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Obtainment of calluses from *Morus alba* L. var. acorazonada with different culture media and explant types

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Abstract

Objective: To evaluate the effect of concentrations of different growth regulators and explant types on the formation of calluses from *M. alba* L. variety acorazonada.

Materials and Methods: The work was conducted in the Plant Biotechnology Study Center of the University of Granma, Cuba. Fifteen treatments were carried out with the combination of three culture media with the explant types and positions. The culture media were complemented with the growth regulators 2,4-dicholorophenoxyacetic acid (2,0 mg L^{-1}), 6-benzylaminopurine (2,0 mg L^{-1}) + naphthaleneacetic acid (0,5 mg L^{-1}) and 6-benzylaminopurine (2,0 mg L^{-1}) + 2,4-dicholorophenoxyacetic acid (0,5 mg L^{-1}). The explant types were leaf blades in abaxial and adaxial position, transversally and longitudinally sectioned stems and petioles. After 15 and 30 days, the beginning of callus formation, the zone in which they were formed, callus percentage, color and consistency, as well as the root formation percentage, were evaluated.

Results: After 15 days, in all the explants and culture media morphological changes occurred. These modifications proved the beginning of callus formation, which reached higher values than 86 % at 30 days of cultivation. The calluses that were formed from sectioned stems in culture media complemented with 6-benzylaminopurine (2,0 mg L^{-1}) + 2,4-dicholorophenoxyacetic acid (0,5 mg L^{-1}) showed better morphogenic characteristics.

Conclusions: Callus formation was obtained in the different culture media and explant types of *M. alba* variety acorazonada, with higher values than 86 %. The highest explant percentage with root formation was recorded from transversally sectioned stems in the culture medium with 2,4-dicholorophenoxyacetic acid, reaching values of 75 %.

Keywords: growth control, *in vitro* culture, mulberry, roots

Introduction

The genus *Morus* has been distributed throughout the world, in temperate as well as tropical areas. The development of sericulture projects in different regions causes that today mulberry (*Morus alba* L.) is present in China, India, Brazil, Japan and other countries (Gahukar, 2015).

Traditionally, the propagation of the species is carried out by cuttings or botanical seeds. This constitutes a limitation, because it reduces even more its availability for animal feeding, due to its growth cycle (Desai *et al*., 2018).

In Cuba, *M. alba* is studied for its utilization in forage production because of its qualities as feedstuff (Noda and Martín, 2017). With this purpose, the country has a germplasm of 21 varieties, introduced from Costa Rica, Ethiopia, Brazil, South Korea, China and Spain (Martín-Martín *et al*., 2015).

The process of *M. alba* introduction and generalization in Cuba has led to the development of diverse studies of evaluation and/or characterization of varieties, among which acorazonada stands out (Martín-Martín *et al*., 2015).

Plant tissue culture is a technique for the cultivation, multiplication and maintenance of plant cells, tissues or organs isolated from the mother plant, under controlled and aseptic conditions (Viquez-Pancho, 2018).

In mulberry, tissue culture has been efficiently applied to improve the potential of yield, resistance to pests and diseases, tolerance to abiotic stresses and resistance to herbicides by the crop. It has also been used with good results for the micropropagation of difficult genotypes, plant regeneration of young leaves, cotyledons, hypocotyl zones and stem segments. Its use in *in vitro* techniques is also referred, in order to develop tetraploids through the use of colchicine (Tikader and Vijayan, 2017) and in molecular markers for germplasm characterization in mulberry (Vijayan *et al*., 2019).

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The basis to develop plant tissue culture techniques in mulberry requires establishing a protocol for callus culture. Agarwal (2002) used hypocotyls and cotyledons as explants for obtaining calluses through the use of different BAP and 2,4-D concentrations. Yew *et al*. (2011) used segments of leaves and of different combinations of BAP, IAA and 2,4-D. For obtaining calluses, Espinosa *et al*. (2012) also used leaf blades, stems and petioles as explants with different concentrations of 2,4-D.

The objective of this work was to evaluate the effect of different concentrations of growth regulators and explant types on the formation of calluses of *M. alba* variety acorazonada.

Materials and Methods

Location. This research was conducted at the Plant Biotechnology Study Center (CEBVEG, for its initials in Spanish) of the University of Granma, Cuba.

Plant material. Cuttings from *M. alba* (mulberry), variety acorazonada, 20 cm long, were cut, collected at the germplasm bank belonging to the CEBVEG.

The cuttings were placed in distilled water for obtaining sprouts. When they reached a size of 10-12 cm, the leaves, stems and petioles were taken as explant sources for the formation of calluses. Disinfection was performed under aseptic conditions of laminar flow cabinet, with 1 % sodium hypochlorite during 20 min. Afterwards, they were washed four times with sterile distilled water.

Basal culture medium. The basal culture medium was composed by salts, vitamins and *Murashige* and *Skoog* solution (Murashige and Skoog, 1962) at concentration of 4,32 g L^{-1}. Furthermore, sucrose (30 g L^{-1}) and agar (6,0 g L^{-1}) with pH 5,8, were added to the culture medium. The growth regulators were used according to the indications by Espinosa *et al*. (2012), who used 2,4-dichlorophenoxyacetic acid (2,4-D). The criteria expressed by Agarwal (2002), who makes reference to the combinations of 6-benzylaminopurine (6-BAP) + naphthaleneacetic acid (NAA) and 6-benzylaminopurine (6-BAP) + 2,4-dichlorophenoxyacetic acid (2,4-D), were followed.

Treatment and experimental design. The treatments were formed by combining the three culture media with the five explant types and positions for a total of 15 treatments (table 1). The explants were placed at a rate of five explants per Petri dish for each of the culture media. A complete randomized design with factorial arrangement was applied.

After 15 and 30 days, the beginning of callus formation, zone on which they were formed, formation percentage, color, consistency and root formation percentage, were evaluated. The calluses were

Table 1. Combinations of different culture media with explant type and position in the formation of *M. alba* calluses

Treatment	Culture media	Explant types and positions
1	2,4-dichlorophenoxyacetic (2,0 mg L^{-1})	TSA
2		TST
3		LAb
4		LAd
5		P
6	6-benzylaminopurine (2,0 mg L^{-1}) + 2,4-dichlorophenoxy-acetic (0,5 mg L^{-1})	TSA
7		TST
8		LAb
9		LAd
10		P
11	6-benzylaminopurine (2,0 mg L^{-1}) + naphthaleneacetic acid (0,5 mg L^{-1})	TSA
12		TST
13		LAb
14		LAd
15		P

TSA: 0,5-cm long stems, longitudinally sectioned with the cut upwards.
TST: transversally sectioned stems, 2-3 mm diameter, and thin cell layers on the culture medium
LAb: leaf blades in abaxial position, of 1 cm^2, with the dorsal side on the culture medium.
LAd: leaf blades in adaxial position, of 1 cm^2, with the ventral side on the culture medium.
P: 0,5-cm long petiole longitudinally placed on the culture medium.

incubated under darkness conditions, at temperature of 25 ± 2 °C and relative humidity between 60 and 65 %.

Statistical analysis. Data such as callus and root formation were expressed in percentages. The proportion comparison analysis using the statistical package CompraPro was applied, according to the indications made by Castillo and Miranda (2014).

Results and Discussion

The results that are shown in table 2, related to the callus formation percentage with regards to the culture medium and explant type, prove that in all the treatments there was callus formation at 15 and 30 days, with higher values than 86 %. This can be considered an adequate physiological response of the explants to different growth regulators, as indicated by the percentages reached in all the treatments.

A similar performance was referred by Bhau and Wakhlu (2001), when forming calluses of *Morus alba* variety Chinese White and Kokuso-27 (100 %) and in Ichinose (95 %) in basal culture medium *Murashige* and *Skoog* supplemented with 2,4-D (1,0 mg L^{-1}) + BAP (0,5 mg L^{-1}).

Callus formation is considered a defense mechanism of the plant to prevent the penetration of alien agents through the cuts that occur in its tissues (Silva *et al*., 2018). At 15 days of cultivation, the beginning of callus formation in the different evaluated explants was observed. In the leaves it became evident when they began to thicken and were separated from the culture medium. In the stem explants the formation of small protuberances could be observed on the edges on which the cuts were made and on the main nervation. In the petioles, the beginning of undifferentiated tissue formation was shown in the healing zones.

These results are similar to the ones reported by Espinosa *et al*. (2012) in the cultivation of mulberry, variety acorazonada in MS medium, with different concentrations of 2,4-D (0,5; 1,0 and 2,0 mg L^{-1}), when using leaf blades, stems and petioles as explants.

The results of this study are higher than those achieved by Agarwal (2002), who obtained 65 % of callus formation on internodal explants, in a combination of BAP (2,0 mg L^{-1}) + NAA (0,5 mg L^{-1}). Meanwhile, in leaf explants, the highest percentage

Table 2. Effect of different culture media and explant types on the formation of mulberry calluses, at 15 and 30 days of cultivation.

Treatment	Culture media	Explant types and position	Callus formation, %	
			15 days	30 days
1	2,4-dichlorophenoxyacetic (2,0 mg L^{-1})	TSA	100^{a}	100^{a}
2		TST	100^{a}	100^{a}
3		LAb	100^{a}	100^{a}
4		LAd	92^{ab}	96^{ab}
5		P	100^{a}	100^{a}
6	6-benzylaminopurine (2,0 mg L^{-1})+2,4-dichlorophenoxyacetic (0,5 mg L^{-1})	TSA	100^{a}	100^{a}
7		TST	100^{a}	100^{a}
8		LAb	100^{a}	100^{a}
9		LAd	100^{a}	100^{a}
10		P	100^{a}	100^{a}
11	6- benzylaminopurine (2,0 mg L^{-1})+ naphthaleneacetic acid (0,5 mg L^{-1})	TSA	100^{a}	100^{a}
12		TST	100^{a}	100^{a}
13		LAb	100^{a}	100^{a}
14		LAd	96^{ab}	96^{ab}
15		P	86^{b}	86^{b}

TSA: 0,5-cm long stems, longitudinally sectioned with the cut upwards.
TST: transversally sectioned stems, 2-3 mm diameter, and thin cell layers on the culture medium
LAb: leaf blades in abaxial position, of 1 cm^2, with the dorsal side on the culture medium.
LAd: leaf blades in adaxial position, of 1 cm^2, with the ventral side on the culture medium.
P: 0,5-cm long petiole longitudinally placed on the culture medium.
Different letters in the same column significantly differ ($p < 0,05$); *$p < 0,05$

(44,05 %) was achieved with BAP (2,0 mg L^{-1}) + NAA (0,5 mg L^{-1}) after five weeks of cultivation. The difference observed with regards to this study can be related to the used variety, which in the work conducted by Agarwal (2002) was M5.

At 30 days of cultivation, it could be observed that the calluses in the leaf explants were little developed, with brown coloring and hard consistency. In the petiole explant, the calluses had cream coloring, and were more evident in the culture media that contained 2,4-D (2,0 mg L^{-1}) and 6-BAP (2,0 mg L^{-1}) + NAA (0,5 mg L^{-1}).

Callus formation in the explants of longitudinally sectioned stems, at 30 days of cultivation in the MS medium with 6-BAP (2,0 mg L^{-1}) + 2,4-D (0,5 mg L^{-1}), showed the best morphological characteristics (figure 1). The amorphous cell mass occupy the entire explant surface, showing light cream coloring with friable consistency.

Agarwal (2002), when evaluating the effect of different combinations of 6-BAP with 2,4-D in leaves and internodal explants after five weeks of cultivation, reported that the best treatment was 6-BAP 2,0 mg L^{-1}+ 2,4-D 0,5 mg L^{-1}, with 52,6 % in internodal explants and 33,7 % in leaves. Those results were lower than the ones in this research, with the variety acorazonada, which can be related to the use of the variety M5 by Agarwal (2002). With regards to the latter, there are no references in Cuba.

Deo *et al.* (2010) indicated that among cultivars of the same species differences can appear regarding the induction of embryogenic tissues. Thus, some genotypes are induced relatively easily; while others do not show favorable responses under the same cultivation conditions. In this case, the cultivation conditions or the medium composition should be modified.

According to Martínez *et al.* (2017), 6-BAP reinforces biochemically the action of 2,4-D by increasing auxin activity. They are in charge of stimulating cell growth and division, increasing the cell formation rate in an organized way from the explant cells. These growth regulators have a fundamental function in sprout formation and growth (Liu *et al*., 2019).

The characteristics shown by the calluses coincide with the observations made by Espinosa *et al*. (2012). In mulberry cultivation, these authors obtained light cream calluses in all the treatments in which they used 2,4-D in the different explant types (leaf blades, petiole and stems). Agarwal (2002) also obtained calluses of friable consistency and brown color in hypocotyl explants in the cultivation of mulberry, variety M5.

Silva *et al*. (2018), when using different explant types (leaves, stems and petiole) of *M. alba*, variety acorazonada, obtained yellowish brown calluses of friable consistency. The calluses were incubated under darkness conditions. All the works reviewed in literature coincide on the fact that callus formation in mulberry occurs in the dark. This is explained because under those conditions the oxidation of auxins, which play an important role in callus formation, is reduced (Sarkar *et al*., 2018).

Correspondingly with the results of this study, it can be stated that the use of leaves, petioles and stems, as explants in the cultivation of mulberry,

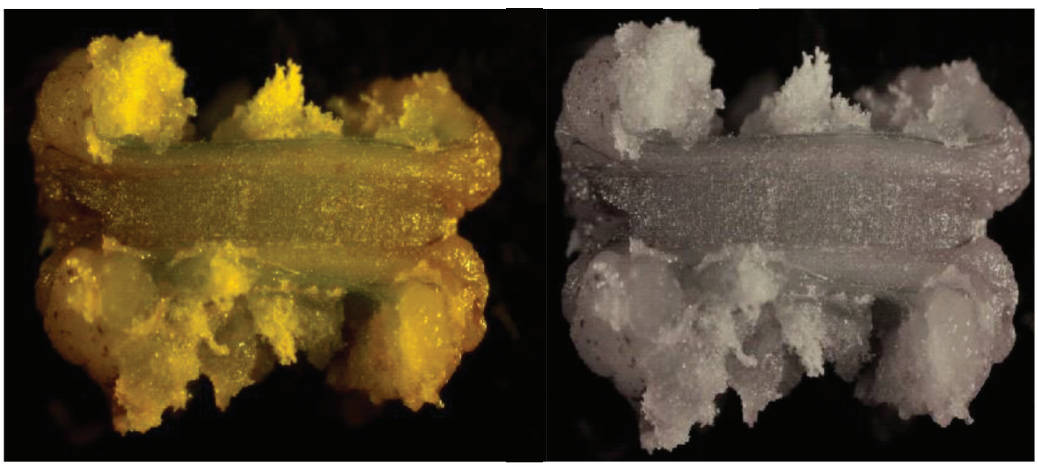

Figure 1. Callus formation in explants of longitudinally sectioned stems in *Murashige* and *Skoog* medium with 6-benzylaminopurine (2,0 mg L^{-1}) + 2,4-dichlorophenoxyacetic (0,5 mg L^{-1}), at 30 days of cultivation.

allows to obtain efficiently calluses and to achieve high values for each of the explant types.

Table 3 shows the results of the effect of different culture media and explant types on the root formation percentage, at 30 days of cultivation. The highest rooting percentage (75 %) was observed in treatment 2, in which explants of transversally sectioned stems in *Murashige* and *Skoog* (MS) medium with 2,4-D (2,0 mg L^{-1}). This treatment showed significant differences with regards to the others. The results prove that 2,4-D exerts a favorable effect, not only on callus formation, but also on the induction of roots in the *in vitro* culture of longitudinal segments in young mulberry stems.

The lowest value in root formation was observed in the culture medium with 6-BAP (2,0 mg L^{-1}) + 2,4-D (0,5 mg L^{-1}). This can be associated to the fact that BAP cytokinin levels were higher than 2,4-D, relation which can decrease root formation (Gil *et al*., 2019).

In the culture medium 6-BAP (2,0 mg L^{-1}) + NAA (0,5 mg L^{-1}) the results were variable, because values of 44 and 27 % were obtained for the positions and explant types LAD and TSA, respectively. However, for the other three treatments, the values were 0 and 4 % which suggests an effect more dependent on the explant type and position in the culture medium.

2,4-D is a strong auxin, which is used in *in vitro* culture for callus formation and induction of somatic embryos. It exerts a rooting effect, and at present it is widely used, due to its low cost and easy access. In diverse plant species it has been used as growth regulator (De-la-Cruz *et al*., 2016).

The plasticity of several explants indicates the possibilities of utilization of these root systems for the production of secondary metabolites, and for the possible cultivation of vesicular arbuscular mycorrhizal fungi (AMF) which need a forced symbiosis. These AMF cannot be obtained in artificial culture media.

Figure 2 shows root formation in explants of transversally sectioned explants at 30 days in MS medium with 2,4-D (2,0 mg L^{-1}). Root growth occurred from the calluses and directly from the explant, which indicates the occurrence of indirect and direct organogenesis, respectively (figure 2). The roots that were formed are thick and long and are densely covered by root hairs.

Espinosa *et al*. (2012) in the species *M. alba* obtained calluses that formed roots from petioles and leaves. These authors reported that in the latter

Table 3. Effect of different culture media and explant types on the root formation percentage at 30 days of cultivation.

Treatment	Culture media	Explant types and position	Root formation, %
1	2,4-dichlorophenoxyacetic (2,0 mg L^{-1})	TSA	34^{bc}
2		TST	75^{a}
3		LAb	38^{bc}
4		LAd	48^{b}
5		P	9^{d}
6	6-benzylaminopurine (2,0 mg L^{-1})+ 2,4-dichlophenoxyacetic(0,5 mg L^{-1})	TSA	4^{d}
7		TST	0
8		LAb	0
9		LAd	0
10		P	4^{d}
11	6-benzylaminopurine (2,0 mg L^{-1}) + naphthaleneacetic acid (0,5 mg L^{-1})	TSA	27^{c}
12		TST	0
13		LAb.	4^{d}
14		LAd	44^{bc}
15		P	0

TSA: 0,5-cm long stems, longitudinally sectioned with the cut upwards.
TST: transversally sectioned stems, 2-3 mm diameter, with thin cell layers on the culture medium
LAb: leaf blades arranged in abaxial position, of 1 cm^2, with the dorsal side on the culture medium.
LAd: leaf blades placed in adaxial position, of 1 cm^2, with the ventral side on the culture medium.
P: 0,5-cm long petiole longitudinally placed on the culture medium.
Different letters in the same column significantly differ ($p < 0,05$); *$p < 0,05$

Figure 2. Root formation on explants of transversally sectioned stems in *Murashige* and *Skoog* medium with 2,4-dichlorofphenoxyacetic (2,0 mg L^{-1}), at 30 days of cultivation.

explant the number of roots was higher in the treatments where the highest concentrations of 2,4-D (1,0 and 2,0 mg L^{-1}).

The formation of roots or sprouts on the calluses depends on the auxin/cytokinins ratio in the medium. Rhizogenesis is thus induced in high presence of IAA or NAA. Meanwhile, when combining 2,4-D with auxins or cytokinins, the formation of calluses with regenerative capacity with sprouts or embryos is favored (Rodríguez-Beraud *et al.*, 2014).

Rodríguez-Beraud *et al.* (2014), when using cotyledon, hypocotyl and leaves of strawberry myrtle (*Ugnimolinae* Turcz), evaluated the *in vitro* induction of callogenesis and indirect organogenesis. The calluses were cultivated in a differentiation medium with diverse concentrations of NAA + 6-BAP. These authors obtained the highest rhizogenic response in leaf calluses with NAA (0,1 mg L^{-1}) + 6-BAP (1,0 mg L^{-1}).

For obtaining roots the use of transversally sectioned stems in the culture media used by Espinosa *et al*. (2012), is recommended.

In this research, higher values than 86 % were reached in callus formation. The best morphological characteristics were obtained in the explants of longitudinally sectioned stems in culture medium with BAP (2,0 mg L^{-1}) + 2,4-D (0,5 mg L^{-1}).

Conclusions

Callus formation was obtained in the different culture media and explant types of *M. alba*, variety acorazonada, with higher values than 86 %. The calluses formed from stems that were longitudinally sectioned in culture medium complemented with 6-benzylaminopurine + 2,4 dichlorophenoxyacetic acid showed better morphogenic characteristics.

The highest percentage of explants with root formation was obtained from transversally sectioned stems in the culture medium with 2,0 mg L^{-1} of 2,4-dichlorophenoxyacetic acid, reaching values of 75 %.

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Authors' contribution

- Yanexis Yayma Fonseca-Carrasco. Information and data collection, besides their analysis and interpretation. Paper writing and final manuscript reading and content approval.
- Lianet Brizuela-Fuentes. Information and data collection, besides their analysis and interpretation. Paper writing and final manuscript reading and content approval.
- Juan José Silva-Pupo. Data interpretation, critical revision of the text and approval of the final version. Final manuscript reading and content approval.

Conflicts of interests

The authors declare that there are no conflicts of interests.

Referencias bibliográficas

Agarwal, S. *Genetic transformation and plant regeneration studies in Morus alba L*. Thesis presented in option to the scientific degree of Doctor

Philosophy, in Biotechnology. India: College of Horticulture, 2002.

Bhau, B. S. & Wakhlu, A. K. Effect of genotype, explant type and growth regulators on organogenesis in *Morus alba* L. *Plant Cell Tiss. Org.* 66:25-29, 2001. DOI: https://doi.org/10.1023/A:1010617212237.

Castillo, Y. & Miranda, I. COMPAPROP: Sistema para comparación de proporciones múltiples. *Revista Protección Vegetal*. 29 (3):231-234, 2014.

De-la-Cruz-Castillo, J.; Mejía-Coico, F.; Mostacero-León, J.; López-Medina, E. & Gonza-Carnero, Aracely. Efecto de la concentración del 2,4-diclorofenoxiacético (2,4-D) en el enraizamiento de estacas de *Rosa* sp., rosa silvestre, en condiciones de invernadero. *Rev. Indes.* 2 (1):37-43, 2016. DOI: http://doi.org/10.25127/indes201.401.004.

Deo, P.; Tyagi, A.; Taylor, Mary; Harding, R. & Becker, D. Factors affecting somatic embryogenesis and transformation in modern plant breeding. *South Pac. J. Nat. Appl. Sci*. 28:27-40, 2010. DOI: https://doi.org/10.1071/SP10002.

Desai, S.; Desai, P.; Mankad, M.; Patel, A.; Patil, G. & Narayanan, S. Development of micropropagation protocol for *Morus nigra* L. (black mulberry) through axillary buds. *IJCS*. 6 (2):585-589, 2018.

Espinosa, A.; Silva, J.; Sariego, S.; Cholo, L. & Delgado, H. Efecto del tipo de explante y la concentración de ácido 2,4-diclorofenoxiacético en la formación de callos en *Morus alba* L. *Pastos y Forrajes*. 35 (4):407-416, 2012.

Gahukar, R. T. Management of pests and diseases of tropical sericultural plants by using plant-derived products: a review. *J. For. Res*. 26 (3):533-544, 2015. DOI: https://doi.org/10.1007/s11676-015-0126-9

Gil, Arlette I.; Ariza, C. A.; Castillo, Lina M.; Salgado, L. E.; Banda-Sánchez, L. & Vanegas, L. E. Inducción de organogénesis *in vitro* con 6-bencilaminopurina en *Cattleya trianae* Linden & Rchb.f. *Rev. UDCA Actual divulg. cient.* 22 (2):e1275, 2019. DOI: http://dx.doi.org/10.31910/rudca.v22.n2.2019.1275

Liu, Q.; Zhang, D.; Liu, F.; Qin, M. & Tian, D. Micropropagation of *Nelumbo nucifera* 'Weishan Hong' through germfree mature embryos. *In Vitro Cell. Dev. Biol.-Plant*. 55:305-312, 2019. DOI: https://doi.org/10.1007/s11627-019-09984-4.

Martín-Martín, G. J.; Noda-Leyva, Yolai; Olivera-Castro, Yuseika & Pentón-Fernández, Gertrudis. Efecto de productos orgánicos en el desarrollo de propágulos de *Morus alba*, L. *Rev. Mex. Cienc. Agríc*. 6 (3):619-625, 2015.

Martínez, S. de J.; Gómez-Kosky, R.; Barbón Rodríguez, R.; Collado, R.; Pérez, Marta; Veitia, N. *et al.* Maduración y germinación de embriones somáticos de *Sorghum bicolor* (L.) Moench cultivar 'CIAP 132R-05. *Biotecnología Vegetal*. 17 (1):33-39, 2017.

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

Noda-Leyva, Yolai & Martín-Martín, G. J. Efecto de la distancia de siembra en el rendimiento de *Morus alba* (L.) var. yu-12. *Pastos y Forrajes*. 40 (1):23-28, 2017.

Rodríguez-Beraud, M. M.; Latsague-Vidal, Mirtha I.; Chacón-Fuentes, M. A. & Astorga-Brevis, Pamela K. Inducción *in vitro* de callogénesis y organogénesis indirecta a partir de explantes de cotiledón, hipocótilo y hoja en *Ugni molinae*. *Bosque (Valdivia)*. 35 (1):111-118, 2014. DOI: https://dx.doi.org/10.4067/S0717-92002014000100011.

Sarkar, T.; Mogili, T.; Gandhi, S. & Sivaprasad, V. Tissue culture in mulberry (*Morus* spp.) intending genetic improvement, micropropagation and secondary metabolite production: a review on current status and future prospects. In: N. Kumar, comp. *Biotechnological approaches for medicinal and aromatic plants*. Singapore: Springer Nature. p. 467-487, 2018.

Silva, J. J.; Fonseca, Yanexis Y.; Bahi, Marisel; Pérez, J. L. & Werbrouck, S. *In vitro* propagation and callus formation of *Morus alba* L. *Proceedings of the Fifth International Conference of the IUFRO Unit 2.09.02 on «Clonal trees in the bioeconomy age: opportunities and challenges».* Coimbra, Portugal: IUFRO. p. 159-164, 2018.

Tikader, A. & Vijayan, K. Mulberry (*Morus* spp.) genetic diversity, conservation and management. In: M. R. Ahuja and S. M. Jain, eds. *Biodiversity and conservation of woody plants. Sustainable Development and Biodiversity.* vol 17. Cham, Switzerland: Springer. p. 95-127, 2017.

Vijayan, K.; Ravikumar, G. & Tikader, A. Mulberry (*Morus* spp.) breeding for higher fruit production. In: J. M. Al-Khayri, S. Jain and D. Johnson, eds. *Advances in plant breeding strategies: fruits*. Cham, Switzerland: Springer. p. 89-130, 2018.

Viquez-Pancho, Rosa A. *Establecimiento de un protocolo de propagación in vitro de morera (Morus sp.) para el suministro de material de siembra en la cadena serícola en el departamento del Cauca-Colombia*. Tesis de Maestría. Palmira, Colombia: Facultad de Ciencias Agrarias, Universidad Nacional de Colombia, Sede Palmira, 2018.