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Indian kino tree (*Pterocarpus marsupium*): propagation, micropropagation, and biotechnology

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Abstract

The Indian kino tree (*Pterocarpus marsupium* Roxb., Fabaceae) is listed in the IUCN red data list as a direct result of the excessive exploitation of its wood. Biotechnology has provided feasible and effective solutions for the tissue culture and mass micropropagation of *P. marsupium*, thus serving as a means to conserve important germplasm. The synthesis of information in this review aims to stimulate further research on *P. marsupium*. Breeding and biotechnological programmes that mass produce and effectively manage *P. marsupium* germplasm *in vitro* are required, using synthetic seed technology, cryopreservation and *in situ* conservation to manage this important wood germplasm. Molecular markers have been used to a limited extent to confirm the genetic stability of *in vitro*-propagated material. Biotechnological advances for this leguminous tree of commercial importance would benefit from research involving photoautotrophic micropropagation for improved rooting, bioreactors for the production of somatic embryos and secondary metabolites, thin cell layers for enhanced micropropagation, and cryoconservation including of synthetic seeds.

Key words: conservation, endangered species, *in vitro* conservation, IUCN red data list, Leguminoseae, medicinal plant, micropropagation, *Pterocarpus marsupium*, somatic embryogenesis.
Abbreviations: BA, 6-benzyladenine; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; MS, Murashige and Skoog; NAA, 1-napthaleneacetic acid.

Introduction

Pterocarpus (Leguminosae) is represented by 66 species globally (The Plant List 2018) that have high economic, medicinal and traditional value. Indian kino tree (*Pterocarpus marsupium* Roxb.), also known as Malabar kino or bijasal, is highly valued as a timber tree and for its pharmaceutically important gum kino (Badkhane et al. 2010; Mishra et al. 2013). It is a native plant of India, Nepal and Sri Lanka (Badkhane et al. 2010). *P. marsupium* is distributed in central, western and southern regions of India (Devgun et al. 2009).

Improtance

The heartwood of *P. marsupium* is an important source of pterostilbene (*trans*-3,5-dimethoxy-4-hydroxystilbene) (Mathew et al. 1977; Adinarayana, Syamasundar 1982; Dama et al. 1982; Manickam et al. 1997; Grover et al. 2005; Gupta, Gupta 2009; 2010; Chakraborty et al. 2010; Gupta, Gupta 2011; Joshi et al. 2012; Mohankumar et al. 2012). Pterostilbene has various clinical applications (Estrela et al. 2013; McCormack, McFadden 2013). In addition to pterostilbene, other secondary metabolites such as epicatechin (Adinarayana, Syamasundar 1982; Sheehan et al. 1983; Chakravarthy, Gode 1985), pterocarpol (Mathew et al. 1984; Mathew, Rao 1984; Rao et al. 1984; Maurya et al. 1985), pterosupin (Dama et al. 1982; Jahromi, Ray 1993; Manickam et al. 1997), pterocarposide (Handa et al. 2000; Maurya et al. 2004; Achari et al. 2012; Mishra et al. 2013) and marsuposides have also been discovered (Grover et al. 2004; Maurya et al. 2004; Gupta, Gupta 2009; Joshi et al. 2012; Mishra et al. 2013). Conventional uses, phytochemical extraction techniques, chemical constituents, pharmacological activity and commercial significance of *P. marsupium* have already been reviewed (Devgun et al. 2009; Badkhane et al. 2010; Hari, Gaikwad 2011), but no systematic review exists on the *in vitro* biotechnology of this species. *P. marsupium* is listed as a vulnerable plant in the IUCN red data list (IUCN 2017). In this review, we highlight how biotechnology, particularly *in vitro* technologies, can be used to preserve and sustainably multiply important germplasm, thus serving as a valuable tool for conservation purposes.

Sexual and vegetative propagation

One of the simplest ways to mass propagate *P. marsupium* is via seed, although this can lead to a genetically heterogeneous population with variable growth characteristsics, which might not be a desired outcome for forestry or secondary metabolite production, which would preferably require clonal, and thus genettically uniform, material. Mishra et al. (2014) showed that seedlings of *P. marsupium* that emerged from large seeds (16 to 17 mm) showed better growth and higher biomass than seedlings derived from medium-sized (13 to 15 mm) and small (10 to 12 mm) seeds. Patel and Patel (2016) reported 96.7% seed germination of *P. marsupium* seeds on Murashige and Skoog (1962) medium when seeds were inoculated horizontally, and 90% of seedlings germinated *in vitro* survived in natural conditions when grown in a substrate of soil and manure (1:1). To provide elite germplasm for desired traits, especially phytochemicals or the wavy nature of the grain, vegetative propagation is desirable, and *in vitro* propagation allows for the production of true-to-type plants via micropropagation such as axillary shoot multiplication or shoot tip culture at a large scale, or somatic emberyogenesis, making plantlets with uniform characteristics continuously available. *In vitro* propagation also allows, in tree biotechnology, for the improvement of desired characteristics such as pathogen resistance or improved wood quality via genetic engineering (Rai, Shekhawat 2014), and *in vitro* propagation is frequently used for the large-scale propagation and conservation of various plants (Kher et al. 2016; Nataraj et al. 2016; Bi et al. 2017; Sanatombi, Sanatombi 2017; Teixeira da Silva et al. 2017). The next section provides an assessment of the progress made on the *in vitro* propagation of *P. marsupium*.

In vitro propagation and tissue culture

In vitro propagation, which has various advantages over vegetative or seed propagation and established micropropagation protocols, can be useful for various biotechnological applications but requires controlled environmental culture conditions. Thus, at each stage of *in vitro* propagation, effective, reproducible and cost-effective methods need to be standardized from cuture initiation to rooting and acclimatization to ensure viable plant production.

Explants

The source of explants, i.e., the mother plant, is a key initial requirement to initiate an *in vitro* propagation protocol. Surface sterilization is used to remove microbes from the surface of explants and thus it is essential to standardize the type of disinfectant, its concentration and the duration of sterilization to avoid infection by endophytic microorganisms that may reappear (Leifert et al. 1994). Information about the explants used for the *in vitro* propagation of *P. marsupium*, as well as surface sterilization protocols, are summarised in Table 1. In general, explants are derived from either seedlings or mature plants (Das, Chatterjee 1993; Chand, Singh 2004; Tiwari et al. 2004; Anis et al. 2005; Husain et al. 2007; 2008; 2010; Tippani et al. 2013a; 2013b).

Basal medium composition and culture conditions

The most prefered basal medium for *in vitro* studies on *P. marsupium* is Murashige and Skoog (1962; MS) medium (Table 2). Chand and Singh (2004) noted that 0.8% agar in a water-based medium was sufficient for germination of *P. marsupium* seed while half-strength MS medium without plant growth regulators was used for *in vitro* seed germination of *P. marsupium* (Husain et al. 2007, 2008, 2010).

In vitro *propagation from predetermined meristems*

Three primary predetermined meristems have been employed in *P. marsupium* tissue culture: shoot tips, cotyledonary nodes and nodes from mature trees. Cotyldenory nodes were sucessfully applied for the *in vitro* propagation of *P. marsupium* (Chand, Singh 2004; Anis et al. 2005; Husain et al. 2007, 2008), most frequently employing 6-benzyladenine (BA), either alone or in combination with other cytokinins or auxins (Table 2).

In vitro *propagation (callogenesis, regeneration and somatic embryogenesis)*

Regeneration from immature *P. marsupium* zygotic embryos was possible on MS medium supplemented with 13.32 µM BA and 2.85 µM indole-3-acetic acid (IAA) (Tippani et al. 2013a). In another study by the same group (Tippani et al. 2013b), when immature cotyledons from nine-day old *in vitro* raised seedlings of *P. marsupium* were cultured on MS medium containing 1-napthaleneacetic acid (NAA), callus formed. When callus was subcultured onto MS medium containing BA and NAA, shoots developed from callus. Only one report is available on somatic embryogenesis of *P. marsupium* induced from hypocotyl segments from 12-day old i*n vitro* seedlings (Husain et al. 2010; Table 2). Somatic embryogenesis is a useful method for obtaining clonal material that can serve as useful propagules for synthetic seed production, bioreactors and cryopreservation, especially for forestry species (Teixeira da Silva, Malabadi 2012).

Rooting and acclimatization

Sucessful roooting of *in vitro* raised plants followed by effective acclimatization and successful transfer of *in vitro* propagated plants to field conditions is the final objective of any micropopagation protocol and care is needed to avoid hyperhydricity in *in vitro*-raised plants, which tend to display poor rooting efficiency (Ruffoni, Savona

Table 1. Explant source, size and surface sterilization procedures for preparation of tissue culture studies of *Pterocarpus marsupium* (chronological listing). No new tissue culture studies have been published after 2015. d, day(s); DW, distilled water; DDW, double distilled water; EtOH, ethyl alcohol (ethanol); $HgCl_2$, mercuric chloride; IZE, immature zygotic embryo; NaOCl, sodium hypochlorite; NR, not reported in the study; RTW, running tap water; s, second(s); SDW, sterilized (by autoclaving) distilled water; SW, sterilized water; y, year(s); TW, tap water

Explant source	Explant type, size and density; culture vessel	Surface sterilization and preparation	Reference
Seeds from the wild → seedlings. Age of mother plant NR.	Cotyledonary node segments (size NR) from 20-d old seedlings. Test tubes/flasks (1 explant/tube).	Pods in DW 24. Seeds: DW 3–4× → 0.1% Tween-20 15 min → 0.06% Savlon 15 min → DW 3–4× → 0.1% $HgCl_2$ 20 min → 70% EtOH 1 min → 4–5× SDW	Chand, Singh 2004
Seeds → *in vitro* seedlings (4–5 cm tall, 35–40-d old).	Nodal segments (1.5–2 cm).	Seeds → seed coat removed mechanically → soaked in SW overnight → 10% NaOCl 10 min → 3× SDW	Tiwari et al. 2004
Seeds → *in vitro* seedlings (5 cm tall, 18-d old).	Seedling-derived cotyledonary nodes, cotyledons, nodal segments, shoot tips (size NR for all explants). Borosil test tubes (1 explant/tube).	Peeled seeds: RTW 30 min → DW 24 h → 5% Teepol (detergent) 5 min → thorough wash in NR → 0.1% $HgCl_2$ 5 min → 4–5× SDW	Anis et al. 2005
Seeds → *in vitro* seedlings (6-, 12-, 18- and 24-d old).	Seedling-derived cotyledonary nodes of 18-d-old seedlings (1–2 cm) (2007; 2008). Hypocotyl segments (0.5 cm) from 12-d-old seedlings 2–4 cm tall (2010). 100-mL Borosil test tubes (1 explant/tube).	Peeled seeds: RTW 30 min → 5% Teepol 10 min → 1% Bavistin (fungicide) 15 min → DW 24 h → 70% EtOH 30 s → 0.1% $HgCl_2$ 5–6 min → 5–6× SDW	Husain et al. 2007; 2008; 2010
Seeds → *in vitro* seedlings (6-, 12-, 18- and 24-d old).	Seedling-derived cotyledonary nodes.	Green pods: RTW 20 min → 1% Laboline 10 min → Tween-20 (conc. NR) 4 min → TW → 0.1% $HgCl_2$ 15 min → 5–6× SDW	Porika et al. 2009
IZEs from seeds in green pods 4–9 w after pollination.	10 × 8.5 cm bottles used.	Pods: RTW 15 min → 2% Teepol 10 min → 5% Tween-20 4 min → 0.1% $HgCl_2$ 6 min → 4–5× SDW	Tippani et al. 2013a
IZEs from seeds in green pods from 30-y-old tree.	9 d after IZE culture, immature cotyledons cut and used as explants. Culture vessels and explant density NR.	Pods: RTW 15 min → 5% Teepol gentle shaking 8 min → 3–4× SDW → 0.1% $HgCl_2$ 8 min → repeated washes SDW. IZEs excised and plated.	Tippani et al. 2013b
Node from 10-y old tree	1 node /100 mL flask (based on photos).	NR in 2013 paper. 2015 paper: Tween-80 5 min → DW time NR → 0.1% Bavistin + 0.05% streptomycin 7 min → DW time NR → 0.1% $HgCl_2$ 7 min → 3–4× SDW	Jaiswal et al. 2013; 2015

2013). Rooting and acclimatization protocols for *in vitro* raised shoots of *Pterocarpus* species are summarized in Table 2. Only a few studies quantified the survival of micropropagated *P. marsupium* plants (Chand, Singh 2004; Husain et al. 2007; 2008; 2010; Tippani et al. 2013a; 2013b). The most effective auxin reported for rooting was indole-3-butyric acid (IBA). Husain et al. (2008; 2007) reported the importance of phloroglucinol – an auxin-like rooting-inducing compound (Teixeira da Silva et al. 2013) – in combination with IBA, for the rooting of *P. marsupium* shoots. Tippani et al. (2013a) pulsed *P. marsupium* shoots first in a solution of IBA for 24 h to induce *in vitro* roots using the same protocol to root putatively transgenic shoots (Tippani et al. 2013b).

Genetic transformation

Only a single report is available on the transient genetic transformation of *P. marsupium* by *Agrobacterium tumefaciens* (Tippani et al. 2013b). Callus was transformed with a hygromycin phosphotransferase (*hpt*) gene (selectable marker) and intron-interrupted uidA (GUS) gene as the reporter gene under the control of the CaMV-35S promoter.

Molecular marker for clonal fiedility and phylogenetic relationships

Molecular markers are useful tools for detecting somaclonal

Table 2. In vitro conditions for tissue culture studies of *Pterocarpus marsupium* (chronological listing). AA, ascorbic acid; ABA, abscisic acid; AdS, adenine sulphate; AS, acetosyringone; AmS, ammomium sulphate; B5 medium, or Gamborg medium (Gamborg et al. 1968); BA, N_6-benzyladenine (BA) is used throughout even though BAP (6-benzylamino purine) may have been used in the original, according to Teixeira da Silva (2012b); cef, cefotaxime; CA, citric acid; CIM, callus induction medium; CWFT, cool white fluorescent tubes; d, day(s); FYM, farmyard manure; hyg, hygromycin; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; ISSR, inter-simple sequence repeat; IZE, immature zygotic embryo; Kn, kinetin (6-furfuryl aminopurine); MS, Murashige and Skoog, (1962) medium; NAA, α-naphthaleneacetic acid; NR, not reported in the study; PG, phloroglucinol; PGR, plant growth regulator; PP, photoperiod; RH, relative humidity; RIM, root induction medium; SE, somatic embryo; SEIM, somatic embryo induction medium; SEMM, somatic embryo multiplication medium; SEM, shoot elongation medium; SG, seed germination; SIM, shoot induction medium; SMM, shoot multiplication medium; w, week(s); WPM, woody plant medium (Lloyd and McCown, 1980). * The original light intensity reported in each study has been represented since the conversion of lux to μmol m^{-2} s^{-1} is different for different illumination (main ones represented): for fluorescent lamps, 1 μmol m^{-2} s^{-1} = 80 lux; the sun, 1 μmol m^{-2} s^{-1} = 55.6 lux; high voltage sodium lamp, 1 μmol m^{-2} s^{-1} = 71.4 lux (Thimijan, Heins 1983). ** Even though calli was used in the original, the term callus has been used here based on recommendation of Teixeira da Silva (2012c). ¶ Claims of somatic embryogenesis without sufficient proof (cytological, histological, genetic), i.e., only photos of macromorphology

Culture medium, PGRs, additives, subcultures	Culture conditions *	Experimental outcome, maximum productivity, acclimatization and variation	Reference
0.8% agar (SG). MS + 4.44 μM BA + 0.26 μM NAA (SIM). ½MS + 9.84 μM IBA (RIM). pH 5.8. 2% sucrose. 0.8% agar.	16-h PP. CWFT. 40 μmol m^{-2} s^{-1}. 24 ± 2 °C. 55–65% RH.	85% of cotyledonary nodes formed shoots (9.5/explant) after 15 w. About 30% of shoots formed roots after 25 d. Acclimatization in autoclaved sand + peat moss + compost (1:1:1) with 52% survival.	Chand, Singh 2004
PGR-free MS (SG, SIM). MS + 13.31 μM BA + 2.69 μM NAA (SEM). pH 5.8. 3% sucrose. 0.8% agar.	8-h PP. Light source NR. 55 μmol m^{-2} s^{-1}. 25 ± 2 °C. RH NR.	Range of SG% (61–100%), maximum on MS, lowest on White's (1963) basal medium and intermediate performance on B5 (78–80%). Although 3.25 shoots/node formed in PGR-free MS, MS + 0.05 μM IBA induced most nodes/shoot (4.95) and longest shoots (2.92 cm). Rooting was not described but successful (>68%) acclimatization was claimed.	Tiwari et al. 2004
½MS (SG). MS + 5 μM BA (SIM). MS + 5 μM BA + 0.25 μM IAA (SEM). Pulse in 200 μM IBA + phenolic acid 5 → ½MS + 0.5 μM IBA (RIM). Subcultures NR. pH 5.8. 3% sucrose. 0.8% agar.	16-h PP. CWFT. ~1200 lux. 25 ± 2 °C. 55–60% RH.	% SG NR. 7.8 shoots/cotyledonary node. 40–50% of shoots rooted. Acclimatized plants shown, but details NR.	Anis et al. 2005
PGR-free ½MS (SG). MS + 0.4 μM TDZ (SIM). Subculture every 21 d. MS + 5 μM BA (SEM). Pulse in 200 μM IBA 4 d paper bridge → ½MS + 0.2 μM IBA + 0.96 μM PG + 2% sucrose (RIM). pH 5.8. 3% sucrose. 0.7% agar.	16-h PP. CWFT. 50 μmol m^{-2} s^{-1}. 25 ± 2 °C. 50–60% RH.	80% SG in 4–5 d. 15.2 shoots/cotyledonary node in 90% of explants. 65% of shoots rooted (chlorogenic acid and salicylic acid were not as effective as PG). 70% survival after acclimatization in autoclaved soil + Soilrite® (1:1) at same conditions as *in vitro* plants for 2 months.	Husain et al. 2007
PGR-free ½MS (SG). MS + 4 μM BA + 0.5 μM IAA + 20 μM AdS (SIM). Subculture every 21 d. MS + 5 μM BA (SEM). Pulse in 100 μM IBA + 15.84 μM PG 7 d → ½MS + 2% sucrose (RIM). pH 5.8. 3% sucrose. 0.7% agar.	16-h PP. CWFT. 50 μmol m^{-2} s^{-1}. 25 ± 2 °C. 55–65% RH.	% SG NR. 8.6 shoots/cotyledonary node in 85% of explants. MS formed more shoots than on B5 or WPM. 70% of shoots rooted. 75% survival after acclimatization in Soilrite® and watered with ¼MS.	Husain et al. 2008
MS + 4.44 μM BA (SIM). ½MS + 49 μM IBA (RIM). pH 5.8. 3% sucrose. 0.8% agar.	16-h PP. CWFT. 40–60 μmol m^{-2} s^{-1}. 25 ± 2 °C. RH NR.	SG not an objective of the study, so NR. 12.9 shoots/immature seed formed on SIM (3.8 cm long). 68% of shoots rooted in RIM (2.1 roots/shoot). 75% plantlets survival after acclimatization in sterilized soil + vermiculite (1:1)	Porika et al. 2009

continued

Table 2. *continued*

Culture medium, PGRs, additives, subcultures	Culture conditions *	Experimental outcome, maximum productivity, acclimatization and variation	Reference
PGR-free ½MS (SG). MS + 5 µM 2,4-D + 1 µM BA (CIM). MS + 2 µM BA (SEIM). MS + 0.5 µM BA + 0.1 µM NAA + 10 µM ABA (SEMM). ½MS + 1 µM BA (SE germination). pH 5.8. 3% sucrose. 0.7% agar.	16-h PP. CWFT. 50 µmol m^{-2} s^{-1}. 25 ± 2 °C. 50–60% RH.	90% of hypocotyls formed callus. 23 globular stage SEs/callus clump after 4 w. 60% survival after acclimatization in Soilrite® and watered with ¼MS.	Husain et al. 2010**¶
MS + 13.32 µM BA + 2.85 µM IAA (SIM). MS + 4.44 µM BA (SEM). Subcultures every 3 w. 14.66 µM IBA 24 h → ½MS (RIM). pH 5.8. 3% sucrose. 0.8% agar.	16-h PP. CWFT. 10–15 µmol m^{-2} s^{-1}. 25 ± 2 °C. 50-60% RH.	93.8% of IZEs formed 17.3 shoots/IZE, or 27.2 shoots/IZE after subculture. BA induced more shoots than Kin. 70.8% of shoots rooted after 4 w. 74% of plantlets survived after acclimatization in sterilized soil + vermiculite (1:1), irrigated with 1/10 dilution of liquid MS, hardened, then transferred to garden soil + sand (1:1). ISSR was used to confirm genetic stability of *in vitro*-derived plantlets relative to mother plants and acclimatized plants.	Tippani et al. 2013a
Untransformed tissue: MS + 0.2–0.9 µM BA (IZE germination). MS + 1.07 µM NAA (CIM). MS + 8.9 µM BA + 1.07 µM NAA (SIM). MS + 4.4 µM BA (SEM). Subcultured every 2–3 w for CIM and every 4 w for SIM/SEM. 19.6 µM IBA 24 h → ½MS + 2.85 µM IBA (RIM). Transformed tissue: MS + 8.9 µM BA + 1.07 µM NAA + 200 µM AS 2 d → MS + 8.9 µM BA + 1.07 µM NAA + 20 mg/L hyg + 250 mg/L cef (SIM). MS + 4.4 µM BA (SEM) then MS + 4.4 µM BA + 15 mg/L hyg + 200 mg/L cef (SEM). Subcultured every 15 d for SIM and every 3 w for SEM. 19.6 µM IBA 24 h → ½MS + 2.85 µM IBA + 20 mg/L hyg (RIM). pH 5.8. 3% sucrose. 0.8% agar.	16-h PP. CWFT. 65 µE m^{-2} s^{-1}. 25 ± 2 °C. RH NR.	Callus formed in 2 w from cotyledons derived from seedlings (*in vitro* germinated IZEs). 60.4% of cotyledons formed callus and 12.2 shoots/callus cluster after 4 w. 75% of shoots rooted after 4 w, forming 4.5 roots/shoot. 95% of plantlets survived after acclimatization in vermiculite + perlite (1:1), irrigated with ½MS. 20.9% transformation efficiency claimed (see text for more details of the transformation experiments).	Tippani et al. 2013b**
MS + Kn (conc. NR) + additives (568 µM AA + 260 µM CA + 605 AmS + 217 µM AdS), pH 5.8. 0.8% agar. Carbon source NR.	16-h PP. CWFT. 1600 lux. 26 ± 2 °C.	About 6 shoots 1.5 cm long obtained from single nodes. All shoots were healthy without any necrosis. Acclimatization NR.	Jaiswal et al. 2013
MS + 13.95 µM Kn + additives (Jaiswal et al. 2013) (SIM). MS + 9.3 µM Kn + 0.54 µM NAA + additives (Jaiswal et al. 2013) (SMM). MS + 4.92 µM IBA (RIM). pH 5.8. 0.8% agar. 3% sucrose.	16-h PP. CWFT. 1600 lux. 26 ± 2 °C.	In SIM, 2.51 shoots/node (1.1–1.9 shoots/node when BA was used) in 64.4% of nodes. In SMM, 5.0 shoots/node. 42.2% of shoots rooted. Acclimatization in sand, soil and FYM (1:1:1).	Jaiswal et al. 2015

variation in *in vitro* culture and have many applications, including the definition of phylogenetic relationships, construction of genetic linkage maps, QTL identification, genome mapping, association mapping and linkage disequilibrium, marker-assisted breeding and genome-wide marker-assisted selection, genetic diversity analysis for crop breeding, comparative genomics and taxonomic classification (Cloutier, Landry 1994; Schlötterer 2004; Teixeira da Silva et al. 2007a; Kalia et al. 2011; Hayward et al. 2015). Despite these strengths, most of these issues have not yet been explored in *Pterocarpus*. Tippani et al. (2013a) used ISSR markers to detect variation in plants raised from immature *P. marsupium* zygotic embryos. Saslis-Lagoudakis et al. (2011) used plastid regions *rbcL* and *matK*, as barcodes for two *Pterocarpus* species, and *ndhF-rpL32* as a plastid marker, as an intergenic spacer to scan the plastid genome; amplified *nrITS2* and *trnL*-F intergenic spacers were useful to elucidate phylogenetic relationships.

Genetic differences in *P. officinalis* populations between island and continental populations were discovered using AFLP markers (Rivera-Ocasio et al. 2002; 2006). Muller et al. (2006) identified chloroplast and nuclear microsatellite markers for *P. officinalis*, which they then used to study genetic diversity and gene flow. Molecular markers have a solid base of use for select *Pterocarpus* species, and can thus serve as a platform for further applied molecular studies.

Conclusions and future perspectives

This review highlights the key advances in the tissue culture-based biotechnology of *P. marsupium*. To date, effective protocols for seed surface disinfection and *in vitro* germination exist. There are also effective protocols for direct shoot regeneration from a range of explants, or through callus induction. Rooting and acclimatization protocols are also well established. Only a single report on somatic embryogenesis – itself without sufficient proof that the structures obtained were in fact somatic embryos – indicates that this area of tissue culture still needs much improvement.

Even though only a single genetic transformation study exists for *P. marsupium*, a reliable and reproducible *in vitro* culture protocol will assist researchers in seeking transgenic strategies to fortify *P. marsupium* germplasm against abiotic and biotic stresses, induce more rapid growth, or resistance to pests and diseases. To fortify current tissue culture efforts, several other strategies can, and should, be attempted: photoautotrophic micropropagation (Xiao et al. 2010), bioreactors for large-scale production of somatic embryos and enhancement of secondary metabolites (Ziv 2005; Sharma, Sharma 2009; Huang, McDonald 2012), thin cell layers to enhance micropropagation and quantitative organogenesis (Nhut et al. 2003; Teixeira da Silva 2003; Teixeira da Silva et al. 2007b; Teixeira da Silva 2012a; Teixeira da Silva, Dobránszki 2013; 2014). The ability to stably produce units that would allow for germplasm conservation would then stimulate the need for cryoconservation (Berjak et al. 2011; Benelli et al. 2013; Kulus, Zalewska 2014; Teixeira da Silva et al. 2015; Bi et al. 2017), including through the application of synthetic seeds (Rai et al. 2009; Sharma et al. 2013).

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Authors' contribution and conflicts of interest statement

All authors contributed equally to all aspects of review development and writing. The authors have no conflicts of interest to declare.

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