Red sandalwood (*Pterocarpus santalinus* L. f.): biology, importance, propagation and micropropagation

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**Abstract** *Pterocarpus santalinus* L. f. (Fabaceae; red sanders) is prized for its wood whose colour and fragrance is due to the presence of santalins that have pharmaceutical and industrial uses. Red sanders is listed as an endangered plant species on the IUCN red data list as a result of the exploitation of its wood and essential oil. This review emphasizes the pollination biology, seed germination, vegetative propagation and micropropagation of *P. santalinus*. Excessive use of *P. santalinus* has also caused the emergence of various adulterants, so accurate identification is essential.

**Keywords** Conservation · Fabaceae · IUCN red data list · Medicinal plant · Micropropagation · Red sanders

**Historical, cultural, medicinal, and economic importance of red sanders as a basis for conservation**

*Pterocarpus santalinus* L. f. (Fabaceae) is most commonly known as red sandalwood in English, but it also has other common names in several languages (Table S1). *Pterocarpus* is derived from the Greek words *pteron* (wing) and *karpos* (fruit), referring to the winged pod, while *santalinus* originates from the Latin *sandal* and *inus* (meaning similar to), i.e., a plant with characteristics similar to Indian sandalwood, *Santalum album* L. (Botanical Survey of India 2012). Like African or Nepalese sandalwood (Teixeira da Silva et al. 2016a) and Indian sandalwood (Teixeira da Silva et al. 2016b), *P. santalinus* is also prized for its hard, dark-purple, bitter heartwood (Navada and Vittal 2014). In India, the natural range of *P. santalinus* used to be a very restricted area of 15,540 km² in the southeast (Sarma 1993). Currently, *P. santalinus* is found exclusively in a well-defined forest tract of Andhra Pradesh in Southern India (Raju and Nagaraju 1999; Prakash et al. 2006; Balaraju et al. 2011), but is also found in the Chinese provinces of Yunnan, Guangdong and Guangxi, and on Hainan Island, where it is referred to as zitan (Kaner et al. 2013).

The colour and fragrance of *P. santalinus* heartwood are derived from santalins while the pleasant aroma is caused by the presence of terpenoids (Kumar et al. 1974). A dye prepared from the heartwood of *P. santalinus* is used as a stain in light microscopy (Banerjee and Mukherjee 1981; Sen Gupta and Mukherjee 1981), as a coloring agent in pharmaceutical preparations, in food, leather and textile industries (Ankalaiah...
et al. 2017), and as a textile dye (Gulrajani et al. 2002). The medicinal properties of *P. santalinus* have been extensively reviewed elsewhere (Navada and Vittal 2014; Azamthulla et al. 2015) and will not be covered in this review. However, multiple uses (Table S2), ethnomedicinal uses (Table S3), and phytochemistry (Table S4) have been provided as supplementary tables to offer a more rounded appreciation of this tree in the context of this review.

The texture and colour differentiate good quality from poor quality trees, with “wavy grain wood texture with intense red color” in the former and “straight grain wood texture with light red color” in the latter (Prakash et al. 2006), and it is the superior quality of *P. santalinus* that makes it popular in the furniture industry (Prakash et al. 2006; Arunkumar et al. 2011; Arunkumar and Joshi 2014; Azamthulla et al. 2015). In Japan, *P. santalinus* is used to make carvings and musical instruments, *shamisen* and *koto* (Kukrety et al. 2013b; Arunkumar and Joshi 2014; Azamthulla et al. 2015; Ramabrahmam and Sujatha 2016), as well as name seals or *hankos*. In Buddhism, *P. santalinus* is considered to be a symbol of holiness, and is thus used for carved statues, as a constituent of incense (Wu et al. 2011), and for cremation (Ramakrishna 1962). In China, *P. santalinus* wood has a long history of use in furniture and other valuable wood products (Berliner 1996; Kaner et al. 2013).

The export of *P. santalinus* from India to Europe started in the 17th century, mainly for fabric dyeing (Vedavathy 2004). The Herbal Folklore Research Centre in Tirupati, India, estimated that from 500 planted trees ha⁻¹, at least 500 kg of heartwood per tree can be obtained after 25 years, thus 25 t ha⁻¹ of wood plantation (Vedavathy 2004). At 2004 prices of Rs. 75 kg⁻¹, such a plantation would yield a return of Rs. 177.5 lakhs ha⁻¹ (US$375,000 ha⁻¹) (Vedavathy 2004). Current market prices are, however, unknown to the authors, although prices are likely to be high since natural *P. santalinus* stands have been in decline as a result of this overexploitation for commercial purposes, earning it an endangered status since 1997 (IUCN 2018).

This review provides an overview of the reproductive biology, seed germination and micropropagation of *P. santalinus* as tools for its conservation and large-scale propagation.

**Basic flowering biology, and sexual and vegetative reproduction**

**Pollination and seedset**

The *P. santalinus* tree flowers in the dry season (Rao et al. 2001; Rao and Raju 2002). The flowers are papilionaceous, bisexual, large and and yellow (Rao and Raju 2002). Flowering is discontinuous, blooming at intervals of 2–5 d (Rao et al. 2001). Flowers open at night and the primary pollinators are *Apis dorsata*, *A. cerana indica* and *A. florea* (Rao and Raju 2002). *P. santalinus*, which shows facultative xenogamy, tends to eliminate growing fruits from self-pollinated flowers, i.e., there is large-scale abortion of flower buds, flowers and fruit (Rao et al. 2001), and has very low fruit set (< 6%), 52% of which set seed (Rao and Raju 2002).

**Seed germination**

Traditional seed propagation of *P. santalinus* yields low germination percentages due to a hard testa, poor viability, and sensitivity to temperature (Kumar and Gopal 1975; Dayanand and Lohidas 1988; Anuradha and Pullaiah 1998; Naidu 2001a, b; Naidu and Rajendrudu 2001). Dried, soaked and scarified *P. santalinus* pods resulted in 49% germination (Kumarasinghe et al. 2003) although seed germination in natural stands or under artificial propagation is generally low (< 30%) (Kumar and Gopal 1975; Dayanand and Lohidas 1988; Kalamuthu and Lakshmanan 1995; Naidu 2001a, b; Naidu and Rajendrudu 2001). Alternate wetting and drying every 48 h enhanced germination, reaching 73% (Vijayalakshmi and Renganayaki 2017). Seed germination, seedling height, and root collar diameter were all significantly stimulated by fire (Kukrety et al. 2013b). Pre-soaking *P. santalinus* pods with 500 mg/L gibberellic acid for 24 h resulted in 66.7% seed germination, as well as improved plant growth and seedling survival relative to other treatments with tap water, lake warm water, gibberellic acid, H₂SO₄ or HCl (Patel et al. 2018).

**Vegetative propagation**

Vegetative propagation of *P. santalinus* by semi-hardwood cuttings, cleft grafting, or air layering is not able to produce stock numbers required for effective preservation or for commercial purposes (Kedharnath et al. 1976; Kesava Reddy and Srivasuki 1990). Relative to seed germination, there are almost no studies on ex vitro vegetative propagation for red sanders. However, to provide elite germplasm with desired traits, such as the wavy grain or phytochemicals such as santalins, vegetative propagation under controlled conditions 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, to continuously produce plantlets with uniform characteristics. In tree biotechnology, such as for Indian sandalwood (Teixeira da Silva et al. 2016b), in vitro propagation also allows for the improvement of desired characteristics such as pathogen resistance or improved wood quality by genetic engineering. The next section assesses the progress of micropropagation of *P. santalinus*. 
Micropropagation

Explants

The explant source (i.e., mother plant) and the procedure to surface disinfect explants are important aspects underlying the success of a tissue culture protocol (Leifert et al. 1994; Teixeira da Silva et al. 2016c). Information about the explants used for the in vitro propagation of *P. santalinus*, as well as surface disinfection protocols, are summarised in Table 1. *P. santalinus* seedlings derived from in vitro seed germination have been a popular source of explants, while in vitro germinated seedlings, shoot tips, cotyledons, hypocotyls, mesocotyl and nodes have also served as popular sources of explants for culture initiation since they do not require surface disinfection (Lakshmi Sita et al. 1992; Anuradha and Pullaiah 1999a,b; Chaturani et al. 2005; Rajeswari and Paliwal 2008; Balaraju et al. 2011; Vipranarayana et al. 2012; Warakagoda and Subasinghe 2013). In terms of ex vitro sources of explants, Prakash et al. (2006) used young terminal shoot cuttings collected from mature trees in winter as the explant; Ashrafee et al. (2014) used leaf segments from 1 to 2 year old plants while Sarita et al. (1988) used nodes and terminal cuttings.

Basal medium

The most commonly used and preferred basal medium for in vitro studies on *P. santalinus* is Murashige and Skoog (1962) (MS) medium (Table 2). Lakshmi Sita et al. (1992) used Gamborg’s B5 medium (Gamborg et al. 1968) with 2% sucrose and 0.8% agar to multiply axillary shoots from shoot tips derived from seedlings germinated in vitro. Chaturani et al. (2006) used Anderson medium (Anderson 1980) and *Vitis* medium (Chee and Pool 1987) to germinate seed. Anuradha and Pullaiah (1999a) employed half-strength B5 medium supplemented with 0.05% activated charcoal to germinate seeds in vitro. Then they used B5 medium with 8.88 μM 6-benzyladenine (BA) for shoot tip culture.

In vitro propagation from predetermined meristems

Three predetermined meristems were employed in *P. santalinus* tissue culture: shoot tips, cotyledonary nodes and nodes from mature trees (Table 2). Shoot tips from in vitro germinated seedlings were used by Lakshmi Sita et al. (1992), Anuradha and Pullaiah (1999a), and Balaraju et al. (2011) for shoot tip culture, with either BA as the most effective cytokinin (Lakshmi Sita et al. 1992; Anuradha and Pullaiah 1999a) or a combination of BA and thidiazuron (Balaraju et al. 2011). Cotyledonary nodes were successfully applied for the in vitro propagation of *P. santalinus* (Anuradha and Pullaiah 1999a; Arockiasamy et al. 2000; Rajeswari and Paliwal 2008; Warakagoda and Subasinghe 2013). BA, alone or combined with other cytokinins or auxins, has frequently been utilized for the micropropagation of *P. santalinus* (Table 2). Prakash et al. (2006) cultured nodes of mature trees directly on filter paper bridges employing liquid MS medium containing 1.16–9.30 μM kinetin or 1.11–8.88 μM BA, as well as an antioxidant, observing that 4.44 μM BA was optimum for bud break and shoot multiplication.

In vitro propagation (calllogenesis, regeneration and somatic embryogenesis)

Leaf, cotyledon, root, internode and nodal segments (presumably with axillary buds) from in vitro *P. santalinus* seedlings formed callus, but shoot regeneration was not reported (Chaturani et al. 2005). Callus was induced from leaves and internodes of *P. santalinus* by Ashrafee et al. (2014) solely to assess antibiotic activity against *Aeromonas* and *Pseudomonas* but regeneration was not assessed. Details of effective plant growth regulator concentrations and combinations, medium composition, and explant type, as well as their effects on morphogenesis are presented in Table 2.

Rooting and acclimatization

Successful rooting 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 micropropagation protocol and care is needed to avoid hyperhydricity in in vitro-raised plants, which tend to display poor rooting efficiency (Ruffoni and Savona 2013; Teixeira da Silva et al. 2017b). Rooting and acclimatization protocols for in vitro-raised shoots of *P. santalinus* are summarized in Table 2. Only a few studies have quantified the survival of micropropagated plants (Lakshmi Sita et al. 1992; Prakash et al. 2006; Rajeswari and Paliwal 2008; Balaraju et al. 2011; Warakagoda and Subasinghe 2013). Among the 12 reports on *P. santalinus* tissue culture, in vitro rooting employed full-strength, half-strength and quarter-strength MS medium (Table 2). According to Arockiasamy et al. (2000), quarter-strength MS medium supplemented with 5.71 μM IAA was effective for 76.2% rooting of shoots derived from cotyledenary nodes, but acclimatization and survival of plantlets were not reported. Vipranarayana et al. (2012) applied a pulse treatment of 7.34 μM IBA in half-strength MS but details about survival were not reported. Rajeswari and Paliwal (2008) achieved 85% plantlet survival ex vitro after a pulse treatment of 5 μM IAA and 1 μM IBA for 25 d. In contrast, Warakagoda and Subasinghe (2013) showed limited success (46%
Table 1: Explant source, size and surface sterilization procedures for preparation of tissue culture studies of *Pterocarpus santalinus* (chronological listing)

| Explant source | Explant type, size and density; culture vessel | Surface sterilization and preparation | References |
|----------------|-----------------------------------------------|---------------------------------------|------------|
| Seeds (soaked and dried for 15 d) → seedlings. Age and source of mother plant NR | Size of shoot tips (15-d-old seedling) NR. 5–8 mm shoot tips and nodes from in vitro grown shoots. Test tubes (15 mL/tube) | Shoot tips: RTW (duration NR) → soap (soap name, duration of treatment and concentration NR) → 0.1% HgCl2 15 min → 3-4X SDW | Lakshmi Sita et al. (1992) |
| Seeds from the wild → seedlings. Age of mother plant NR | Roots, hypocotyls, mesocotyls, cotyledons, shoot tips, nodes, leaves, internodes (size NR for all explants) from 15-d old seedlings 6–7 cm tall with 3–4 nodes. Test tubes (1 explant/tube) | Pods: 50% HCl + 50% EtOH 2–3 h → TRW → dried for 2 d → pods opened and seeds sown directly in vitro | Anuradha and Pullaiah (1999a) |
| Seeds from the wild → seedlings. Age of mother plant NR | Hypocotyls (1 cm), epicotyls (1 cm), cotyledons (1.5 cm), shoot tips (0.5 cm), internodes (0.5 cm), axillary nodes (0.5 cm) from 20-d old seedlings. Test tubes (1 explant/tube) | Seeds: 5% Teepol (duration NR) → RTW 2 h → 0.1% HgCl2 + 0.1% sodium dodecyl sulfate 10 min → 5X DDW | Arockiasamy et al. (2000) |
| Pods (<3 to >5 cm) including wing. Age of mother plant NR | Seeds from pods of various sizes. Pods stored at 28 ± 5°C for 1–4 weeks. Culture vessel NR | Seeds and nodes: RTW 30 min → testa removed → 2% Bavistin (15 min for nodes, 30 min for seeds) → 70% EtOH 2 min → 0.1% HgCl2 (15 min for nodes, 12 min for seeds) → 4-5X SDW | Chaturani et al. (2006) |
| 10-y-old tree, sampled in Nov.-Jan. | Mature nodes from terminal shoots (7–8 cm long). 25 × 150 mL test tubes (1 explant/tube) | Shoots: 1% Teepol 30 min → cut into nodal segments 2–3 cm long → 70% EtOH 2 min → 0.1% HgCl2 + 0.1% Tween-20 7 min → 5X SDW | Prakash et al. (2006) |
| Mature pods and nodes (forest and campus culture) | Pods scarified in boiling water (5 min) or 5% H2SO4 (10 min). Culture vessel NR | Seeds and nodes: RTW 30 min → testa removed → 2% Bavistin (15 min for nodes, 30 min for seeds) → 70% EtOH 2 min → 0.1% HgCl2 (15 min for nodes, 12 min for seeds) → 4-5X SDW | Padmalatha and Prasad (2007, 2008) |
| Seeds → seedlings. Age and source of mother plant NR | Seedling-derived cotyledonal nodes (1.5 cm long), nodal segments (1 cm long). Test tubes (1 explant/tube) | Explants from 30-d-old seedlings: RTW 1 h → 0.025% Tween-20 10 min → 3X DW → 0.1% HgCl2 10 min → 3X SDW | Rajeswari and Paliwal (2008) |
| Seeds from the wild → seedlings | Shoot tips of 20-d-old in vivo seedlings. 50-mL Borosil test tubes (1–2 explants/tube) | Peeled seeds: 1% Bavistin (fungicide) 10 min → wash with H2O → 50–300 ppm GA3 24 h. Apical meristem explants: RTW 10 min → DW + some drops Tween-20 5 min → SDW 2-3X → 1% Bavistin 5 min → SDW → 70% EtOH 30 s → SDW 2-3X → 0.1% HgCl2 3 min → 4X SDW | Balaraju et al. (2011) |
| In vitro seedlings (age NR) | Nodal segments; one explant per test tube (150 mm × 25 mm) | Seeds: RTW 30–40 min → 5% Teepol-B-300 (wetting agent) stirring 15 min → 1% Bavistin Carbandazim (fungicide) 10 min → DW 30 min → 70% EtOH 30 s → 0.05% HgCl2 5–10 min → 4X SDW | Vipranarayana et al. (2012) |
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Table 1 continued

| Explant source | Explant type, size and density; culture vessel | Surface sterilization and preparation | References |
|----------------|---------------------------------------------|-------------------------------------|------------|
| 1. Seeds from green-brown pods from 25-y-old trees | 1. Mesocotyl segments, cotyledonary nodal segments, shoot tips, from 20-d-old in vitro germinated seedlings and seedlings | Seeds: 10% Clorox® (fungicide) 20 min → 70% EtOH 2 min → rinse NR Shoot segments: 5% Teepol 5 min → RTW 30 min → 0.3% topsin solution 1 h → 2X SDW → Clorox® + 2 drops Tween-20 (10, 15, 20% for 10, 15, 20 min each) → 70% EtOH 2 min → 2X DW. Whole process repeated twice | Warakagoda and Subasinghe (2013) |
| 2. Age of mother plants in greenhouse NR. Greenhouse conditions NR. Terminal bud removed and sprayed with 10 mg/l BA at 2-w intervals. 70% thiophanate methyl (topsin) sprayed at 100 mg/l 24 h before collecting explants. Plants treated with 200 mg/l Albert’s solution (liquid fertilizer) at 2-w intervals | 2. Immature and semi-hard shoot segments | Culture vessel and other conditions for 1 and 2 NR | |
| 1–3-y-old trees | Leaves and internodes. Size and density NR; test tubes | Leaves: wash in DW → 70% EtOH 30 s → 0.1% HgCl2 3 min. Internodes: 70% EtOH 2 min → 0.1% HgCl2 → 2–3 drops Tween-20 7 min. No rinses described for both explants | Ashrafee et al. (2014) |

$d$ day(s), $DW$ distilled water, $DDW$ double distilled water, $EtOH$ ethyl alcohol (ethanol), $GA_3$ gibberellic acid, $HgCl_2$ mercury 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)

... of plantlets rooted) ex vitro with 49,000 μM IBA, possibly because of the excessively high concentration of this auxin.

### Variability in quality and quality control

There is a problem with the adulteration and falsification of plant material in the *P. santalinus* market. The heartwood of *Adenanthera pavonina* Willd. (*Mimosaceae*), known as ‘Ranjana’ and ‘Raktakambal’ in West Bengal and ‘Bari Gumchi’ in the northern parts of India, is often sold as a fake substitute for *P. santalinus*, while artificially colored wood shavings and the sawdust of some other trees are also sold on the market as cheap substitutes (Botanical Survey of India 2012). In China, the manufacture of furniture utilizes *Dalbergia louvelii* R. Vig. (violet rosewood) as a substitute for *P. santalinus* since both plants have a very similar appearance and anatomical characteristics, and cheaper *D. louvelii* is often illegally used to impersonate the valuable *P. santalinus* (Zhang et al. 2014). Zhang et al. (2014) used conventional infrared spectroscopy (FT-IR), second derivative infrared (SD-IR) spectroscopy and two-dimensional correlation infrared (2DCOS-IR) spectroscopy to differentiate furniture made of *P. santalinus* wood from furniture made from *D. louvelii*. They observed that *P. santalinus* wood had a higher holocellulose content than *D. louvelii* wood while *D. louvelii* had more NaOH- and benzyl-alcohol-based extracts than *P. santalinus*.

The size and age of trees affects the heartwood content and wood density of *P. santalinus* (Suresh et al. 2017). Woody anatomy such as grain waviness can be used to delimit and identify *P. santalinus* (Rawat and Uniyal 1996; Gasson and MacLachlan 2010). The Botanical Survey of India (2012) used various anatomical methods such as maceration, scanning electron microscopy, exo- and endomorphic features, and fluorescence analysis to correctly identify *P. santalinus* wood samples.

Molecular markers are regularly utilized to measure the degree of genetic variation within natural or breeding populations, and have been extensively used in Indian sandalwood research (Teixeira da Silva et al. 2017a). In *P. santalinus*, RAPD (random amplified polymorphic DNA)-based marker analysis was used to detect variations in micropropagated plants raised from shoot tips, verifying that in fact no variation existed (Balaraju et al. 2011). RAPD was also used by Usha et al. (2013) to detect variation among nursery-grown plants. Variation in genetic distance among natural accessions, detected by RAPD markers, reflected a high level of DNA polymorphism due to outcrossing (Padmalatha and Prasad 2007; Usha et al. 2013). Jhansi Rani and Usha (2013) developed a sequence characterized amplified region (SCAR) marker to differentiate wavy from straight-grained plants at the seedling stage. Jyothi et al. (2014) reported differences in the quantity of genomic DNA in samples collected from different locations in Andhra Pradesh, India.

Therefore, quality control, as assessed by anatomical or chemical methods, is essential to verify the originality of *P. santalinus* wood while molecular methods serve to confirm genetic stability.
Table 2 In vitro conditions for tissue culture studies of *Pterocarpus santalinus* (chronological listing)

| Culture medium, PGRs, additives, subcultures | Culture conditions | Experimental outcome, maximum productivity, acclimatization and variation | References |
|---------------------------------------------|-------------------|--------------------------------------------------------------------------|------------|
| **B**<sub>5</sub> + 0.88 µM or 4.44 µM B<sub>A</sub> + 0.46 or 4.6 µM Kin (SIM), MS + 5.71–28.59 µM IAA or 4.9–24.42 µM IBA (RIM), pH 5.6–5.8, 2% sucrose, 0.8% agar. Subculture every 4–5 w | 16-h PP, CWFT. 1200 lx, 25 °C. RH NR | Shoot tips from in vitro seedlings produced 4–5 cm shoots with 4–5 nodes. Shoot tips from in vitro grown shoots produced up to 8 shoots 3–5 cm long on **B**<sub>5</sub> + 4.44 µM BA + 4.65 µM Kin within 4–6 w. Nodal explants produced 1 shoot/explant, 80% rooting on IAA vs. 30–40% on IBA in RIM. 5.71–11.43 µM IAA produced adventitious roots but 28.59 µM IAA produced a thick tap root. Acclimatization in sterilized soil + sand (1:1) with 50% survival. 60% survival when kept in liquid RIM for 2 w then transferred to test tubes with only water for 1 w and the finally to plastic covered pots | Lakshmi Sita et al. (1992) |
| **½B**<sub>5</sub> + 0.05% AC + 0.44 µM BA (SG), B<sub>5</sub> + 8.88 µM BA (SIM). ½MS + 0.57 µM IAA + 0.49 µM IBA + 0.53 µM NAA (RIM), pH 5.7–5.8. 3% sucrose. Gelling agent NR | NR | 40–70% germination in 24 h. Only shoot tips, nodes and mesocotyls formed shoot buds and multiple shoots; the five other explants induced callus. Mesocotyls formed maximum shoots/explant (5–8). MS and WPM not as effective as B<sub>5</sub> as SIM basal medium. Acclimatization claimed in sand + soil, but not quantified | Anuradha and Pullaiah (1999a) |
| MS + 2% sucrose + 0.6% agar (SG), MS + 0.53 µM NAA + 4.44 µM BA + 4.65 µM Kin (SIM). ½MS + 5.71 µM IAA (RIM). Carbon source, gelling agent, pH NR | 16-h PP, Light source NR, 1200 lx, 26 °C. RH NR | 10.4 shoots/cotyledon on SIM. No shoots formed from epicotyls, hypocotyls or internodes. 76.2% of cultures rooted. Acclimatization claimed in sterilized garden soil + sand (1:1) and watered with RIM for 1 w, but not quantified | Arockiasamy et al. (2000) |
| MS, WPM, Anderson or *Vitis* basal medium + AC conc. NR or without AC (SG), pH NR, 3% sucrose. 08% agar | 12-h PP, Light source NR, 23 ± 2 °C. RH NR | 90% germination after 15 min exposure to 0.1% HgCl<sub>2</sub> <3 cm pods were either seedless or with fragile seeds. About 95% of seeds from pods >5 cm in size germinated. No correlation between size and germination period. 96% germination in pods stored for 1 w at 28 ± 5 °C. Storage period and browning were inversely proportional to germination efficiency. Rapid (within 6 d) germination (92%) with 10 mm hypocotyls was possible from seeds cultured on Anderson medium without AC. | Chaturani et al. (2006) |
| Liquid MS + 4.4 µM BA + 2.2 µM TDZ (SIM, SMM) with 6-w subcultures on paper bridges. ½MS + 4.9 µM IBA (RIM), pH 5.8, 3% sucrose. 0.8% agar (RIM only) | 16-h PP, CWFT. 50 µE m<sup>−2</sup> s<sup>−1</sup>, 25 ± 2 °C, 65% RH | Subculture on paper bridges improved nodal explant survival more than all antioxidants tested but 80% of cultures showed browning. 74–75% of nodes formed axillary shoots. An 8.3-fold increase in shoots by the 6th subculture. 70% survival after acclimatization in autoclaved soil + FYM (4:1) after 5 months | Prakash et al. (2006) |
| Culture medium, PGRs, additives, subcultures | Culture conditions | Experimental outcome, maximum productivity, acclimatization and variation | References |
|--------------------------------------------|-------------------|------------------------------------------------------------------------|------------|
| MS + 13.3 µM BA (SIM, nodes). MS + 4.44 µM BA + 9.30 µM Kin (SIM, seeds). MS + 4.44 µM BA (SMM, nodes, seeds). Auxin-free MS + 0.25% phytagel (RIM). pH NR. 3% sucrose. 0.6% agar | 16-h PP. CWFT, 83.6 µE m⁻² s⁻¹. 25 ± 2 °C. 60-70% RH | 17 shoots/seed explant on SIM, then SMM, with 90% explant response. Rooting data NR. Low (20%) survival of acclimatized plantlets in Soilrite® + manure + sand (1:1:1) | Padmalatha and Prasad (2008) |
| MS + 2.5 µM BA + 2 µM 2iP (SIM, SMM). Dip in 5 µM IAA + 1 µM IBA (ex vitro RIM). pH NR. 3% sucrose. 0.8% agar | 16-h PP. CWFT, 60 µmol m⁻² s⁻¹. 24 ± 2 °C. RH NR | Max. of 4/4 shoots/cotyledonic node in 2nd subculture in 95% of cotyledonic nodes. 82.5% of shoots induced roots. 95% survival of acclimatized plantlets in coarse sand + clay + FYM (1:1:1). Tissue-cultured plants showed better morphological performance than seedlings | Rajeswari and Paliwal (2008) |
| MS + 1 mg/l BA + 0.45 µM TDZ (SIM). Subculture every 4 w. MS + 2.22 µM BA + 0.28 µM GA₃ (shoot elongation). MS + 0.49 µM IBA (RIM) using 3–4 cm long shoots with 4–5 leaves. After 4 w, transfer to PGR-free MS (root elongation). pH 5.8. 2% sucrose. 0.8% agar | 16-h PP. CWFT, 35–50 µmol m⁻² s⁻¹. 22 ± 1 °C. RH NR | % SG NR. 83% of shoot tips formed new shoots, with 11 buds/explant after 45 d. 60% of shoots rooted. 73.3% survival of acclimatized plantlets in organic manure and garden soil + sand (1:1) under in vitro culture conditions. RAPD used to confirm lack of variation | Balaraju et al. (2011) |
| MS + 2 µM GA₃ (SG). MS + 1 mg/l BA + 0.5 mg/l NAA (SIM). Pulse in 1.5 g/l IBA → ½MS (RIM), pH 5.8. 3% sucrose. 0.8% agar | 16-h PP. CWFT, 50 µmol m⁻² s⁻¹. 25 ± 2 °C. 65% RH | 85% of adventitious shoots elongated. 8.8 shoots/shoot tip. 85% rooting after 4 w in soil + manure (1:1) (survival NR) | Vipranarayana et al. (2012) |
| MS, WPM or B₅ 4–12 µM BA + 0.5–2 µM NAA (SIM). 25–2500 mg/l IBA pulse treatment 12 h → ½ MS + 0.5 µM IBA (RIM), pH 5.8. 3% sucrose. 0.01% myo-inositol. Gelling agent NR | 16-h PP. CWFT, 1220 lx. 23 ± 2 °C. 60% RH | 80% survival from immature cuttings when surface sterilized with 15% Clorox® for 10 min. 0.1% AC with WPM was best interaction. No significant difference in interaction between media and explant type. Maximum number of shoot buds (= 4.95) cotyledonary nodal explant on B₅ medium supplemented with 8 µM BA and 2 µM NAA. Longest roots in 25 mg/L pulse treatment. Ex vitro rooting using 1000 mg/L IBA produced 40% rooting. Acclimatization performed in sand + coir dust (1:1) was 80% when kept in humid conditions and weak light for the first 4 w then for 6 w in greenhouse | Warakagoda and Subasinghe (2013) |
Conclusions and future perspectives

This review highlights key advances in the tissue culture-based biotechnology of economically important *Pterocarpus santalinus*. 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. In most cases, explants are derived from seeds or seedlings which are not suitable for clonal propagation (Table 1). Therefore, a clonal method should be developed from vegetative tissues of elite germplasm. Rooting and survival of micropropagated plants remain a major limitation to the success of *P. santalinus* tissue culture and should be optimized in the future, for example by using CO₂ enrichment and vessels that allow for maximized aeration without impacting relative humidity levels within the culture vessel (Teixeira da Silva et al. 2005). The ability to stably produce units that allow for germplasm conservation would then stimulate the need for cryoconservation (Teixeira da Silva and Engelmann 2017; Bi et al. 2017), including through the application of synthetic seeds (Sharma et al. 2013). Analytic hierarchy, which is a multicriteria decision-making tool, is valuable for incorporating the perceptions of stakeholders when planning the conservation and restoration of a *P. santalinus* population (Kukrety et al. 2013a, c). The micropropagation and biotechnology of another commercially important tree in this genus, *P. marsupium* (Indian kino tree), have recently been reviewed (Teixeira da Silva et al. 2018).

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