Biotecnología Vegetal Vol. 20 No. 2: 75 - 82, abril - junio, 2020
Instituto de Biotecnología de las Plantas. UCLV. MES.
eISSN 2074-8647, RNPS: 2154

Lavandula angustifolia L. plants regeneration from *in vitro* leaf explants-derived callus as conservation strategy

Leelavathi Devasigamani[1] https://orcid.org/0000-0003-1777-8065
Raajasubramanian Devarajan[2,3] https://orcid.org/0000-0001-6295-7770
Ramu Loganathan[1] https://orcid.org/0000-0003-2336-2031
Haseena Rafath[2,4] https://orcid.org/0000-0002-0753-3454
Midhila Padman[5] https://orcid.org/0000-0003-2153-5701
GovindaRaju MV[6] https://orcid.org/0000-0001-5393-6556
Lavanya Giridhar[2] https://orcid.org/0000-0003-4057-8651
Chetan HC[7] https://orcid.org/0000-0002-1853-3353
Narendra Kuppan[2*] https://orcid.org/0000-0001-5637-4106

[1]MES Degree College of Arts, Commerce and Science. Bengaluru. Karnataka. India.

[2]Department of Botany, Annamalai University. Chidambaram. Tamil Nadu. India.

[3]Department of Botany, Thiru A Govindasamy Government Arts College. Tindivanam. Tamil Nadu. India.

[4]Goverment Arts College, C Muttur. Chidambaram. Tamil Nadu. India.

[5]Deptof Biotechnology, Sahrdaya College of Engineering and Technology. Kodakara. Thrissur. Kerala. India.

[6]Sanjeevini, Karnataka State Rural Lively hood Promotion Society. Sheshadripuram. Bangalore. Karnataka. India.

[7]Centre for Conservation of Natural Resources, The University of Trans-Disciplinary Health Sciences and Technology. Yelahanka. Bengaluru. Karnataka. India.

[*]Corresponding author email:narendrakuppan@gmail.com

ABSTRACT
Lavandula angustifolia L. is an aromatic and medicinal herb with multiple industrial applications. Nevertheless, the over exploitation of wild plantation attempts against to its conservation as natural resource. The aim of this paper was to regenerate plants of *L. angustifolia* from *in vitro* leaf explants by indirect organogenesis as conservation strategy. Leaves sections from *in vitro* plants were cultured in MS with 6-Benzylaminopurine (BAP) combined with Naphthalene acetic acid (NAA) or 2,4-Dichlorophenoxy acetic acid (2,4-D) to induce callus formation. The callus growth was categorized into three types according to the explant area covered by callus and the fresh and dry weigh (mg) per callus were determined. Then, callus were subcultured on MS with BAP and Kinetin combined with NAA or 2,4-D for shoots regeneration. Shoots were cultured in MS with BAP 8.88 µM, indole butyric acid (IBA) 4.92 µM and 2.68µM NAA for rooting. Plantlets were acclimatized and after 50 days of hardening the plants were transferred to the soil. Explants in all treatments formed calli. The higher percentage of callus formation with abundant growth was achieved in MS with 8.88 µM BAP + 5.36 µM NAA (92%). In MS with 4.44 µM BAP + 4.64 µM Kn+ 2.68 µM NAA, 93% of calli regenerating shoots and25-30 multiple shoots were obtained after 63 days of subculture. Shoots developed roots and plants were successfully acclimatized with 93 percentage of survival. *In vitro* leaf of *L. angustifolia* is a suitable explant for plants regeneration.

Keywords: auxin, cytokinin, lavender

Regeneración de plantas de *Lavandula angustifolia* L. a partir de callos formados de explantes de hojas *in vitro* como estrategia de conservación

RESUMEN

Lavandula angustifolia L. es una hierba aromática y medicinal con múltiples aplicaciones industriales. Sin embargo, la sobreexplotación de las plantaciones silvestres atenta contra su conservación como recurso natural. El objetivo de este trabajo fue regenerar plantas de *L. angustifolia* a partir de explantes foliares *in vitro* mediante organogénesis indirecta como estrategia de conservación. Se cultivaron secciones de hojas de plantas *in vitro* en MS con 6-bencilamino purina (BAP) combinada con ácido naftalen acético (ANA) o ácido 2,4-diclorofenoxiacético (2,4-D) para inducir la formación de callos. El crecimiento de los callos se clasificó en tres tipos de acuerdo con el área de explante cubierta por el callo y se determinó la masa fresca y seca (mg) por callo. Luego, se subcultivaron los callos en MS con BAP y Kinetina (Kn) combinados con ANA o 2,4-D para la regeneración de los brotes. Los brotes se cultivaron en MS con 8.88 µM de BAP, 4.92 µM de ácido indol butírico (AIB) y 2.68 µM de ANA para su enraizamiento. Las plántulas se aclimatizaron y después de 50 días de endurecimiento se transfirieron a suelo. Los explantes en todos los tratamientos formaron callos. El mayor porcentaje de formación de callos con crecimiento abundante se logró en MS con 8.88 µM de BAP + 5.36 µM de ANA (92%). En MS con 4.44 µM de BAP + 4.64 µM de Kn + 2.68 µM de ANA, el 93% de los callos regeneraron brotes y se obtuvieron de 25-30 brotes múltiples después de 63 días de subcultivo. Los brotes desarrollaron raíces y las plantas se aclimatizaron con éxito con un alto porcentaje de supervivencia 93% La hoja *in vitro* de *L. angustifolia* es un explante adecuado para la regeneración de plantas.

Palabras clave: auxina, citoquinina, lavanda

INTRODUCTION

In nature, environment plays a pivotal role on plant species development. It may cause changes in genetic diversity, losses of many native wild species and their economically important phytochemicals. Thus, loss in genetic diversity, inbreeding and un-erupted risk is resulted in the reduction in population size of a species. In this context, the conservation of wild species is very important for genetic diversity because their extinction rate is increasing directly and indirectly in response to anthropogenic activities and by environmental factors (Frankham *et al.*, 2002). Unavailability of agricultural land in global scenario of increase in human population and urbanization is a threat for plant diversity and agriculture. In the case of aromatic and medicinal plants, the augmented demand of biomass and metabolites attempt against the plants population growing in natural conditions or cultivated. The use of bio-technological techniques can be a suitable alternative for conservation of these plants and supply the necessity of its use as genetically pure lines in large-scale production (Parkash and Singh, 2013).

Lavandula angustifolia L. is a widely distributed aromatic and medicinal herb worldwide (Cavanagh and Wilkinson, 2002; Hajhashemi *et al.*, 2003). It is commonly known as Lavender or English Lavender. It belongs to the family Lamiaceae and cultivated in the Mediterranean areas. Its essential oil displays carminative, antiflatulence, and anticolic properties. Aroma therapists use it in the holistic approach (Hajhashemi *et al.*, 2003). This essential oil acts as a central nervous system depressant, anticonvulsant, sedative, spasmolytic agent, local anesthetic, antioxidant, antibacterial, and mast cell degranulation inhibitor (Cavanagh and Wilkinson, 2002). Lavender is comprised of over 100 constituents, among which the primary components are linalool and linalyl acetate, á-pinene, limonene, 1,8-cineole, cis-andtrans-ocimene, 3-octanone, camphor, caryophyllene, terpinen-4- ol, and flavonoids (Hajhashemi *et al.,* 2003).

Vegetative propagation of *Lavandula* species by conventional methods is limited by low multiplication rate, poor rooting ability of the stem cuttings, as well as the lack of selected clones (Gonçalves and Romano, 2012). In this regard, the use of *in vitro* culture under controlled environmental conditions have been reported as alternative to overcome lavender propagation problems. Nevertheless, to

produce large quantities of plants with high genetic and phytosanitary qualities, effective protocols for the *in vitro* propagation are required.

Different types of explants such as hypocotyl sections, apical shoots, nodal explants, leaf segments, seedlings from *in vitro* germinated seeds, cotyledonary buds and meristem have been used in *Lavandula* spp. *in vitro* culture (Segura and Calvo, 1991; Nobre, 1996; Dronne *et al.*, 1999; Tsuro *et al.*, 2000; Zuzarte *et al*., 2010; Miclea and Chifor, 2018; Rana *et al*., 2018). Among that, nodal explants are the most common explant type (Gonçalves and Romano, 2012).

Several authors referred that leaf segments of lavender plants are suitable explant for callus formation in *L. vera* (Tsuro *et al.,* 2000), *Lavandula* × *intermedia* Emeric ex Loiseleur (Dronne *et al.,* 1999) and *L. angustifolia* (Falk *et al.,* 2009; Guadarrama-Flores *et al.,* 2012). Despite of, the *in vitro* microbial contamination of explants taken from plants grown in field or in greenhouse is one of the problems that limited the successful of plants regeneration by organogenesis (De Bona *et al.*, 2011).The use of explants from *in vitro* plants would result in regenerated plants without visible microbial contamination. Additionally, it would facilitate the employ of the protocol development previously for rapid clonal propagation of *L. angustifolia* (Leelavathi and Kuppan, 2013), and to decrease the losses by this concept. The aim of this paper was to regenerate plants of *L. angustifolia* from *in vitro* leaf explants by indirect organogenesis as conservation strategy.

MATERIAL AND METHODS

Plant material

L. angustifolia plants were chosen and collected from the Department of Horticulture G.K.V.K., Bengaluru, India. The plants were maintained in the greenhouse at Plant Biotechnology Unit, Department of Botany, Bangalore University.

L. angustifolia was *in vitro* established from axillary and apical buds (Leelavathi, 2010). The apical section of leaves from *in vitro* plants were used as explants.

Callus formation

Nine leaves sections were inoculated in test tubes (25mm X 150 mm) with the abaxial surface in contact with the medium (Dronne *et al*., 1999). Culture tubes were plugged with non-absorbent cotton wool wrapped cheesecloth. The tubes containing 15 ml of basal media Murashige and Skoog (Murashige and Skoog, 1962) (MSBM) (Sigma) supplemented with 6-Benzylaminopurine (BAP) (8.88 µM) combined with Naphthalene acetic acid (NAA) (2.68, 5.36, 8.05, 10.74, 13.42µM) or 2,4-Dichlorophenoxy acetic acid (2,4-D) (2.26, 4.52, 6.78, 9.04, 11.30 µM) to induce callus formation. The pH of the media was adjusted to 5.8 before sterilization in autoclave at 121 °C and 0.1 MPa for 20 min.

Each explant was used as an experimental unit for observations and data collection and 10 replicates were utilized per treatment. After 18 days of culture, the number of explants with callus was quantified and the percentage of response was calculated.

After that, the callus were subcultured on vessels (117 mm X 271 mm, 510 ml capacity with polypropylene caps) with 40 ml of the same medium for 18 days more (36 days of culture). The growth of observed calli were categorized into three types and described as abundant (+++), moderate (++) or scanty (+) according to the explant area covered by callus. Besides, the fresh and dry weight (mg) per callus in each treatment were determined. For dry weight the callus were dried at 27 ºC during 48 hours.

Plant regeneration

Callus were subcultured on MSBM supplemented with BAP (2.22, 4.44, 6.66, 8.88, 11.11 µM) and Kinetin (2.32, 4.64, 6.96, 9.28, 11.60 µM) combined with 2.68 µM NAA or 2.26 µM 2,4-D for shoots regeneration. The number of callus that regenerated shoots were quantified and the percentage was calculated. Besides, the total number of shoots per vessels was recorded.

After 42 days of culture, shoots were separated and cultured five per vessel (510 ml capacity) with 40 ml of MS basal medium supplemented with 8.88 µM BAP, 4.92 µM indole butyric acid (IBA) and 2.68 µM NAA for

rooting. The pH of the media was adjusted to 5.8 before sterilization by autoclave at 121 °C and 0.1 MPa for 20 min.

All the cultures in callus formation and plant regeneration stages were maintained at 25±2 ºC with photon flux density of 30-50 $\mu Em^{-2} s^{-1}$ under photoperiodic regime of 16 h light and 8 h dark cycles.

Hardening

The well-developed plantlets were carefully taken out from the culture bottles and washed thoroughly with water to remove the traces of agar. The plantlets were then transferred to plastic pots (Hardening pot capacity 2 liters with 17 cm in diameter at the top, 12 cm diameter at base and 13 cm pot height) containing potting mixture peat: perlite: vermiculate in the ratio 1:1:1 (v/v). The potted plants were covered with polythene covers to maintain humidity. These plantlets were maintained at 25 ± 2 ºC and 90-95% Relative Humidity (RH). After 15 days, the covers were removed and the plants were gradually exposed to less humid conditions. After 50 days of hardening the final survival (%) was recorded and the plants were transferred to the soil.

Statistical analysis

The data obtained from the results of all experiments were analyzed statistically by one-way analysis of variance (ANOVA) to determine the variation between the treatments and Critical Difference (CD). To find out variations within the treatments, the overall hypothesis of difference between more than two groups were tested while making only on statistical decision using ANOVA.

RESULTS AND DISCUSSION

The study showed that explants in all treatments formed calli at the leaf cut ends surface after 18 days of culture. The percentage of response ranged between 46 and 92%.The best response of explants (over 75%) was recorded for the combination of BAP and NAA in MS basal medium. The higher percentage of callus formation with abundant growth was achieved in MS supplemented with 8.88 µM BAP + 5.36 µM NAA (92%) (Figure 1 b, Table 1). Despite of small *in vitro* leaves size its morphogenetic capacity was well demonstrated. In this sense, Yegorova *et al.* (2019) found that leaves of *in vitro* regenerated plants of *L. angustifolia* possessed differentiated mesophyll from the early stages of *in vitro* culture and it were characterized by a dark-green color, a lancet-like shape, and the level of necrotic tissue in them did not exceed 5-7%.

The use of *in vitro* leaf explants avoided the explants disinfection and reduced the incidence of microbial contaminants. In this study, the contamination percent was minimal (under 2%) and it was related to manipulation of the explants in the lab, and not associated to the explants.

After 36 days of subculture on the same medium, large amount of profuse, greenish brown compact callus was observed (Figure 1 c, d). In the present study, *in vitro*leaf were chosen for retrieval of plants through callus phase. The advantage of using aseptically derived tissue was essential because the callus were free from phenolic compounds in the media. The accumulation of these compounds may inhibit organogenesis in many plants, including *L. angustifolia* as described by De Bona *et al.* (2011). The results was in accordance with reports that have used *in vitro* leaf explants for callus induction in aromatic or medicinal plants (Janarthanam and Seshadri, 2008; Anjusha and Gangaprasad, 2017).

The ANOVA analysis revealed a highly significant effect for the treatment factor ($p<0.05$). The highest amount of callus was observed on MSBM + BAP (8.88 µM) + NAA (5.36 µM) with fresh weight 23.30 g and dry weight 4.00 g. It was observed that the increase in concentration of NAA to 8.05 µM resulted in decrease of callus formation. Besides, the combination of BAP and 2,4-D did not reached better results than BAP + NAA (Table 1).

The results were in agreement with previous report of Donne *et al.* (1999) who demonstrated that callogenesis in lavandin (*Lavandula* × *intermedia* Emeric ex Loiseleur) was stimulated by the simultaneous addition of BAP and auxins in leaves explants. They informed in cultivar Grosso 2 the best results on MS medium containing 9 µM BA and 4.5 µM NAA.

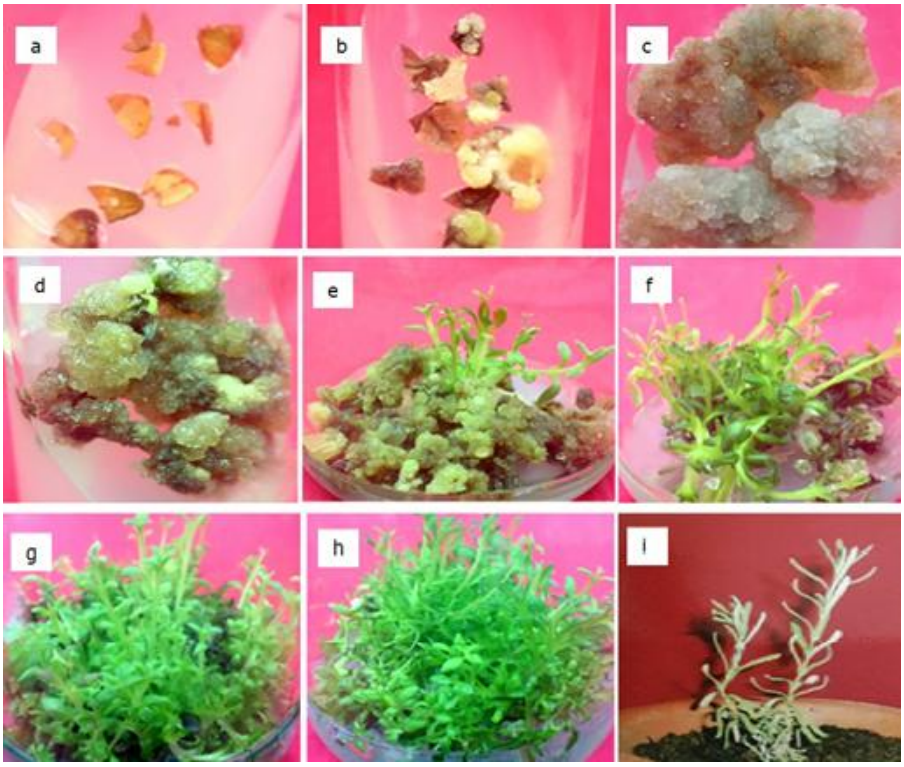

Figure 1. *In vitro* propagation of *Lavandula angustifolia* L. (a) Leaf explants from *in vitro* cultured plants inoculated on MS Basal Medium, (b) Explant showing callus initiation, (c) Callus, (d) greenish brown compact callus after 36 days of culture, (e) Callus showing shoot initiation, (f) Culture showing multiple shoots at 52 days of culture, (g) shoots after 63 days of culture, (h) Shoots in rooting stages, (i) plants hardening.

Table 1. Effect of BAP with NAA or 2,4-D in MS basal medium on *Lavandula angustifolia* leaf callus formation and growth after 18 days of culture.

Treatment	Callus formation (%)	Callus growth	Fresh weight (mg)	Dry weight (mg)
8.88 µM BAP + 2.68 µM NAA	81	+	20.29±1.53	2.50±0.56
8.88 µM BAP + 5.36 µM NAA	92	+++	23.30±1.52	4.00±0.64
8.88 µM BAP + 8.05 µM NAA	78	++	19.69±1.09	2.39±0.40
8.88 µM BAP + 10.74 µM NAA	75	++	18.98±1.14	2.14±0.14
8.88 µM BAP + 13.42 µM NAA	69	++	17.04±1.10	1.80 ±1.44
8.88 µM BAP + 2.26 µM 2,4-D	59	+	14.71±2.05	1.92 ±0.33
8.88 µM BAP + 4.52 µM 2,4-D	67	++	16.88±1.19	2.50±0.56
8.88 µM BAP + 6.78 µM 2,4-D	59	++	14.88±0.67	1.97±0.20
8.88 µM BAP + 9.04 µM 2,4-D	50	++	12.52±1.03	1.84± 0.77
8.88 µM BAP + 11.30 µM 2,4-D	46	+	11.50±1.44	1.62±0.33

Callus growth: + scarcy, ++ moderate, +++ abundant. Values of media ± standard deviation. n=10

After 21 days of subculture, shoot initiation was observed on all treatments with varying frequencies of response (43 - 93%). The highest frequency of response (93%) of calli regenerating shoots was noted for those induced on the medium with 4.44µM BAP + 4.64 µM Kn+ 2.68 µM NAA (Table 2).

Shoots that formed in calli developed the green color progressively with growth after 28 days of culture (Figure 1 e and f). Two to five shoots developed only from greenish brown compact callus. Five to six multiple shoots per explant were noticed after 36 days of subculture, 10-15 after 52 days of subculture (Figure 1 f) and 25 - 30 well developed elongated multiple shoots after 63 days of subculture (Figure 1 g).

The results of the study particularly underscore the potential of *in vitro* leaf explants to produce many plants in short time and space. Maximum number of multiple shoots were obtained in medium MS with 4.44 µM BAP + 4.64 µM Kn+ 2.68 µM NAA (Table 2). The average number of shoots per callus was seven in this treatment with the most efficient shoot regeneration.

It was found that composition of medium had an influence on the callus regeneration capacity. In the same way of the results in callus formation stage, the addition of 2,4-D in the media do not increase the callus response. In relation with this, Dronne *et al.* (1999) informed that higher level of 2,4-D in callus induction medium was unsuitable for further shoot regeneration. Well-developed elongated multiple shoots developed roots when cultured on MS basal medium with 8.88 µM BAP + 4.92 µM IBA + 2.68 µM NAA after 30 days of culture (Figure 1 h). In contrast, De Bona *et al.* (2011) indicated that only in media without growth regulator or with BAP + GA_3 (gibberellic acid) they observed the roots induction in *L. angustifolia* organogenesis.

At the end of the experiment, *L. angustifolia* plants were successfully acclimatized (Figure 1 i) with high percentage of surviving (98%).

The propagation through *in vitro* leaf explants-derived callus is a reliable method for the rapid multiplication of lavender, allowing the selection, propagation and conservation of superior clones. Nevertheless, studies about the genetic stability of these plants will be developed.

On the other hand, another example of application would be the *in vitro* production of metabolites (Andrys *et al.*, 2018). In this sense, Guadarrama-Flores *et al.* (2012) reports the presence of terpenoids linalool and linalyl acetate in callus from immature leaf explants of *L. angustifolia.* The extraction of valuable metabolites in lavender plants propagated by tissue culture will contribute to avoid the exploitation of wild populations (Gonçalves and Romano, 2012) and to their conservation.

Table 2. Effect of different concentrations of cytokinin and auxin for shoots regeneration in callus derived from *in vitro* leaf explants of *Lavandula angustifolia.*

Treatments	Callus with shoots (%)	No. of shoots per vessel
2.22 µM BAP + 2.32 µM Kn+ 2.68 µM NAA	62	20
4.44 µM BAP + 4.64 µM Kn+ 2.68 µM NAA	93	30
6.66 µM BAP + 6.96 µM Kn+ 2.68 µM NAA	75	24
8.88 µM BAP + 9.28 µM Kn+ 2.68 µM NAA	62	20
11.11 µM BAP + 11.60 µM Kn+ 2.68 µM NAA	56	18
2.22 µM BAP + 2.32 µM Kn+ 2.26 µM2,4-D	43	18
4.44 µM BAP + 4.64 µM Kn+ 2.26 µM2,4-D	68	22
6.66 µM BAP + 6.96 µM Kn+ 2.26 µM2,4-D	62	20
8.88 µM BAP + 9.28 µM Kn+ 2.26 µM2,4-D	56	16
11.11 µM BAP + 11.60 µM Kn+ 2.26 µM2,4-D	50	14

There is a great need for the conservation of the plant species mainly, rare and imperiled, which are economically and medicinally important. Genetic diversity is one of the key for the survival of the plant species in their natural domain. Its disappearance may lead to decrease in ability to cope with changing environment and demographic fluctuations both in short and long term. Once plant species disappear from their natural habitat, they cannot be regenerated and it is very difficult to re-establish their previous rich diversity. Therefore, these biotechnological tools should be applied for the conservation (Parkash and Singh, 2013) and genetic improvement of *L. angustifolia*.

CONCLUSIONS

In vitro leaf of *L. angustifolia* plants is a suitable explant for plants regeneration by indirect organogenesis as conservation strategy. It has advantage such as a reduction in microbial contamination and less phenolic oxidation.

ACKNOWLEDGEMENT

The first author is thankful to the UGC (University Grants Commission), Government of India for providing salary during the tenure of the research. UGC (University Grants Commission) had no participation in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare. The work is genuinely under taken and all the data in the paper is never published or in consideration for publication in any other journal.

Author contributions

Conceptualization MP, Data curation GR, Formal analysis RL, Funding acquisition LD, Investigation LD, Methodology CHC, Project administration RD, HR, Resources LD, Supervision MP, Validation RD, HR, MP, Visualization NK, LG, Writing – original draft NK, Writing – review & editing NK.

REFERENCES

Andrys D, Adaszyñska-Skwirzyñska M, Kulpa D (2018) Essential oil obtained from micropropagated lavender, its effect on HSF cells and application in cosmetic emulsion as a natural protective substance. Nat Prod Res 32(7): 849-853

Anjusha S, Gangaprasad A (2017) Callus culture and *in vitro* production of anthraquinone in *Gynochth odesumbellata* (L.) Razafim. & B. Bremer (Rubiaceae). Industrial Crops and Products 95: 608-614; doi: 10.1016/j.indcrop.2016.11.021

Cavanagh HM, Wilkinson JN (2002) Biological activities of lavender essential oil.Phytother Res 16: 301-308

De Bona CM, Reinhart V, Biasi LA, Zanette F (2011) *Lavandula dentata* and *Lavandula angustifolia in vitro* organogenesis. Plant Cell Cult Micropropag 7(2): 66-70

Dronne S, Jullien F, Caissard JC, Faure O (1999) A Simple and efficient method for *in vitro* shoot regeneration from leaves of Lavandin (*Lavandula* x *intermedia* Emeric ex Loiseleur). Plant Cell Report 18: 429-433

Falk L, Biswas K, Boeckelmann A, Lane A, Mahmoud SS (2009) An efûcient method for the micropropagation of lavenders: regeneration of a unique mutant. J Essent Oil Res21: 225-228

Frankham R, Ballou JD, Briscoe DA (2002) Introduction to Conservation Genetics. Cambridge University Press, London

Gonçalves S, Romano A (2012) *In vitro* culture of lavenders (*Lavandula* spp.) and the production of secondary metabolites. Biotechnol Adv 31: 166–174; doi: 10.1016/j.biotechadv.2012.09.006

Guadarrama-Flores B, Buendía-González L, Orozco-VillaFuerte J, Estrada-Zúñiga ME, Cruz-Sosa F (2012) Producción en cultivos *in vitro* de los componentes principales del aceite esencial de *Lavandula angustifolia*. Rev Latinoamer Quím 40(2): 65-73

Hajhashemi V, Ghannadi A, Sharif B (2003) Anti-inflammatory and analgesic properties of the leaf extracts and essential oil of *Lavandula angustifolia* Mill. J Ethnopharmacol 89: 67-71

Janarthanam B, Seshadri S (2008) Plantlet regeneration from leaf derived callus of *Vanilla*

planifolia Andr. *In Vitro* Cell Dev Biol Plant 44: 84–89; doi: 10.1007/s11627-008-9123-4

Leelavathi D (2010) *Ex–situ* conservation of *Lavandula angustifolia* using *in vitro* techniques. Wetlands, Biodiversity and change, Bangalore

Leelavathi D, Kuppan N (2013) Protocol for rapid clonal multiplication using *in vitro* apical bud of *Lavandula angustifolia* IOSR Journal Of Pharmacy And Biological Sciences 7(3): 96-98

Miclea I, Chifor R (2018) Germination, *in vitro* propagation and acclimatization in *Lavandula angustifolia*. Bull UASVM Anim Sci Biotechnol 75: 106-109; doi: 10.15835/buasvmcn-asb:2018.0017

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-97

Nobre J (1996) *In vitro* cloning and micropropagation of *Lavandula stoechas* from field-grown plants. Plant Cell Tissue Organ Cult 46: 151-155

Parkash V, Singh H (2013) *Lavandula angustifolia* L. (Lavender): an important aromatic medicinal shrub and its *in vitro* micro-propagation for conservation. Journal of Agricultural Technology 9(3): 91-702

Rana N, Khadka S, Rajbahak S (2018) *In vitro* propagation of Lavender (*Lavandula angustifolia* Mill.). J Pl Res 16(1): 112-118

Segura J, Calvo MC (1991) *Lavandula* spp. (Lavender): *in vitro* culture, regeneration of plants, and the formation of essential oils and pigments. In: Bajaj YPS (Eds.) Biotechnology in Agriculture and Forestry, pp. 283-310, Springer Cham, Dordrech

Tsuro M, Koda M, Inoue M (2000) Efficient plant regeneration from multiple shoots formed in the leaf-derived callus of *Lavandula vera*, using the open culture system. SciHort 86: 81-88

Yegorova NA, Mitrofanova IV, Brailkoa VA, Grebennikovaa OA, Paliya AE, Stavtseva IV (2019) Morphogenetic, Physiological, and Biochemical Features of *Lavandula angustifolia* at Long-Term micropropagation *in vitro*. Russian Journal of Plant Physiology 66(2): 326-334

Zuzarte MR, Dinis AM, Cavaleiro C, Salgueiro LR, Canhoto JM (2010) Trichomes, essential oils and *in vitro* propagation of *Lavandula pedunculata* (Lamiaceae). Ind Crop Prod 32: 580-587

Recibido: 02-10-2019
Aceptado: 03-02-2020