which impacts plant development (Buah *et al.*, 1999). Plants receive direct physical touch with nutrients from gelling agents, which fosters development (Nery *et al.*, 2021). Plant development is directly influenced by the composition of gelling agents since it favors the binding of some nutrients over others. Also, it has been previously documented that the same gelling agent, at different doses, has a significant impact on water

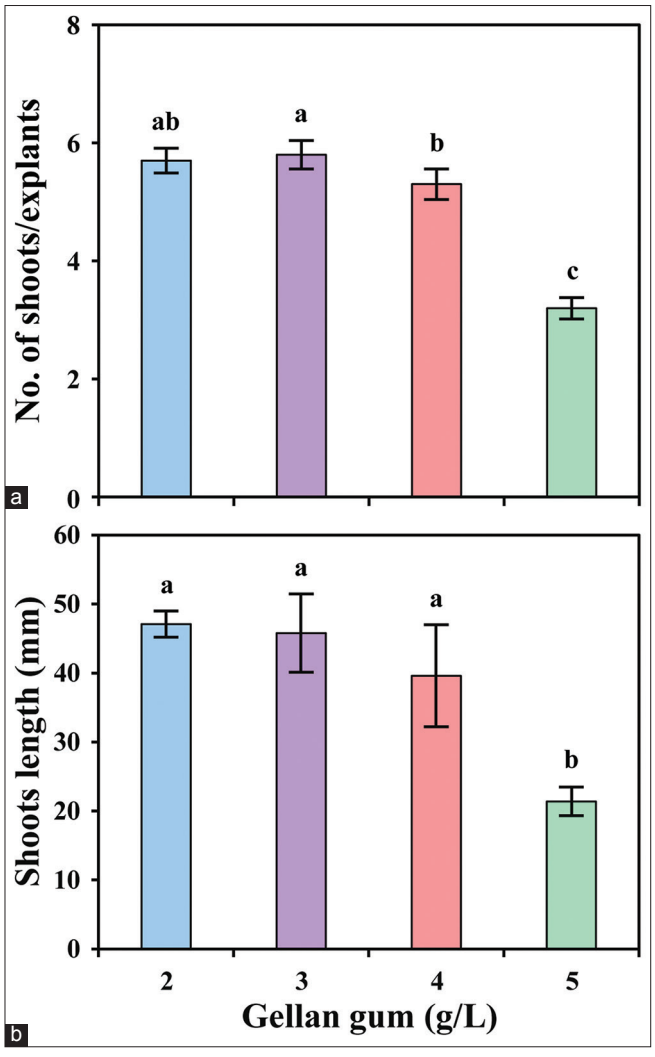

Figure 4: (a and b)The effect of gellan gum on shoot regeneration and growth from meristem cultures of *L. platyphylla* after 6 weeks of culture

retention and the control of the medium's moisture regime (Repalli *et al.*, 2019). This justifies adding gelling agents at the proper concentration is necessary to satisfy various demands at various phases of plant tissue culture.

Compared to plant agar, gellan gum performed well in terms of shoot regeneration and shoot length. The superior performance of gellan gum (gelrite and phytagel) over agar products may be due to the impurities found in Agar. Agar is typically employed as a common gelling agent, however, its usage as a propagation medium is constrained by issues such as batch variability, vitrification, the presence of contaminants, and substances that hinder growth (Stolz, 1971, Debergh *et al.*, 1992). These drawbacks may be solved by using gellan gums (gelrite and phytagel), which have a high ash content, few impurities, and more consistency (Huang *et al.*, 1995). Agar that includes agropectins and other organic contaminants can prevent the development and proliferation of an explant. Being a water-soluble anionic polysaccharide, gellan gum is a very pure and reliable natural gelling agent. Hence, it doesn't include any of the contaminating contaminants present in agar (Mohamed *et al.*, 2021). To build an effective regeneration system, the choice of gelling agent is therefore a crucial component of the research.

CONCLUSIONS

From this study, we found that this protocol can be effectively used to optimize and enhance the regeneration of a large number of plants, especially *L. platyphylla*. Among various media 1/2 MS medium is best for shoot regeneration and subsequent shoot growth. Compared to plant agar, gellan gum is better for promoting shoot organogenesis and elongation frequency in this species. This finding can potentially provide basic information for the mass micropropagation of *L. platyphylla*.

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REFERENCES

Bhatia, P., & Ashwath, N. (2005). Effect of medium pH on shoot regeneration from the cotyledonary explants of tomato. *Biotechnology, 4*(1), 7-10. https://doi.org/10.3923/biotech.2005.7.10

Boudaoud, A. (2010). An introduction to the mechanics of morphogenesis for plant biologists. *Trends in Plant Science*, *15*(6), 353-360. https://doi.org/10.1016/j.tplants.2010.04.002

Brown, D. C., & Thorpe, T. A. (1986). Plant regeneration by organogenesis. In I. K. Vasil (Ed.), *Cell Culture and Somatic Cell Genetics of Plants* (pp. 49-65) New York, USA: Academic Press.

Buah, J. N., Kawamitsu, Y., Sato, S., & Murayama, S. (1999). Effects of different types and concentrations of gelling agents on the physical and chemical properties of media and the growth of banana (*Musa* spp.) in vitro. *Plant Production Science*, *2*(2), 138-145. https://doi.org/10.1626/pps.2.138

Debergh, P., Aitken-christie, J., Cohen, D., Grout, B., Arnold, S.V., Zimmerman, R., & Ziv, M. (1992). Reconsideration of the term 'vitrification' as used in micropropagation. *Plant Cell, Tissue and Organ Culture, 30,* 135-140. https://doi.org/10.1007/BF00034307

Fleming, A. (2006). Metabolic aspects of organogenesis in the shoot apical meristem. *Journal of Experimental Botany, 57*(9), 1863-1870. https://doi.org/10.1093/jxb/erj178

Gamborg, O. L., Miller, R. A., & Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research, 50*(1), 151-158. https://doi.org/10.1016/0014-4827(68)90403-5

Han, J. H., Yoon, Y. H., Kang, D. J., & Lee, Y. S. (1993). Studies on improvement of cultural practices in *Liriope platyphylla* WANG et TANG-(3)-stimulation of seed germination and effects of seedling age on growth and tuber yield. *Korean Journal of Medicinal Crop Science*, *1*(2), 120-124.

Huang, L.-C., Kohashi, C., Vangundy, R., & Murashige, T. (1995). Effects of common components on hardness of culture media prepared with gelrite. *In Vitro Cellular & Developmental Biology - Plant, 31*, 84-89. https://doi.org/10.1007/BF02632242

Hur, J., Lee, P., Kim, J., Kim, A. J., Kim, H., & Kim, S.Y. (2004). Induction of nerve growth factor by butanol fraction of *Liriope platyphylla* in C6 and primary astrocyte cells. *Biological & Pharmaceutical Bulletin*, *27*(8), 1257-1260. https://doi.org/10.1248/bpb.27.1257

Hur, J., Lee, P., Moon, E., Kang, I., Kim, S.-H., Oh, M. S., & Kim. S.Y, (2009). Neurite outgrowth induced by spicatoside A, a steroidal saponin, via the tyrosine kinase A receptor pathway. *European Journal of Pharmacology*, *620*(1-3), 9-15. https://doi.org/10.1016/j.ejphar.2009.08.016

Jiang, T., Huang, B.-K., Zhang, Q.-Y., Han, T., Zheng, H.-C., & Qin, L.-P. (2007a). Effect of *Liriope platyphylla* total saponin on learning, memory and metabolites in aging mice induced by D-galactose. *Journal of Chinese Integrative Medicine, 5*(6), 670-674. https://doi.org/10.3736/jcim20070614

Jiang, T., Huang, B.-K., Zhang, Q.-Y., Han, T., Zheng, H.-C., & Qin, L.-P. (2007b). Studies on chemical constituents of *Liriope platyphylla*. *Zhong Yao Cai*, *30*(6), 1079-1081.

Kim, S. W., Oh, S. C., In, D. S., & Liu, J. R. (2000). High frequency somatic embryogenesis and plant regeneration in zygotic embryo cultures of *Liriope platyphylla* Wang et Tang. *Plant Cell, Tissue and Organ Culture, 63*, 227-229. https://doi.org/10.1023/A:1010783120412

Kim, S.-W., Chang, I.-M., & Oh, K.-B. (2002). Inhibition of the bacterial surface protein anchoring transpeptidase sortase by medicinal plants. *Bioscience, Biotechnology, and Biochemistry, 66*(12), 2751-2754. https://doi.org/10.1271/bbb.66.2751

Kim, W. K., Pyee, Y., Chung, H.-J., Park, H. J., Hong, J.-Y., Son, K. H., & Lee, S. K. (2016). Antitumor activity of spicatoside A by modulation of autophagy and apoptosis in human colorectal cancer cells. *Journal of Natural Products, 79*(4), 1097-1104. https://doi.org/10.1021/acs.jnatprod.6b00006

Mo, X. R., Zhu, C., Ren, X. M., Tang, Y. L., & Qian, P. (2000). The tissue culture of *Liriope platyphylla* Wang et Tang var. variegata Hort. *Journal of Plant Resources and Environment, 9*, 27-29.

Mohamed, G. M., Amer, A. M., Osman, N. H., Sedikc, M. Z., & Hussein, M. H. (2021). Effects of different gelling agents on the different stages of rice regeneration in two rice cultivars. *Saudi Journal of Biological Science, 28*(10), 5738-5744. https://doi.org/10.1016/j.sjbs.2021.06.003

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3), 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nery, L. A., Batista, D. S., Rocha, D. I., Sérgio, H. S. F., Matheus da Costa, Q., Priscila, O. S., Marília, C. V., & Wagner, C. O. (2021). Leaf development and anatomy of in vitro-grown *Polygala paniculata* L. are affected by light quality, gelling agents, and sucrose. *Vegetos, 34*, 19-28. https://doi.org/10.1007/s42535-021-00192-3

Park, C. H., Morgan, A. M. A., Park, B. B., Lee, S. Y., Lee, S., Kim, J. K., & Park, S. U. (2019). Metabolic analysis of four cultivars of *Liriope platyphylla*. *Metabolites*, *9*(3), 59. https://doi.org/10.3390/metabo9030059

Park, H. R., Lee, H., Park, H., Jeon, J. W., Cho, W.-K., & Ma, J. Y. (2015). Neuroprotective effects of *Liriope platyphylla* extract against hydrogen peroxide-induced cytotoxicity in human neuroblastoma SH-SY5Y cells. *BMC Complementary and Alternative Medicine*, *15*, 171. https://doi.org/10.1186/s12906-015-0679-3

Park, W. T., Kim, Y.-K., Kim, Y. S., Park, N.-I., Lee, S.-Y., & Park, S. U. (2011). In vitro plant regeneration and micropropagation of *Liriope platyphylla*. *Plant Omics*, *4*(4), 199-203.

Phillips, G. C., & Hubstenberger, J. F. (1995) Micropropagation by proliferation of axillary buds. In O. L. Gamborg & G. C. Phillips (Eds.), *Plant cell, tissue and organ culture: fundamental methods* (pp. 45-54) Berlin, Heidelberg: Springer. https://doi.org/10.1007/978-3-642-79048-5_4

Prust, R., Awasthi, O. P., Singh, S. K., & Kumar, K. (2022). In vitro shoot organogenesis in sweet orange (*Citrus sinensis* L.) cv. Mosambi and the effect of ethylene adsorbents on micro-shoot quality. PREPRINT (Version 1) available at Research Square. https://doi.org/10.21203/rs.3.rs-1730471/v1

Schenk, R. U., & Hildebrandt, A. C. (1972). Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Canadian Journal of Botany*, *50*(1),199-204. https://doi.org/10.1139/b72-026

Stolz, L. P. (1971). Agar restriction of the growth of excised mature Iris embryos. *Journal of American Society of Horticultural Science*, *96*(5), 618-684. https://doi.org/10.21273/JASHS.96.5.681

Wang, H.-C., Wu, C.-C., Cheng, T.-S., Kuo, C.-Y., Tsai, Y.-C., Chiang, S.-Y., Wong, T.-S., Wu, Y.-C., & Chang, F.-R. (2013). Active Constituents from *Liriope platyphylla* Root against Cancer Growth In Vitro. *Evidence-Based Complementary and Alternative Medicine*, 857929. https://doi.org/10.1155/2013/857929

Watanabe, Y., Sanada, S., Ida, Y., & Shoji, J. (1983). Comparative studies on the constituents of ophiopogonis tuber and Its congeners. I. studies of the constituents of the subterranean part of *Liriope platyphylla* Wang et Tang. *Chemical and Pharmaceutical Bulletin*, *31*(6), 1980-1990. https://doi.org/10.1248/cpb.31.1980