In vitro propagation from axillary buds of the endangered tree *Dalbergia congestiflora* Pittier (Fabaceae)

Alejandra Hernández-García1,2, Enrique Ambriz-Parra2, Pablo López-Albarrán2, José Cruz-de León2, Rafael Salgado-Garciglia*

1 Instituto de Investigaciones Químico-Biológicas, Edif. B3, Universidad Michoacana de San Nicolás de Hidalgo, Ciudad Universitaria, Ave. Francisco J. Múgica S/N, C.P. 58030, Morelia, Michoacán México; 2 Facultad de Ingeniería en Tecnología de la Madera, Universidad Michoacana de San Nicolás de Hidalgo, Ciudad Universitaria, Ave. Francisco J. Múgica S/N, C.P. 58030, Morelia, Michoacán México

*E-mail: rsalgadogarciglia@gmail.com  Tel: +52-443-2002091  Fax: +52-443-3265790

Received July 27, 2021; accepted September 1, 2021 (Edited by T. Kobayashi)

**Abstract**  *Dalbergia congestiflora* Pittier is a woody plant species grown in Mexico and Central America and widely used as timber wood and medicinal material. Since *D. congestiflora* is an endangered species, an in-vitro micropropagation technique is needed for mass propagation of *D. congestiflora* plantlets. Nodal segments of *D. congestiflora* stem cuttings grown in greenhouse conditions were disinfected with an appropriate protocol and in vitro established on Murashige and Skoog medium (MS) supplemented with 0.05 mg l⁻¹ benzylaminopurine (BA). The explants showed 10% contamination with 90% survival, and the initial shoot was regenerated in 90% of them. Axillary buds of 45-day-old initial shoots were cultured on MS containing BA (0, 0.05, 0.1, 0.5, 1, 1.5 and 2 mg l⁻¹) singly or in combination with α-naphthaleneacetic acid (NAA) (0, 0.1, 0.5 and 1 mg l⁻¹). A higher shoot number (9.6 shoots/explant) was obtained on MS with 1 mg l⁻¹ BA and 0.1 mg l⁻¹ NAA. Rooting was investigated using half-strength MS, 2% sucrose and different concentrations of indole butyric acid (IBA) (0, 0.1, 0.5 and 1 mg l⁻¹). After 30 days of culture, developing shoots were elongated and rooted in culture medium without IBA, with production of 3.2 roots/shoot. Micropropagated plantlets of *D. congestiflora* were successfully transplanted and acclimatized to a mixture of peat moss and perlite (2:1) with 100% relative humidity in greenhouse conditions with 80% survival at 30 days of culture. This micropropagation protocol will contribute to the conservation of *D. congestiflora*, and assure the mass propagation for sustainable usage of this species.

**Key words:**  *Dalbergia congestiflora*, direct organogenesis, micropropagation, nodal segments, shoot regeneration.

**Introduction**

The genus *Dalbergia* (Fabaceae) is represented in Mexico by twenty species, six of which are endemic. *Dalbergia congestiflora* Pittier is an endangered tree commonly known as campincerán in Mexico. According to the official ecological regulations of Mexico (NOM-059-SEMARNAT-2010), its management and use are regulated by the General Law of Wildlife, and this species is listed in Appendix II of CITES (CITES 2017; Linares and Sousa 2007; Ricker et al. 2013; SEMARNAT 2010).

Although *D. congestiflora* is distributed in the region of Central and South Mexico to Guatemala and El Salvador, their populations are declining from overexploitation due to the attractiveness and durability of the tree's wood and habitat loss and fragmentation (Miles et al. 2006). The wood of *D. congestiflora* has a wide purple heartwood and sapwood of contrasting color, used for the production of musical instruments such as guitars and crafts and to obtain dyes (Barragán et al. 1999; Pittier 1922). Likewise, the heartwood of this species is a source of phytochemicals, mainly medicarpin, a pterocarp phytoalexin that has antifungal activity against the xylophagous fungus *Trametes versicolor* (Martínez-Sotres et al. 2012) as well as osteogenic and cytotoxic properties (Bhargavan et al. 2012; Trivedi et al. 2014). The wood is also a source of isoflavan-cinnamylphenol pigments with cytotoxic activity in HeLa cell lines (Barragán et al. 1999; Barragán-Huerta et al. 2004; Perez-Gutierrez and García-Baez 2013).

In response to the difficulty in sexually and asexually propagating *D. congestiflora* with conventional techniques (Hernández-García et al. 2016), micropropagation has been used as an alternative for mass propagation of this tree. In vitro propagation is very useful in the production of many endangered plants and species with unique features, such as tropical hardwood trees (Pijut et al. 2012; Sarasan et al. 2006).
Some species of the genus Dalbergia, such as D. melanoxylon, D. latifolia, D. lanceolaria, D. retusa and D. sissoo, have been micropropagated (Kiondo et al. 2014; Manikandan et al. 2017; Valverde-Cerdas and Alvarado-Guzmán 2004). D. sissoo has been the species most studied for its high ecological value and commercial timber, and micropropagated plantlets have been produced by shoot regeneration from cotyledon explants, nodal and internodal segments, shoot tips and callus on Murashige and Skoog (MS) medium with benzylaminopurine (BA) and α-naphthaleneacetic acid (NAA) or indoleacetic acid (IAA) and rooting on complete and half-strength MS medium with indole butyric acid (IBA) (Ali et al. 2012; Bari et al. 2008; Chand and Singh 2005; Singh et al. 2002). An efficient method for micropropagation of D. sissoo was reported from nodal shoot segments derived from rejuvenated shoots of adult trees, obtaining multiple differentiated from nodal shoot segments derived from rejuvenated method for micropropagation of D. sissoo (Chand and Singh 2005; Singh et al. 2002). An efficient acid (NAA) or indoleacetic acid (IAA) and rooting on α and callus on Murashige and Skoog (MS) medium with explants, nodal and internodal segments, shoot tips been produced by shoot regeneration from cotyledon of BA and 0.44 mg l⁻¹ of thidiazuron (TDZ) (Vibha et al. 2014). However, there are no reports on the in vitro establishment, shoot induction and complete plant regeneration of D. congestiflora. The asexual propagation of this species by stem cuttings of adult plants was established by our working group; however, with only 2.5% rooting, this was considered a procedure yielding a low number of plants (Hernández-García et al. 2016). Therefore, the development of an efficient micropropagation protocol would be a practical way to achieve mass propagation of this endangered tree. In the present investigation, we established an efficient system of micropropagation of D. congestiflora, starting from the in vitro establishment of nodal segments from adult plants stem cuttings grown in a greenhouse, and analyzed the effect of the combination of BA and NAA for the propagation of adventitious shoots, as well as the production of suitable plantlets for greenhouse cultivation.

Materials and methods

Plant materials

Stem cuttings of basal leafy branches obtained during spring from adult plants of D. congestiflora collected in Carácuaro, Michoacán, Mexico (18°59′17.17″N, 101°6′18.56″W) were kept in greenhouse conditions until shoot development and used as a source of explants (Hernández-García et al. 2016). Plants were botanically identified in the Herbarium of the Faculty of Biology of the Universidad Michoacana de San Nicolás de Hidalgo.

In vitro nodal segment establishment

Nodal segments from shoots formed on stem cuttings (3–4 cm long) were surface sterilized following the same methodology used by Cortés-Rodriguez et al. (2011) as follows: immersion of nodal segments for 30 min in distilled water followed by dipping in a solution of ethanol (70%) (v/v) for 2 min and then in a 1.2% (available chlorine) solution of commercial sodium hypochlorite plus 1% thiabendazole fungicide (Tecto® 60, Syngenta, México) (w/v) for 20 min with gentle stirring. Under aseptic conditions, sterilized explants were rinsed three times in sterile distilled water, and leaves and basal portions of nodal segments were removed. Nodal segments (explants) 1 cm in length with axillary buds were cultured in 125 ml baby food jars (polypropylene-covered) with 20 ml of MS culture medium (Murashige and Skoog 1962) basal salts supplemented with 3% sucrose (w/v) (Sigma-Aldrich, Mexico), 0.1% myo-inositol (Sigma-Aldrich, Mexico) and solidified with 0.8% agar (w/v) (Sigma-Aldrich, Mexico); in addition, 0.05 mg l⁻¹ BA was added, and the pH was adjusted to 5.7 with KOH or HCl (0.1 N).

The culture flasks used in explant establishment were kept in the dark (4°C) for the first five days to prevent browning. Then, all the cultures were maintained under a 16 h photoperiod with 36 μmol m⁻² s⁻¹ irradiance, provided by white fluorescent lamps and a temperature of 25 ± 1°C. Browning, contamination, survival, initial shoot formation and shoot length were evaluated at 45 days of culture.

Shoot regeneration of axillary buds

Explants (1 cm in length) of 45-day-old initial shoots with axillary buds were cultured under the conditions indicated above on MS containing BA (0, 0.05, 0.1, 0.5, 1.0, 1.5 and 2.0 mg l⁻¹) alone or in combination with NAA (0, 0.1, 0.5 and 1.0 mg l⁻¹). Shoot induction percentages, number and length of shoots per explant were recorded after 45 days of culture.

Shoot-rooting and plantlet development

Actively growing shoots (2–3 cm length) with a single node were used for rooting. Rooting medium consisted of half-strength MS (½ MS) supplemented with 2% sucrose (w/v), 0.8% agar (w/v), and different concentrations of IBA (0, 0.1, 0.5, and 1.0 mg l⁻¹). Cultures were maintained in a growth room under the above-described conditions. Rooting percent, root number and root length (cm) were recorded after 30 days.

Transplanting and acclimatization

After 45 days of in vitro culture, plantlets cultured on ½ MS without growth regulators were transplanted into 250-ml plastic pots filled with a mixture of peat moss and perlite (2:1 v/v). Each pot was covered with a clear, plastic dome, and acclimatization was realized under greenhouse conditions (Agrosystem, Mexico) with constant irrigation and kept in controlled light (50% shade) and temperature conditions (23±5°C). In the first two weeks of transplantation, plantlets were kept under a relative humidity of 100%, and during the subsequent two weeks, the domes were removed by gradually reducing the humidity to 70%. The percentage of surviving plants was recorded after 30 days, the plants were transplanted after acclimatization into 1.5l plastic pots with a commercial substrate (Professional soil mix, Vigoro®, México) and grown under greenhouse conditions. Plant survival.
was also recorded 180 days later.

Statistical analysis
Statistical analyses were performed with JMP 9 software (Windows version; SAS Institute Inc., USA). All experiments consisted in completely randomized design with twenty-five replicates per treatment (n=25). One explant was cultured per flask. One-way analysis of variance was applied, and data were expressed as the means±standard error (SE) and compared using Tukey’s test. Differences were considered statistically significant at p<0.05.

Results
After 150 days of greenhouse culture of stem cuttings from adult plants of *D. congestiflora* under appropriate conditions, the development of shoots that ranged 8–10 cm in length with 3–5 nodal segments was observed. Segments 3–4 cm in length (Figure 1A) were removed from these shoots to establish 1-cm-long explants with axillary buds in vitro (Figure 1B). After 30 days of in vitro establishment, the explants did not show contamination, but 10% presented browning and mortality, while 90% survived. Bud break occurred after 21–30 days of culture, and initial shoots were formed that measured 2.5 cm in length with 3 nodes without the presence of leaves, roots or adventitious shoots.

Shoot regeneration
After 45 days of culture of *D. congestiflora* initial shoots with axillary buds on MS containing BA and NAA, 100% of explants in treatment T1 (0.05 mg l⁻¹ BA without NAA) and T9 (1.0 mg l⁻¹ BA and 0.1 mg l⁻¹ NAA) showed shoot regeneration. Percentages of shoot regeneration in a range of 52–84% were observed in treatments containing low concentrations of BA without auxin (T2 and T3) and low concentrations of NAA (T7, T8 and T10). However, low percentages (0–32%) of shoot regeneration were observed for treatments without growth regulators (T0) or those with a higher concentration of BA and NAA (Table 1).

Treatments with BA and without auxin (T1, T2 and T3) showed more than 1.7 shoots/explant, but in the other treatments with shoot responding explants, the number of shoots/explant was less than one. Longer shoots (1.10–2.15 cm length) were observed in the T1, T2 and T9 treatments, which had the highest number of shoots, and explants cultured in 1.0 mg l⁻¹ BA and 0.1 mg l⁻¹ NAA (T9) produced the largest number of adventitious shoots (9.6 shoots/explant) with a length of 1.64 cm (Figure 1C; Table 1).

In treatments with concentrations of 0.5 mg l⁻¹ BA and higher, callus formation was observed, and incipient callus induction (10%) at the base of explants was...
micropropagation from axillary buds

Dalbergia congestiflora

T13 (1.5 mg l⁻¹ dedifferentiated explants were obtained with treatment 50% with a higher development of callus, while 100% as T12, T14 and T15, the level of dedifferentiation was 25). Differences were considered statistically significant at p<0.05 (plants with optimal growth and development registered 80% survival (Figure 2C)).

Discussion

Approximately 90% of the explants responded to MS 0.05 mg l⁻¹ BA, and the regenerative potential at the establishment stage was influenced by the presence of cytokinin, as reported during in vitro apple shoot development (Magyar-Tábori et al. 2010). The explants had no microbial contamination because they originated from young shoots produced from stem cuttings under greenhouse conditions. In addition, a thiabandazole fungicide (Tecto® 60) was used for superficial sterilization. Cortés-Rodríguez et al. (2011) used this protocol of disinfection of axillary buds from developing shoots of field-grown Mexican race avocado plants, reducing the contamination of established explants from 70 to 30%. Explants with a smooth epidermis and axillary buds in the early stage of growth allow for better disinfection because they have fewer contaminating agents (Mantovani et al. 2013). Similar nodal segments of adult plants of Gingko biloba (Mantovani et al. 2013) and Citrus limon (Rathore et al. 2007) were disinfected and successfully established in vitro.

To avoid or reduce browning of Dalbergia congestiflora explants, those were treated with darkness at low temperature (4°C) for five days, which led to reduced release of phenolic compounds and the consequent death of nodal segments. This has been reported to be a successful method during the in vitro culture of nodal segments of shoots of apple root stocks (Dalal et al. 2006), and during in vitro shoot tips from offshoots of superior male date palm (Mustafa et al. 2013).

Shoot-rooting and plantlet formation

Root formation in micropropagated shoots of D. congestiflora occurred in all IBA treatments, with 100% rooting observed at 8 days of culture. At 30 days of cultivation, the number of roots was proportional to the length of these roots; a longer length was observed in ½ MS without auxin, such as T12, T14 and T15, the level of dedifferentiation was 50% with a higher development of callus, while 100% dedifferentiated explants were obtained with treatment T13 (1.5 mg l⁻¹ BA and 0.5 mg l⁻¹ NAA) (Table 1).

Table 2. Effect of indole butyric acid in ½ MS medium on the in vitro rooting of Dalbergia congestiflora micropropagated shoots after 30 days of culture.

| IBA (mg l⁻¹) | Rooting (%) | No. roots/shoot | Root length (cm) |
|-------------|-------------|-----------------|-----------------|
| 0           | 100         | 3.2±0.11a       | 3.6±0.38a       |
| 0.1         | 100         | 3.8±0.27b       | 4.4±0.32b       |
| 0.5         | 100         | 5.2±0.35c       | 5.7±0.44c       |
| 1.0         | 100         | 4.8±0.31b       | 5.4±0.47b       |

IBA, indole butyric acid; each value represents the mean±SE, and each mean value followed by the same letter does not differ significantly according to Tukey’s test; differences were considered statistically significant at p<0.05 (n=25).

Transplanting, acclimatization and greenhouse culture

D. congestiflora micropropagated plantlets cultured for 45 days in ½ MS without auxin were transplanted and cultivated in greenhouse conditions for 30 days to determine their survival. At this time, the plants showed a survival of 80% (Figure 2B). After 180 days under greenhouse conditions, D. congestiflora micropropagated plants with optimal growth and development registered 80% survival (Figure 2C).

Figure 2. A) Rooting shoot of Dalbergia congestiflora after 30 days on ½ MS without IBA. Arrows indicate newly formed roots; B) D. congestiflora micropropagated plant 30 days after transplantation and cultivation under greenhouse conditions; C) D. congestiflora plants 180 days after acclimatization.

shown in treatments T3, T4, T7, T8 and T9, with a slight increase (25%) in treatments T5, T6, T10, T11, T16 and T17, without affecting the differentiation of the explants. However, in treatments with 1.5 and 2.0 mg l⁻¹ BA, such as T12, T14 and T15, the level of dedifferentiation was 50% with a higher development of callus, while 100% dedifferentiated explants were obtained with treatment T13 (1.5 mg l⁻¹ BA and 0.5 mg l⁻¹ NAA) (Table 1).

Shoot-rooting and plantlet formation

Root formation in micropropagated shoots of D. congestiflora occurred in all IBA treatments, with 100% rooting observed at 8 days of culture. At 30 days of cultivation, the number of roots was proportional to the length of these roots; a longer length was observed in ½ MS 0.5 mg l⁻¹ IBA, which produced 5.2 roots/shoot with a root length of 5.7 cm; in addition, roots of 3.6 cm in length were produced in culture medium without auxin (3.2 roots/shoot) (Table 2; Figure 2A). These plantlets showed better growth than that obtained with IBA; the plantlets showed greater height (4.5 cm) with a stem thickness of 2.4 mm and 3–4 branches without leaf loss, contrary to the observations in plantlets grown with IBA.

Transplanting, acclimatization and greenhouse culture

D. congestiflora micropropagated plantlets cultured for 45 days in ½ MS without auxin were transplanted and cultivated in greenhouse conditions for 30 days to determine their survival. At this time, the plants showed a survival of 80% (Figure 2B). After 180 days under greenhouse conditions, D. congestiflora micropropagated plants with optimal growth and development registered 80% survival (Figure 2C).
A high cytokinin/auxin ratio induces shoot regeneration during in vitro culture (Puliamamakkal et al. 2014) and has been critical in shoot induction of different species of *Dalbergia*. Valverde-Cerdas and Alvarado-Guzmán (2004) obtained optimal shoot formation in hypocotyls of *D. retusa* (cocobolo) cultured on ½ MS with 2 mg l⁻¹ BA. Cytokinins plus auxins have shown good results for the induction of shoots of *D. sissoo*. Singh et al. (2002) reported 4.1 shoots/explant for cotyledons in MS medium with 1 mg l⁻¹ BA and 0.05 mg l⁻¹ NAA within 15–20 days, and Chand and Singh (2005) obtained 7.5 shoots per clump of callus after 15 weeks of culture in MS with 2 mg l⁻¹ BA and 0.25 mg l⁻¹ NAA. Additionally, in nodal segments of this species cultured in MS medium supplemented with 1.5 mg l⁻¹ BA and 0.5 mg l⁻¹ IAA, 2.4 shoots/explants were obtained (Bari et al. 2008). The results of the present study showed that 1 mg l⁻¹ BA and 0.1 mg l⁻¹ NAA was the most effective for the regeneration of *D. congestiflora* shoots, which coincides with that described by Singh et al. (2002) for the shoot regeneration of *D. sissoo*.

Callus formation occurs in some explants when grown in BA and NAA combination and has been observed in some *Dalbergia* species. Similar to the response shown in *D. congestiflora* explants, the callus formation during shoot differentiation was observed in cotyledon explants of *D. sissoo* cultured in 5 mg l⁻¹ BA and 0.05 mg l⁻¹ NAA (Singh et al. 2002). Callus induction offers a promising way to study indirect organogenesis to achieve adventitious shoot formation in this species.

The best rooting response in micropropagated shoots of *D. congestiflora* was achieved in a medium reduced in salts without auxin (IBA). Reducing salt and sucrose levels to increase in vitro root induction has proven useful in micropropagated shoots of many leguminous species, and IBA is the most common auxin for the induction of root formation because it is much more potent than IAA or synthetic auxins (Dewir et al. 2016; Bhargavan B, Singh D, Gautam AK, Mishra JS, Kumar A, Goel A, Dixit M, Pandey R, Manickavasagam L, Dwivedi SD, et al. 2012) Medicarpin, a legume phytoalexin, stimulates osteoblast differentiation and promotes peak bone mass achievement in rats: Evidence for estrogen receptor β-mediated osteogenic action of medicarpin. *J Nutr Biochem* 65: 925–928.

Bhargava B, Singh D, Gautam AK, Mishra JS, Kumar A, Goel A, Dixit M, Pandey R, Manickavasagam L, Dwivedi SD, et al. (2012) Medicarpin, a legume phytoalexin, stimulates osteoblast differentiation and promotes peak bone mass achievement in rats: Evidence for estrogen receptor β-mediated osteogenic action of medicarpin. *J Nutr Biochem* 23: 27–38.

Bianchetti RE, de Resende CF, Pacheco VS, Dornellas FF, de Oliveira AMS, Freitas JCE, Peixoto PHP (2017) An improved protocol for in vitro propagation of the medicinal plant *Mimosa pudica* L. *Afri J Biotechnol* 16: 418–428.

Biswas A, Roy M, Miah M, Bhadra S (2005) Plant regeneration from semi-mature zygotic embryos of *Dalbergia sissoo* Roxb. *Indian J Biotechnol* 4: 78–81.

CITES (2017) The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Appendices I and II. Valid from 4 October 2017. https://cites.
micropropagation from axillary buds

Dalbergia congestiflora

Mustafa NS, Rania AT, Hassan SAM, Nagwa SMZ, Mustafa EA

Murashige T, Skoog F (1962) A revised medium for rapid growth

Miles L, Newton AC, Defries RS, May I, Blyth

Ludwig-Müller J (2000) Indole-3-butyric acid in plant growth and

Manikandan G, Thiri-Bhuvaneswari R, Arputha-Ramya

In vitro

Mantovani NC, Grando MF, Xavier A, Otoni WC (2013) In vitro

Dalbergia sissoo

Gulati A, Jaiwal PK (1996) Micropropagation of

In Vitro Cell Dev Plant

Dalbergia congestiflora

Kiondo F, Feyissa T, Ndakidemi PA, Seth M (2014) Overcoming phenolic accumulation of date palm

Physiol Mol Biol Plants

Dalbergia retusa

Singh AK, Chand S, Pattnaik S, Chand PK (2002) Adventitious

Rev Mex Biodivers

Dalbergia sissoo Roxb.

Ricker M, Hernández-Macias HM, Sousa-Sánchez M, Ochoterena H (2013) Tree and tree-like species of Mexico: Asteraceae, Leguminosae, and Rubiaceae. Rev Mex Biodivers 84: 439–470

San José MC, Romero L, Janeiro LV (2012) Effect of indole-3-butyric acid on root formation in Alnus glutinosa microcuttings. Silva Fenn 46: 643–654

Sarasas V, Cripps R, Ramsay MM, Atherton C, Michmich M, Prendergast G, Rowntree JK (2006) Conservation in vitro of threatened plants-progress in the past decade. In Vitro Cell Dev Biol Plant 42: 206–214

SEMARNAT (2010) Norma Oficial Mexicana NOM-059-SEMARNAT-2010, Protección ambiental-Especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-Lista de especies en riesgo. https://www.profepla.gob.mx/innovaportal/ file/435/1/NOM_059_SEMARNAT_2010.pdf (accessed: 4 June 2021).

Siddique I, Bukhari NAW, Perveen K, Siddiqui I (2015) Influence of plant growth regulators on in vitro shoot multiplication and plantlet formation in Cassia angustifolia Vahl. Braz Arch Biol Technol 58: 686–691

Singh AK, Chand S, Pattnaik S, Chand PK (2002) Adventitious shoot organogenesis and plant regeneration from cotyledons of Dalbergia sissoo Roxb., a timber yielding tree legume. Plant Cell Tissue Organ Cult 68: 203–209

Swamy BVR, Himabindu K, Sita GL (1992) In vitro micropropagation of elite rosewood (Dalbergia latifolia Roxb.). Plant Cell Rep 11: 126–131

Trivedi R, Maurya R, Mishra DP (2014) Medicarpin, a legume through axillary shoot proliferation and ex vitro rooting. Physiol Mol Biol Plants 20: 81–87

Pijut PM, Beasley RR, Lawson SS, Palla KJ, Stevens ME, Wang Y (2012) In vitro propagation of tropical hardwood tree species: A Review (2001–2011). Propag Ornamentals Plants 12: 25–5

Pittier H (1922) On the species of Dalbergia of Mexico and Central America. J Wash Acad Sci 12: 54–64

Prakash E, Khan PSSV, Rao TJVS, Meru ES (2006) Micropropagation of red sanders (Pterocarpus santalinus L.) using mature nodal explants. J For Res 11: 329–335

Pulianmacal AJ, Kareem AV, Durugprasad K, Trivedi ZB, Prasad K (2014) Competence and regulatory interactions during regeneration in plants. Front Plant Sci 5: 142

Rajanna LN, Sharanabassappa G, Seetharam YN, Aravind B, Mallikharjuna PB (2011) In vitro regeneration of cotyledonal node explant of Bauhinia racemosa. Bot Res Int 4: 75–80

Rathore JS, Rathore MS, Singh M, Singh RP, Shekhawat NS (2007) Micropropagation of mature tree of Citrus limon. Indian J Biotech 6: 239–244

Rehman HM, Rana IA, Ijaz S, Mustafa G, Joyta FA, Khan IA, Pijut PM (2012) In vitro regeneration of Dalbergia sissoo Roxb. and the potential for genetic transformation. Nat Bot. Horti Agrobot Chaj-Napoca 40: 140–147

Copyright © 2021 Japanese Society for Plant Biotechnology