Research Article
Journal of Agricultural Biotechnology (JOINABT) 1(1), 12-17, 2020
Recieved: 04-Dec-2020 Accepted: 18-Dec-2020

A Research on Micropropagation of *Loropetalum chinense*

Neslihan BABALI[1*], Taki DEMİR[2]
[1] Sakarya University of Applied Sciences, Faculty of Agriculture, Department of Horticulture, Sakarya/Turkey
[2] Sakarya University of Applied Sciences, Faculty of Agriculture, Sakarya/Turkey

ABSTRACT

The ornamental plant sector has been increasing rapidly in the world and in Turkey in recent years. It is aimed to develop micropropagation protocols of *Loropetalum chinense* (Chinese fringe flower), which are increasingly used in our country and are difficult to produce with traditional methods. For this purpose, from the MS and DKW media which are used commonly; MS-Mod and DKW-Mod media prepared considering the soil requirements of the plants were used for this plant. In *Loropetalum chinense* media experiments, the best number of shoots per explant (1.76 shoots/explant) was produced on DKW with 1 mg L^{-1} of BAP. Also It was determined that the MS-Mod media with 1.00 mg L^{-1} BAP (1.63 shoots/explant) is suitable for *Loropetalum chinense* micropropagation. According to the results obtained in the study, the nutrient media that can be used for micropropagation were determined for the plant *Loropetalum chinense* (Chinese fringe flower).

Keywords: Chinese fringe flower, Clonal propagation, *Loropetalum chinense*, Micropropagation, Plant media, Tissue culture.

Loropetalum chinense Mikroçoğaltım Araştırması

ÖZ

Süs bitkileri sektörü son yıllarda Dünya'da ve Türkiye'de hızla artmaktadır. Ülkemizde kullanımı gittikçe yaygınlaşan ve geleneksel yöntemlerle üretilmesi zor olan *Loropetalum chinense* (Çin püskülü) mikroçoğaltım protokollerinin geliştirilmesi hedeflenmektedir. Bu amaçla yaygın olarak kullanılan MS ve DKW ortamından bitkilerin toprak ihtiyaçları dikkate alınarak hazırlanan MS-Mod ve DKW-Mod besiyeri kullanılmıştır. *Loropetalum chinense* ortam denemelerinde, eksplant başına en iyi sürgün sayısı (1.76 sürgün/eksplant) 1 mg L^{-1} BAP ile DKW ortamında görülmüştür. Ayrıca 1.00 mg L^{-1} BAP (1.63 sürgün/eksplant) içeren MS-Mod ortamının *Loropetalum chinense* mikroçoğaltım için uygun olduğu belirlenmiştir. Araştırmada elde edilen sonuçlara göre *Loropetalum chinense* (Çin püskülü) bitkisi için standart ortamlar dışında da mikroçoğaltım için kullanılabilecek besi ortamları tespit edilmiştir.

Anahtar Kelimeler: Çin püskülü, Klonal çoğaltım, *Loropetalum chinense*, Mikroçoğaltım, Besi ortamı, Doku kültürü.

1. Introduction

Loropetalum chinense (Chinese Fringe Flower), evergreen shrub that belongs to the family of *Hamamelidaceae* [1]. Its natural distribution area covers the area extending from the south of China to the east of India, especially with Hunan province being the center in China [2, 3, 4, 5]. After being

* Corresponding Author's email: neslihanb@subu.edu.tr

cultivated in other provinces of China except Hunan, it was promoted in countries such as America and Japan, and quickly entered the ornamental plants sector [3]. The leaves, flowers and roots of the plant are known to be used in traditional Chinese medicine for the treatment of many diseases. It is known to have antipyretic, hemostatic and detoxifying properties. It is also used in the treatment of bleeding disorders, burn treatment, skin infections, various gynecological diseases, dysentery and diarrhea. It has various properties such as antioxidant, anti-inflammatory and bacteriostatic with the tannins it contains [1, 4, 6]. The most widely used method in the production of perennial ornamental plant species is vegetative production method. Irregular collection of plants from natural flora by humans, shrinkage of ecological area due to urbanization, global warming and increase in global transportation threaten plant species. For this reason, it is very important to spread the plant tissue culture method instead of traditional production [7]. Today, many plant species are cultivated for these purposes and grown as ornamental plants [8]. Tissue culture; It is defined as the production of a new plant, tissue or various secondary metabolites from the cell, tissue or organ taken from the plant in sterile and controlled conditions, in artificial nutrient media [9]. It is widely used because it provides faster production compared to traditional plant breeding techniques [10]. Thus, the reproduction of endangered, endemic, economically valuable, genetically and productively superior individuals is provided. Quality, fast and virus-free, disease-free plants can be produced [11]. *Loropetalum chinense* is a very difficult plant that can be grown by seed or vegetative methods. *Loropetalum chinense* last 5 years with different specifications required in Turkey and is therefore one of the most imported ornamental plants. However, the difficulties in its production can be overcome with tissue culture [12]. In this study, it is aimed to develop *Loropetalum chinense* tissue culture and micropropagation protocols. For this purpose, the effect of different nutrient media in the number of shoots per explant and the average growth performance of growing shoots on plant reproduction were investigated.

2. Research Methodology

In this study, *Loropetalum chinense* plant in Sakarya University Plant Tissue Culture Research and Production Laboratory was used in March 2020. The nutrient media used in the study and their contents are given in Table 1. The nutrients were sterilized in autoclave at 121°C for 20 minutes (1 atm). Growth conditions and soil requirements of the plant were taken into account in determining the content of the nutrient media. In order to encourage shoot reproduction, 1.00 mg L^{-1} BAP was added to each experimental media considering the literature information. Explants taken from the mother plant were first washed under running tap water for 20 minutes and pre-sterilized with antifungal (Benomyl) for 10 minutes. It was then washed with 20% by volume sodium hypochlorite (ACE) for 15 minutes. Then, after washing with sterile distilled water for 5 minutes and 3 repetitions, they were planted in nutrients. Planting was done with 5 plants in each group. It was cultured for 45 days at 24 ± 2 °C in a 16-hours light photoperiod. All plant trials were set up with 5 replicates and were subjected to Duncan multiple comparison and variance analysis in SPSS statistical program.

3. Results and Discussion

In this study, MS-Mod and DKW-Mod nutrient media, which were modified by considering the soil requirements of the plants, based on the commonly used MS and DKW media, were used. In addition, 1.00 mg L^{-1} BAP was added to the nutrient media for the plant to propagation.

Table 1. *Nutrient media and ingredients (mg L^{-1}).*

Ingredients	MS	MS-modified	DKW	DKW- modified
NH_4NO_3	1650	500	1416	1416
KNO_3	1900	2000	-	-
$Ca(NO_3.4H_2O)$	-	1200	1968	1968
K_2SO_4	-	-	1559	1559
$MgSO_4$	181	370	740	740
$CaCl_2$	333	-	147	147
KH_2PO_4	170	170	259	259
$FeSO_4.7H_2O$	27.8	33.80	42.25	-
Na_2EDTA	37.26	45.40	56.75	-
EDDHA-Fe	-	-	-	168
Na_2MoO_4	0.25	0.39	0.40	0.40
$CuSO_4.5H_2O$	0.25	0.25	0.25	0.25
H_3BO_3	6.2	4.8	12.4	12.4
$Zn(NO_3)7H_2O$	16.9	17	26.7	26.7
$MnSO_4.2H_2O$	8.6	33.5	33.8	33.8
$NiSO_4.6H_2O$	5	5	5	5
Glycine	2	2	2	2
Nikotinik acid	0.5	1	1	1
Thiamine HCl	0.1	2	2	2
My-inositol	1	1	1	1
L-glutamine	1	-	1	-

Table 2. *Loropetalum chinense shoot per explant development.*

Plant media	Shoots per explant (shoots/explant)	Shoots length (mm)	Stem length (mm)	Internode (mm)	Leaf per explant (leaves/explant)
MS	0.48 ^{b}	4.36 ^{a}	9.45 ^{c}	2.89 ^{ns}	4.26 ^{b}
MS-Mod	1.63 ^{a}	4.26 ^{a}	11.81 ^{a}	2.91 ^{ns}	5.80 ^{a}
DKW	1.76 ^{a}	4.07 ^{a}	10.48 ^{b}	2.78 ^{ns}	6.25 ^{a}
DKW-Mod	0.69 ^{b}	1.34 ^{b}	9.49 ^{c}	2.62 ^{ns}	4.14 ^{b}

a-c: The difference between the samples in the same column is statistically significant (P<0.01).

In Table 2, the highest number of shoots per explant was obtained from DKW media with 1.76. It was determined that there was no significant difference between DKW and MS-Mod media in terms of the number of shoots per explant. The highest shoot length per explant was 4.36 mm from MS media. It was determined that there was no significant difference between MS, MS-Mod and DKW media in terms of shoot size per explant. As the number of shoots per explant increased, shoot length decreased (Figure 1).

In Table 2, the highest body length with 11.81 mm was obtained from MS-Mode media. No statistically significant difference was found between environments in terms of internode length. The number of plant leaves was obtained from DKW media with maximum 6.25 pieces. It was determined that there was no significant difference between MS-Mod and DKW media in terms of leaf number. The most suitable growth media for *Loropetalum chinense* was determined as DKW media. Since their development in the MS-Mod media is close to the desired level, it has been determined that this media can be used as an alternative in reproduction (Figure 2-5).

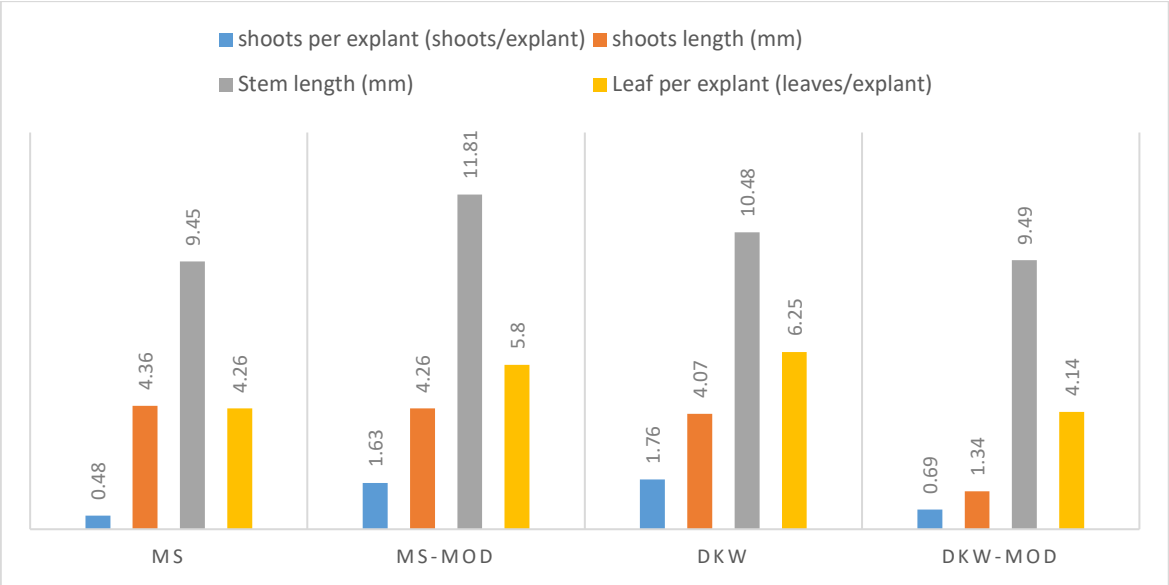

***Figure 1**. Loropetalum chinense plant and shoot development per explant.*

[13], in their study on the pollen germination percentage of *Loropetalum chinense*, they found that B^{+3} and Ca^{+2} increased by 58.47% in tissue culture media. In this study, both shoot and plant growth of the explant was high in the nutrient media with high B^{+3} and Ca^{+2} amounts. [14], it has been reported that in *Loropetalum chinense* callus culture, the most suitable media is MS media containing 2.00 mg L^{-1} NAA and 0.50 mg L^{-1} BA. However, it has also been reported that there is better callus development in MS media containing 1.50 mg L^{-1} NAA in dark environmental condition. This study does not show similarity with the test results used for meristem culture. [15], *Loropetalum chinense* in the vitrification studies, BA, NAA, activated carbon, sugars, have found that reducing the vitrification of heavy use. In this study, no vitrification problem was observed in any of the plants due to the use of BAP as cytokinin. [16], it has been reported that the most suitable media for *Loropetalum chinense* haploid callus culture is Gamborg B5 media containing 2.50 mg L^{-1} NAA, 0.50 mg L^{-1} BA and 30 g L^{-1} sucrose. The plant showed different growth in different methods because of micropropagation method used in our research.

At the end of the culture period, the plants and shoots were extended for 15 days in MS media containing 0.10 mg L^{-1} BAP and 20 g L^{-1} sugar. Then it was rooted for 7 days in 1/2 MS media containing 1.00 mg L^{-1} IBA. When rooting started, it was planted in copeat plates (Figure 5). And pre-conditioning was done in a plant tissue culture greenhouse.

4. Conclusions

Successful results have been obtained from modified nutrient media prepared by considering soil requirements. This shows that it is important to meet the natural requirements of the plant in micropropagation. Accordingly, instead of standard nutrient media, the media prepared according to the plant's requirements for each plant species give more successful results in micropropagation.

Acknowledgements

All studies in this research were carried out in the Sakarya University Plant Tissue Culture Research and Production Laboratory as part of the Studies On Micropropagation Of *Nandina domestica* (Heavenly bamboo) and *Loropetalum chinense* (Chinese fringe flower) master's thesis study.

***Figure 2**. Loropetalum chinense is in 60 ml MS media containing 1.00 mg L-1 BAP (Orginal, March 2020).*

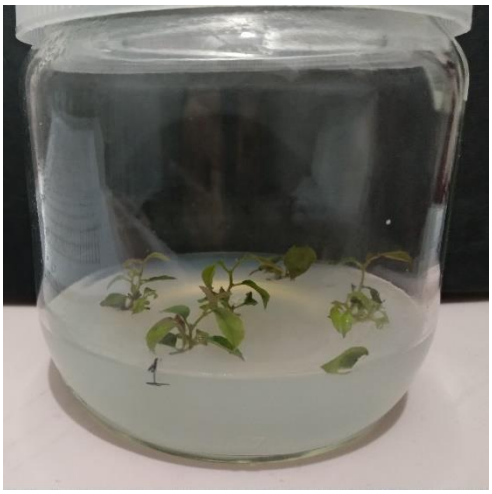

***Figure 3**. Loropetalum chinense is in 60 ml MS-Mod media containing 1.00 mg L-1 BAP (Orginal, March 2020).*

***Figure 4.** Loropetalum chinense is in 60 ml DKW media containing 1.00 mg L-1 BAP (Orginal, March 2020).*

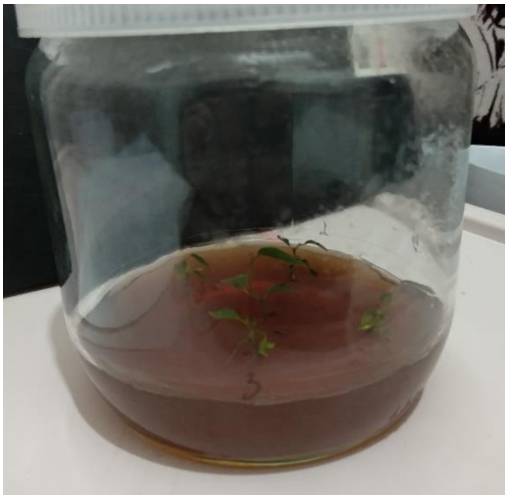

***Figure 5.** Loropetalum chinense is in 60 ml DKW-Mod media containing 1.00 mg L-1 BAP (Orginal, March 2020).*

***Figure 6.** Loropetalum chinense is in cocopeat plate. (Orginal, May 2020).*

References

[1] Chen, H. L. (2018). Isolation And Identification Of The Anti-Oxidant Constituents From *Loropetalum chinense (R. Brown) Oliv*. Based On UHPLC–Q-TOF-MS/MS. Molecules, 23(1720), 2-15.

[2] Gawel, N. J. (1996). Identification Of Genetic Diversity Among *Loropetalum chinense var. rubrum* Introductions. J. Environ. Hort., 14(1), 38-41.

[3] Bao, Z. C. (2007). Variation In Morphological Traits Among *Loropetalum chinense var. rubrum* Accessions. HORTSCIENCE, 42(2), 399-402.

[4] Zhang, Q. F. (2013). Isolation Of New Flavan-3-Ol And Lignan Glucoside From *Loropetalum chinense* And Their Antimicrobial Activities. Fitoterapia, 90, 228-232. doi:10.1016/j.fitote.2013.08.003

[5] Gong, W. L. (2016). From Glacial Refugia To Wide Distribution Range: Demographic Expansion Of *Loropetalum chinense (Hamamelidaceae)* In Chinese Subtropical Evergreen Broadleaved Forest. Org Divers Evol, 16, 23-38.

[6] Zhou, X. X. (2011). A New Lignan From The Leaves Of *Loropetalum chinensis*. Chemistry Of Natural Compounds, 47(5), 690-692.

[7] Demirbaş, A. R. (2010). Süs Bitkileri Yetiştiriciliği. Samsun: Samsun Tarım İl Müdürlüğü Çiftçi Eğitimi ve Yayım Şubesi.

[8] Aygün, G. (2015). Bitki Büyüme Düzenleyicilerinin *Anthurium andreanum L.* Türünün İki Çeşidinin Mikroçoğaltımı Üzerindeki Etkileri. Yüksek Lisans Tezi. Çanakkale Onsekiz Mart Üniversitesi Fen Bilimleri Enstitüsü**.**

[9] Babaoğlu, M. G. (2001). Bitki Biyoteknolojisi 1 Doku Kültürü ve Uygulamaları. Selçuk Üniversitesi Vakfı Yayınları.

[10] Hamidi Birecikli, A. (2018). Balcı Aspir (*Carthamus tinctorius L.*) Çeşidinin Mikroçoğaltımı. Yüksek Lisans Tezi. Batman Üniversitesi Fen Bilimleri Enstitüsü.

[11] Düzer, E. (2010). *Origanum Onites* Ve *Origanum Majorana* Bitkileri İle Meristem Kültürü Kullanılarak Stres Fizyolojisi Çalışması. Anadolu Üniversitesi Fen Bilimleri Enstitüsü.

[12] Bajpai, V. K. (2019). Antioxidant And Antimicrobial Efficacy Of A Biflavonoid, Amentoflavone From *Nandina Domestica In Vitro* And In Minced Chicken Meat And Apple Juice Food Models. Food Chemistry, 239-247.

[13] Tang, Q. Z. (2005). Factors Effecting Pollen Germination Percentage Of *L.chinensis var rubrum* And *L.chinensis*. Hunan Forestry Science And Technology, 4.

[14] Wang, H. T. (2006). Studies On Callus Inducing Of *Loropetalum chinense var.rubrum.* Journal Of Yueyang Vocational Technical College, 6.

[15] Yin, H. T. (2008). A Preliminary Study On The Tissue Culture To Overcome The Vitrification Of *Loropetalum chinense Oliver var rubrum* Yieh. Journal Of Jiangsu Forestry Science & Technology, 3.

[16] Li, Y. Y. (2011). Induction And Cultivation Of Haploid Callus Of *Loropetalum chinense var. rubrum*. Journal Of Hunan Agricultural University, 37(6), 632-636.