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Pongamia pinnata: An Untapped Resource for the Biofuels Industry of the Future

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Abstract *Pongamia pinnata* (L.) Pierre is a fast-growing leguminous tree with the potential for high oil seed production and the added benefit of the ability to grow on marginal land. These properties support the suitability of this plant for large-scale vegetable oil production required by a sustainable biodiesel industry. The future success of *P. pinnata* as a sustainable source of feedstock for the biofuels industry is dependent on an extensive knowledge of the genetics, physiology and propagation of this legume. In particular, research should be targeted to maximizing plant growth as it relates to oil biosynthesis. This review assesses and integrates the biological, chemical and genetic attributes of the plant, providing the basis for future research into *Pongamia*'s role in an emerging industry.

Keywords Legume · Biodiesel · Sustainability · *Pongamia*

Introduction

Pongamia pinnata (L.) Pierre, an arboreal legume, is a member of the subfamily Papilionoideae, more specifically the Millettieae tribe. This medium-size tree is indigenous to the Indian subcontinent and south-east Asia, and has been successfully introduced to humid tropical regions of the world as well as parts of Australia, New Zealand, China and the USA. Historically, this plant has been used in India and neighbouring regions as a source of traditional medicines, animal fodder, green manure, timber, fish poison and fuel. More importantly, *P. pinnata* has recently been recognized as a viable source of oil for the burgeoning biofuel industry. The sustainable production of plant oils for biodiesel production from a tree crop such as *P. pinnata*, which can be cultivated on marginal land, has the potential to not only provide a renewable energy resource but in addition will alleviate the competitive situation that exists with food crops as biofuels and associated arable land and water use. Finally, *P. pinnata* has been identified as a resource for agroforestry, urban landscaping (Fig. 1e), and the bioameloriation of degraded lands. Here we describe the current state of knowledge of the biology, taxonomy, and biogeography of *P. pinnata*, and the extent to which humans have exploited it as a valuable commodity. Further, the degree to which extensive propagation of *P. pinnata* and the extraction and processing of oil from seeds may contribute to the success of a sustainable biofuels industry is discussed.

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A Legume Little Known Outside the Indian Sub-continent

Pongamia pinnata is reported to be a native of India, Myanmar, Malaysia and Indonesia. It is a nitrogen-fixing tree and therefore a member of the family Leguminosae. More detailed taxonomic description places it in the subfamily Papilionoideae and the tribe Millettieae. This plant has been synonymously known as *P. pinnata* Merr., *Pongamia glabra* Vent., *Derris indica* (Lam) Bennett and

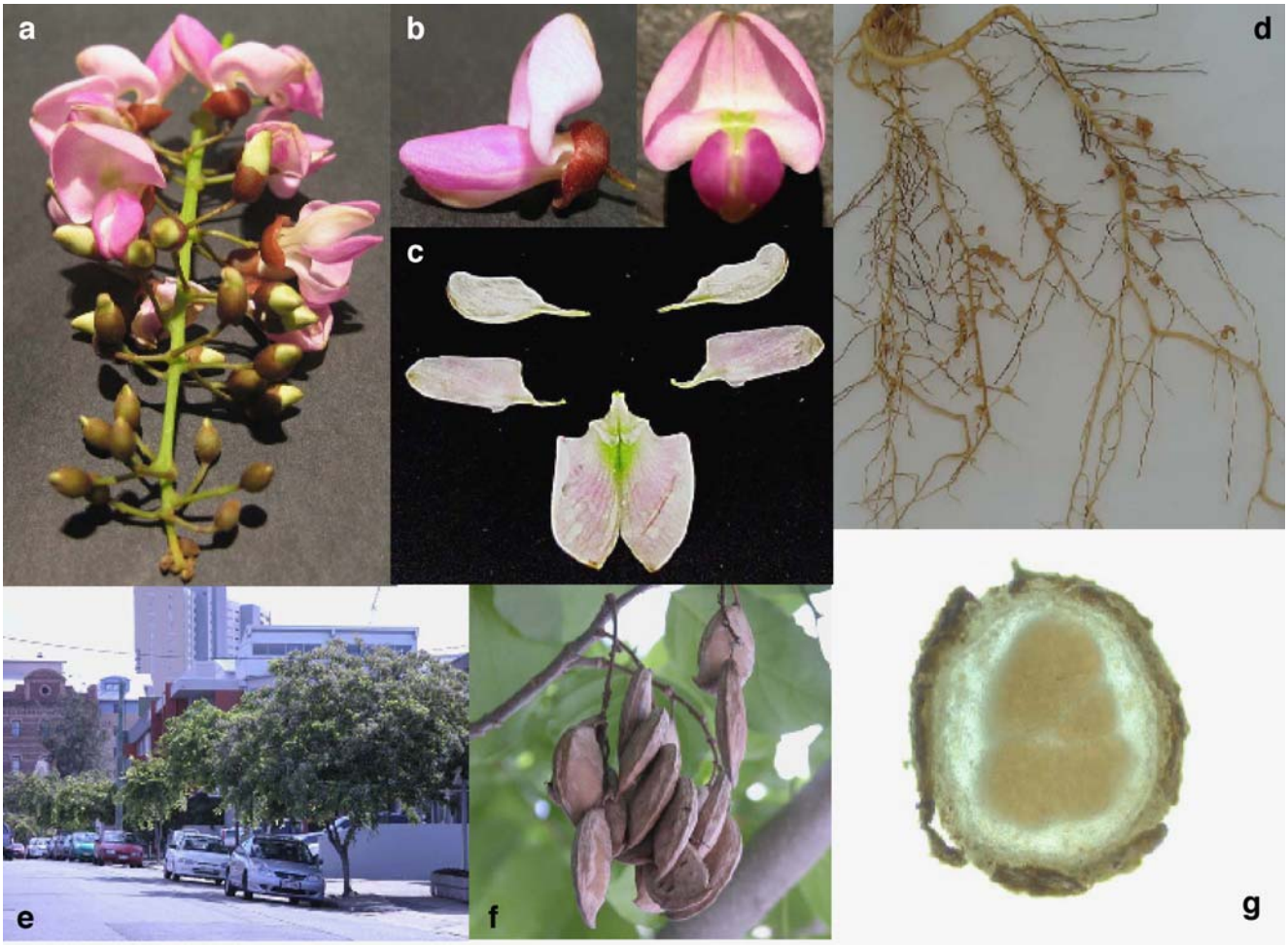

Fig. 1 Botanical characteristics of *Pongamia pinnata*: **a** *P. pinnata* inflorescence showing the ornamental advantages. **b** Characteristic legume (pea) flower morphology. **c** Dissected flower showing standard petal, two identical wings and two identical keel petals. **d** Nodulated root system of *P. pinnata* seedling. **e** *P. pinnata* as a shade street tree in Brisbane, Australia (27°25′ S 153°9′ E). **f** *P. pinnata* seed cluster. In general about 25–35% of flowers set seed. Flowering in Brisbane occurs in mid-November. Seed maturation takes about 10 months. Each seed weighs about 2 g and the dried pod wall also weighs about 2 g. **g** Hand-section through a *P. pinnata* nodule induced by *Bradyrhizobium japonicum* strain CB1809 demonstrating the reddish-brown nitrogen fixation zone and the general spherical (determinate) nature of the nodule

Millettia novo-guineensis Kane & Hat. Depending on the language and location, common names for *P. pinnata* include Indian-beech, poonga-oil-tree, pongam tree, karanja tree, karum and kanji. The plant has been introduced to several countries with humid tropical lowlands as well as parts of Australia, New Zealand and the USA. An introduction to Australia may have taken place early in the history of human habitation, as there are reports of *P. pinnata* being used by the aboriginal people of northern Australia as a fish poison, a source of timber for the building of tools, and the timing of flowering used as a seasonal cue for the construction of stone fish traps [12, 18, 77]. With northern Australia in such close proximity to south-east Asia, the question may be asked as to whether *P. pinnata* may also be regarded as a native plant of Australia.

While there is a substantial pool of general descriptive information on *P. pinnata* that can be accessed from the world wide web (http://www.worldagroforestrycentre.org; http://ecoport.org; http://www.ars-grin.gov; http://www.winrock.org), technical reports and selected monographs [47], there is a considerable gap in the range of detailed scientific publications. If this plant is to become an emerging crop and plantation species grown on extensive tracts of land, then comprehensive studies encompassing its physiology, agronomy, propagation, genetics and molecular biology are needed. In reviewing the available literature, it is clear that *P. pinnata* has a long history of association with indigenous populations, primarily villagers and small land-holders [64]. What are now required are detailed studies providing information that will enable the successful cultivation and management of well-defined elite varieties of *P. pinnata*. Such studies would include the clonal propagation of high oil content and high yielding individual trees, already seen in natural populations, as well as the

discovery of agronomic parameters such as growth in water-deficient and/or saline conditions.

From previous publications, the vast majority of which come from universities and research institutes in India, it is known that *P. pinnata* is a plant well-suited to "marginal lands". Growth is seen best from sea level to an altitude of approximately 1,200 m and an optimal annual rainfall of 500 to 2,500 mm. Further, *P. pinnata* is regarded as both a saline and drought tolerant species. Tomar and Gupta [78] examined the survival of 16 tree species, including *P. pinnata*, under variable soil salinity and moisture in both field and pot trials. In the field, 6-month old saplings were planted at a subsurface depth of 30 cm and over a 12 month period, experiencing salinity in the range 12 to 19 dS m^{-1}, showed 13% of the 48 saplings surviving. In pot trials under controlled moisture of 4.6 and 26.0 dS m^{-1} the *P. pinnata* saplings displayed moderate and poor tolerance, respectively. A longer trial over 4 years was undertaken by Patil et al. [54]. For this study of 23 tree species, the survival rate and growth characteristics, namely the increases in height and collar diameter, were determined in a sand, silt and clay mix of salinity 10 to 12 dS m^{-1}, and a water table within 0.5 m during the monsoon season and to 0.8 m during the summer season. At the end of this trial *P. pinnata* had a survival rate of 62%, an increase in height from 71.8 cm at the end of the first year to 218.5 cm at the end of the fourth year, and an increase in collar diameter from 1.65 cm to 4.61 cm for the same period. Further, *P. pinnata* had a positive bioameliorative effect with contributions to soil nitrogen, phosphorous, potassium and organic carbon.

Much of what we know regarding *P. pinnata* is its contribution as a source of valuable commodities for agriculture and medicine, and to a lesser extent some features of its agronomy and interactions with other organisms as a legume. Areas of research that require particular attention are the physiology and reproduction of *P. pinnata*. The structural and biochemical features of the flowers and the mechanism of pollination have already been described [27, 59]. The flowers are white and pink to purple, and arise in a raceme-like inflorescence (Fig. 1a–c). Anatomically, the flowers resemble a typical legume flower, with two keel and two wing petals, and a single standard petal. Pollination is insect-mediated, most often by bees, of which *P. pinnata* is recognized as an important source of nectar [37]. The abundant pollen, enclosed in the keel petals, is released from flowers by an explosive mechanism triggered by the predominantly nectar feeding bees, but also by wasps. Mature flowers will open for a single day, with nectar secretion coinciding at this time. The volume of nectar is approximately 1 to 3.5 μl per flower and contains up to 60% sugar, principally glucose, fructose and sucrose. In the pods of *P. pinnata* it is usual for only one of the two ovules to develop into a seed. Seed abortion of the peduncular seed is thought to result from successful competition for resources by the stigmatic seed [6].

Biotic Interactions

In promoting the benefits of legumes in agriculture, the obvious advantage that legumes have over other plants is the formation of nodules resulting from a symbiotic relationship with nitrogen-fixing bacteria. Despite being a legume, relatively little is known regarding the nodulation of *P. pinnata*. Dayama [19] noted the nodulation of *P. pinnata* grown in sandy loam soil and the stimulatory effect of foliar applied sucrose on nodule number and plant growth. Siddiqui [66] reported the nodulation and associated nitrate reductase activity of *P. pinnata* seedlings grown on locally derived garden soil, sand and farm manure. In both these studies the nodulation of plants was reliant on the presence of endogenous rhizobia and their ability to nodulate *P. pinnata*. In a more comprehensive study, the plant host range was tested for *Rhizobium* sp. strain NGR234 and *Rhizobium fredii* USDA257 against a comprehensive list of more than 450 species of legumes, including *P. pinnata*, which failed to form nodules with either strain [56]. Interestingly, in preliminary studies we have been able to demonstrate the effective nodulation of *P. pinnata* (Fig. 1d and g) with three strains of rhizobia; *Bradyrhizobium japonicum* strain CB1809, a strain more commonly associated with Glycine max; *Bradyrhizobium* sp. strain CB564, a strain previously isolated in Australia from *P. pinnata*; and *Rhizobia* sp. strain NGR234, the same strain previously reported unable to form nodules on *P. pinnata* [56]. While it appears from our preliminary studies and those previously reported that *P. pinnata* can readily form nodules, there is a clear need to characterize in more detail the spectrum of rhizobia that can form an effective symbiotic relationship with *P. pinnata*, as well as the ontogeny of nodule formation.

The World Agroforestry Centre (http://www.worldagroforestrycentre.org) cites both insect pests and fungal diseases of *P. pinnata*. For example *Parnara mathias*, *Gracillaria* spp., *Indarbela quadrinotata*, *Myllocerus curvicornis*, and *Acrocercops* spp. are noted as insects pests with *Ganoderma lucidum* and *Fomes merilli* identified as fungal pathogens of root and shoot tissues, respectively. Our observations of *Pongamia* show genetic variation in susceptibility to insect pests and microbial infection on young and mature leaves. Clearly selection of superior germplasm for the traits of insect and disease resistance, and clonal propagation are essential steps towards crop improvement.

Sandal (*Santalum album* L.), a native of India, is a hemiparasite of the roots of more than 300 host plant species. It

is noteworthy as a source of valuable timber (sandal wood) and essential oils. Among nine selected host species, including *Wrightia tinctoria*, *Tectona grandis*, *Atrocarpus integrefolia*, *Swietenia mahogany*, *Azardirachta indica*, *Eucalyptus camaldulensis*, and *Acacia auriculiformis*, *P. pinnata* together with *Casuarina equisetifolia* were shown in both pot and field trials to be the hosts capable of supporting the greatest biomass production of sandal timber [46]. In an earlier study Subbarao et al. [74] demonstrated that in the case of *P. pinnata* and *Cajanus cajan* L., haustorial connections by *S. album* were not only with the root tissue of the host but also via direct contact to nodules. Not surprisingly, *S. album* haustoria connections with root nodules are established at the expense of the host plant in such aspects as nodule number and plant fresh weight.

Molecular and Cytogenetics

To date there are very few genomic regions of *P. pinnata* that have been sequenced and characterised. The regions that have been characterised are exon 1 of the phytochrome A-like gene [38], the 5.8S rRNA gene and internal transcribed spacer regions 1 and 2 [25], the 18S rRNA, maturase and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit genes [65], and a NBS-LRR class resistance protein gene (Ramasubramanian et al. unpublished data). Following BLAST analysis of either the nucleotide or translated protein sequences, it was found that the phytochrome A-like protein is 93% identical to the phytochrome A proteins from *Pisum sativum* and *Medicago truncatula* over 210 amino acids; the maturase protein is 97% identical to the same protein from *Glycine soja* over 563 amino acids; the NBS-LRR class resistance protein is 68% identical to G. max NBS-LRR disease resistance protein RPG1-B over 174 amino acids; the 18S rDNA is 99% identical to the corresponding region from *P. sativum*, *M. truncatula* and *G. max*; and the 5.8S rRNA gene and internal transcribed spacer regions 1 and 2 were 94% and 93% identical to the corresponding regions from *Millettia pulchra* and *Fordia splendidissima*, respectively. The sequence data for all but the NBS-LRR class resistance protein gene have been used for the purposes of molecular phylogenetic studies.

In addressing the issue of the phylogeny determined by molecular methods, it is clear that *P. pinnata* is a member of the "core Millettieae" group [25, 38]. The conclusions from these DNA sequence-based studies were more recently supported by a RAPD-PCR study of nine species from the Millettieae [1]. In this study, 18 10-mer oligonucleotides were used to assess the genetic relationship between *Tephrosia pumilla*, *Tephrosia purpurea*, *Tephrosia villosa*, *Derris trifoliata*, *Derris scandens*, *Millettia peguensis*, *Millettia racemosa*, *Piscidia piscipula* and *P. pinnata*. A dendrogram constructed on the basis of 347 polymorphic bands showed that *P. pinnata* was most closely related to the *Millettia* spp.

The only other reported studies on any aspect of *P. pinnata* genetics are those addressing gross chromosome organization. Cytogenetical studies have suggested a chromosome number for *P. pinnata* of either $2n=20$ [7] or $2n=22$ [53, 57, 63]. In a preliminary study we have used a DNA fingerprinting protocol (DAF) that employs PCR amplificiation using short arbitrary primers [15] to address the question of the genetic diversity of *P. pinnata* (Fig. 2b). Shown in this figure are the profiles of three trees with some common but many polymorphic DNA amplification products. This diversity is also reflected through variation in leaf (five and seven pinnate) and overall plant structure, flowering time and growth vigour. These initial studies with germplasm from Australia and India, suggesting wide genetic diversity in this species, may be of great benefit for the development of superior trees. Such superior germplasm will then require vegetative propagation by either rooted cuttings or in vitro approaches.

Initial experiments in our laboratory demonstrated the feasibility of vegetative propagation by cuttings, resulting in viable clonal material now planted in a field site. However, propagation by rooted cuttings may not generate the required replication rate needed for large scale *Pongamia* plantations. For example, to satisfy the existing 18 billion litre annual diesel consumption of Australia an estimated area of 7,000 km^2 would need to be harvested, assuming a planting density of 350 trees per hectare, a yield of 20,000 seeds per tree, 1.8 g per seed and 40% fatty acid/triglyceride/oil recovery.

Propagation of *P. pinnata*

The successful introduction and subsequent expansion of plantings of any new crop species is reliant on the ability to develop simple and reliable methods for the propagation of large numbers of plants. Furthermore, the long-term viability of tree crop species such as *P. pinnata* is dependent on good pruning management practices. In addressing this second issue, coppicing and pollarding have been reported as successful means of agroforestry management practices for *P. pinnata* [42]. With respect to mass propagation, *P. pinnata* can be propagated easily from seed [23, 69]. To this, Manonmani et al. [41] found that there was a direct relationship with seed size and germination efficiency, but only with fresh seeds. Germination and plant vigour began to decrease following storage of seeds for 3 months or more.

Despite the successes seen in germination trials, in the context of the development and continued vegetative propagation of superior genotypes, other protocols are

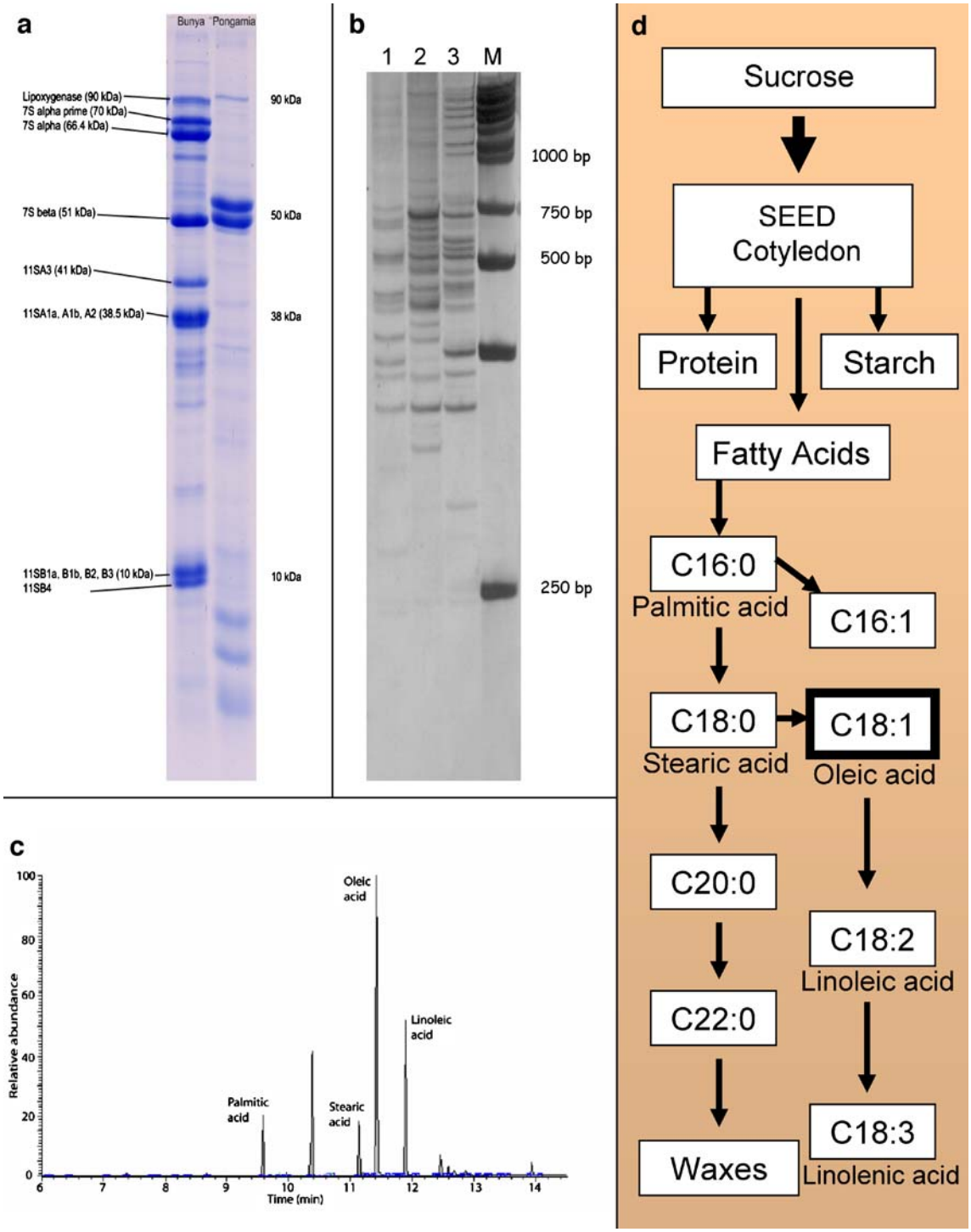

Fig. 2 General molecular characteristics of *Pongamia pinnata*: **a** One dimensional PAGE gel (14.2%) after Coomassie Blue staining of *P. pinnata* seed proteins compared to a parallel isolate form soybean (*Glycine max*) cultivar Bunya. **b** DNA amplification profile using arbitrary primed PCR (DAF method; [15]) separated on a thin, plastic-backed polyacrylamide gel (5%) stained with silver [10, 11]. Three samples of leaf-extracted DNA were amplified with primer PP-UQ-57. **c** GC-MS separation of fatty acids from a single *P. pinnata* seed referenced against the internal C17:0 standard (*unlabelled peak*). Palmitic, stearic, oleic and linoleic acids comprised 11.3%, 12.9%, 41.4% and 26.7% of the total fatty acids, respectively. **d** Scheme demonstrating the biochemical/developmental decisions made during fatty acid biosynthesis in oil seeds [76]

required. Palanisamy et al. [52] reported the development of adventitious roots in shoot cuttings from *P. pinnata*. The formation of adventitious roots, which was promoted by the three auxins, indole acetic acid (IAA), indole butyric acid (IBA) and naphthyl acetic acid (NAA), was most prominent in association with the development of new shoots on the planted cuttings. IBA was the most effective inducer. Karoshi and Hegde [30] also undertook a study examining the propagation of stem cuttings and looked at the potential of softwood grafting. Similarly to Palanisamy et al. [52], IBA (at 2,500 ppm) was found to promote rooting of *P. pinnata* and, in addition grafting had a 95% success rate. In a third study, Ansari et al. [5] examined the effect of dipping semi-hardwood coppice shoot cuttings in $KMnO_4$, KCl, $KHPO_4$, KH_2PO_4 or K_2SO_4 on both IAA ionization and adventitious rhizogenesis. At an equimolar K concentration of 5 mM, the S and P salts had a significantly positive effect on the percentage of cuttings that produced new sprouts and roots, the number of roots per cutting, and the root length, while at the same time reducing the amount of IAA ionization. In contrast, the Mn salt decreased adventitious rhizogenesis while the chloride salt had no effect.

Sujatha and Hazra [75] report on a method for the micropropagation of *P. pinnata* from mature-tree-derived axillary meristems. Pretreatment of the explant material in media containing the cytokinin-like compound thidiazuron increased the mean number of shoots over explants grown in the absence of this plant growth regulator. However, the development of new shoots required that the explants be sub-cultured in thidiazuron-free tissue culture media. Thidiazuron enhances the production of meristematic cells but inhibits their differentiation, as evidenced by the formation of meristematic domes when cultured under continuous thidiazuron treatment. Other plant growth regulators, benzylaminopurine, kinetin and zeatin, failed to induce the formation of multiple shoots from the same explant material. Finally, Srinivas and Rao [71], at a recent meeting of the Society for In Vitro Biology, reported on what they claim to be an efficient and reproducible system for the regeneration of *P. pinnata* from immature embryo derived cotyledonary explant.

Biomedical and Biocidal Properties

There is a long tradition of *P. pinnata* being used as a medicinal plant, particularly with the Ayurvedha and Siddha medicine systems of India [43]. More recently, the effectiveness of *P. pinnata* as a source of biomedicines has been reported, specifically as both an antimicrobial agent and as a therapeutic agent targeting host pathways and processes. For example, Brijesh et al. [13] were able to demonstrate that while a leaf decoction did not have biocidal activity against *Giardia lamblia*, rotavirus, or strains of *Escherichia coli*, *Vibrio cholerae* or *Shigella flexneri*, it significantly reduced the production of cholera toxin by *V. cholerae* and the invasion of HEp-2 cells by *E. coli*. In contrast, a crude seed extract of *P. pinnata* was able to completely inhibit the growth of herpes simplex virus type 1 and type 2 in Vero cells [21]. Bark, leaf, and to a lesser extent, seed extracts, inhibited the activity of the malaria parasite *Plasmodium falciparum* in an in vitro assay using parasitized erythrocytes [68]. Both aqueous and alcohol leaf and fruit extracts of *P. pinnata* possessed antifilarial activity against the cattle parasite *Setaria cervi* [79]. These extracts acted both on whole worm and nerve-muscle preparations. Using a rat model, Srinivasan et al. [72] demonstrated that an alcohol extract of *P. pinnata* leaves had significant anti-inflammatory activity. Further, this extract did not induce ulcers in rats, indicating its potential as an anti-inflammatory therapeutic agent. The same research group was also able to show that an identical leaf extract had antinociceptive (reduction in sensitivity to painful stimuli) and antipyretic (reduction in fever) activity, once again using mice and rat models [73]. Interestingly, the seed oil of *P. pinnata* has been reported to have spermicidal activity, with obvious implications in contraception [9].

Extracts of *P. pinnata* have also been shown to have applications in agriculture and environmental management, with insecticidal and nematicidal activity. Kabir et al. [28] reported that a petroleum ether extract of leaf tissue had insecticidal activity towards the American cockroach *Periplaneta americana* (L.). Similarly, the larvae of three mosquito species, *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi*, were susceptible to a petroleum ether extract of seed tissue [22]. Kumar et al. [36] tested extracts from bark, leaves and seeds against the cluster caterpillar *Spodoptera litura* and the insect pests of stored products *Trogoderma granarium* and *Tribolium castaneum*. A methanolic fraction from the seed oil exhibited the greatest toxicity towards *S. litura* and *T. granarium*, while a leaf extract was most toxic towards *T. castaneum*. Nematicidal activity from seed extracts has been demonstrated against the root-knot nematode *Meloidogyne incognita*. Yadav et al. [81] were able to show that *P. pinnata* oil cake, as part of a seed soaking treatment, inhibited the reproduction of nematodes while simultaneously having beneficial effects on the growth characteristics of chickpea. Similarly, Khurma and Mangotra [31] tested the effectiveness of seed extracts from 15 leguminosae towards the juveniles of root-knot nematode and found that *P. pinnata* caused high mortality.

While all the reports above describe the biocidal effects of various *P. pinnata* tissue extracts, there was no detailed characterization of the active constituents in these extracts.

This is an obvious area for future research if these active constituents are to be produced on a commercial scale. There are reports dating back more than 50 years of natural products chemistry with detailed descriptions of the identification and synthesis of complex organic compounds, particularly flavone-like molecules [3, 4, 14, 17, 39, 55, 60, 67, 80, 82]. Importantly, some of the studies have also described the biomedical efficacy of these compounds. For example, Alam et al. [4] demonstrated the bacteriocidal activity of pongaglabol to the human pathogens *S. flexneri*, *Salmonella typhi*, *Staphylococcus aureus*, and *Streptococcus haemolyticus*. Similarly, Simin et al. [67] demonstrated both antifungal and antibacterial activity of two *Pongamia* derivatives pongarotene (a rotenoid) and karanjin (a flavonol) against a wide range of human and animal pathogens. Another interesting outcome of these natural products chemistry studies is the synthesis of a non-toxic polyesteramide from seed oil that has applications as an anticorrosive coating material [2].

Pongamia as an Animal Feed Supplement

Of all the by-products of *P. pinnata* with commercial applications, much of the reported research has been associated with the use of deoiled cake as a feed supplement for cattle, sheep and poultry [16, 32–35, 48–51, 62, 70]. Deoiled cake is the leftover component of *P. pinnata* seeds following solvent extraction and as a by-product containing up to 30% protein has the potential to provide a sustainable animal feed supplement. Preliminary work in our laboratory has shown that the seed storage proteins of *P. pinnata* are dominated by two proteins of approximately 51 kDa (Fig. 2a) perhaps related to the 51 kDa 7S beta seed storage protein of soybean (variety Bunya shown here). The characteristic 11S and other 7S components are not very abundant, suggesting lesser nutritional quality of *P. pinnata* seed cake protein.

While the deoiled cake may be a source of protein, it contains a number of toxic and unpalatable components, including the furanoflavones karanjin and pongamol, and other polyphenolic compounds in the residual oil. To overcome this undesirable characteristic, the toxic components may be reduced by soaking the cake in water, autoclaving, alkali treatment and/or ether extraction. *P. pinnata* oil cake also contains protease inhibitors, the activity of which can be eliminated by firstly autoclaving the cake with lime, refluxing with 2% HCl and then neutralizing with NaOH [40]. This treatment strategy also improves the protein digestibility of the seed cake. In cattle the deoiled cake has been trialed as a protein supplement replacing that provided by groundnut cake. Modest weight gains were obtained at levels up to 50% replacement of groundnut cake. For poultry and sheep the poor palatability and toxic effects lead to deleterious effects on mortality, weight gains and other relevant performance indicators (e.g. egg production).

There are reports that leaf material of *P. pinnata* also possesses properties that make it suitable as a potential animal feed supplement. Ramana et al. [61] evaluated the nutritive value of the leaf material of five nitrogen-fixing trees, including *P. pinnata*, and five non-nitrogen-fixing trees, for such properties as dry matter, organic matter, crude protein, neutral and acid detergent fibre, cellulose and hemicellulose, lignin, total phenolics, and tannins. This study was carried out by simple chemical analysis of dried leaves, but also included an in sacco experiment whereby a leaf concentrate was administered to steers fitted with cannulae in order to determine the digestibility of the leaf material in the rumen. In addition to its use as a feed supplement, the leaf material has value as organic or green manure. Muthukumar and Udaiyan [44] evaluated the effect of organic soil amendments, including sunnhemp, cow dung, sheep manure and leaves of *P. pinnata*, on arbuscular mycorrhizal numbers, the formation of mycorrhizae, and growth and yield of cowpea (*Vigna unguiculata*). As an organic amendment, *P. pinnata* had positive effects on soil N, P and K, mycorrhizal formation on cowpea roots, root and shoot dry weight, nodulation, and pod and seed number.

Pongamia pinnata, a Renewable and Sustainable Source of Biodiesel

At a time when society is becoming increasingly aware of the declining reserves of oil for the production of fossil fuels, it has become apparent that biofuels are destined to make a substantial contribution to the future energy demands of the domestic and industrial economies. *Pongamia pinnata* will impact most significantly through the extraction of seed oil for use in the manufacture of biodiesel. The potential of *P. pinnata* oil as a source of fuel for the biodiesel industry is well recognized [8, 20, 29]. Moreover, the use of vegetable oils from plants such as *P. pinnata* has the potential to provide an environmentally acceptable fuel, the production of which is greenhouse gas neutral, with reductions in current diesel engine emissions [58]. Importantly, the successful adoption of biofuels is reliant on the supply of feedstock from non-food crops with the capacity to grow on marginal land not destined to be used for the cultivation of food crops (c.f., [24]). In this regard *P. pinnata* is a strong candidate to contribute significant amounts of fuel feedstock, meeting both of these criteria.

Existing feedstocks such as palm oil and canola are costly (~€600 per ton and ~€550 per ton, respectively),

making the production of biodiesel economically marginal (in Australia diesel currently has a retail price of €0.9 per litre). Sources such as tallow and waste oil from food outlets are seen as variable in availability and/or of low quality. For example, the dominant fatty acid of tallow is $C_{18:0}$ leading to a high cloud-point diesel, necessitating B5 or B10 mixtures with crude oil diesel. In Australia, the unmet demand for a reliable feedstock has lead to a "mothballing" of industrial production facilities and placed the ailing biodiesel industry in a precarious position. Clearly, there is an opportunity for *Pongamia* to fill this unmet demand, not only in Australia but in comparable agricultural regions. Table 1 summarises the biological and agronomic features of *Pongamia* that characterize the suitability of this tree crop for biodiesel production.

Fatty acids are the products of seed cotyledon metabolism, which takes sucrose derived from photosynthesis and converts it into three major storage components, namely protein, starch and fatty acids. Fatty acids are synthesised by a well defined pathway involving two carbon elongation (by elongases) and bond desaturation (Fig. 2d). Oleic acid is viewed as an optimal fatty acid for biodiesel production as it generates a low cloud-point fuel. Palmitic and stearic acids increase the cloud point, as these molecules have less mobility. More unsaturated C18 acids (C18:2 and C18:3) are less desirable as oxidation occurs. The seeds of *P. pinnata* contain 30% to 40% oil [8, 45], which can be converted to biodiesel (fatty acid methyl esters; FAMEs) by esterification with methanol in the presence of KOH. The predominant fatty acid is oleic acid ($C_{18:1}$; 40% to 55%) with palmitic acid ($C_{16:0}$; 5% to 15%), stearic acid ($C_{18:0}$; 5% to 10%) and linoleic acid ($C_{18:2}$; 15% to 20%), and to a much lesser extent arachidic acid ($C_{20:0}$), eicosanoic acid ($C_{20:1}$), behenic acid ($C_{22:0}$) and lignoceric acid ($C_{24:0}$; Fig. 2c). The composition of the seed oil and the properties of the FAMEs meet North American and European industry standards [8, 29]. These properties include the saponification number (196.7 for *P. pinnata*), which indicates the relative fatty acid chain length of the FAMEs; the iodine value (80.9 for *P. pinnata*), which is a measure of the total number of double bonds amongst the respective fatty acids; and the cetane number (55.84 for *P. pinnata*), which gives an indication of ignition quality of the fuel. Other important properties of *P. pinnata* FAMEs are the viscosity (3.8 to 4.8 mm^2/s at 40°C), flash point (135 to 150°C), pour point (2.1°C), and cloud point (8.3°C). Of these, the pour point, which is the lowest temperature at which oil will flow, and the cloud point, which is the temperature that will lead to separation of dissolved solids from the oil, are critical to the implementation of biodiesel use in temperate and cold climates. In the case of biodiesel from *P. pinnata* the values for the pour and cloud points are satisfactory for tropical and some temperate regions. However, if this product is to find a market in cool and cold regions there needs to be improvement in these properties. Nonetheless, with respect to the cloud point of biodiesel derived from other sources *P. pinnata* compares favourably with palm oil (10°C) and beef tallow (13°C) but less so with soybean (−1°C), rapeseed (−7°C) and sunflower (1°C) [26]. Improving the physico-chemical properties of biodiesel derived from the oil of *P. pinnata* will require a comprehensive understanding of seed oil biosynthesis and the probable modification of seed oil composition through genetic manipulation.

Table 1 Agronomic predictions for *Pongamia* biodiesel production

	Agronomic predictions
Biological	~40% seed oil content
	~50% $C_{18:1}$ content
	~20,000 seeds per year (10 year old tree)
	~1.8 g per seed
	~1.8 g pod wall (for biomass applications)
	~25% protein/starch meal (for biomass or animal feed supplement)
	~20 tons CO_2 sequestered per hectare
	~5 m tall trees within 5 to 7 years
Farm management	Tree spacing of 5 m
	350 trees per hectare
	Mechanical harvesting of seed pods
	Establishment/production costs ~€600 per hectare
	Maintenance costs ~€60 per hectare per annum
	Oil extraction ~€48 per ton

In meeting the future demands for biodiesel it will be important to establish extensive plantations comprising elite varieties of trees. Azam et al. [8], in discussing the potential of seed oils for biodiesel production on wasteland in India, calculated the area of land required for sufficient production to replace the demand met by current fossil fuel supplies. In comparison with *Azadirachta indica* (4.10×10^6 ha), *Calophyllum inophyllum* (2.33×10^6 ha), *Jatropha curcas* (4.38×10^6 ha), and *Ziziphus mauritiana* (7.98×10^6 ha), *P. pinnata* compares very favourably, requiring 1.99×10^6 ha to meet 10% replacement of fossil fuel derived biodiesel.

The challenging task of establishing *P. pinnata* as a premium feedstock crop for the emerging biofuels industry will require tools in the fields of genetics, molecular biology, plant propagation and agronomy that will enable this now important legume to be fully characterized, and for optimal yields of oil to be achieved. At the ARC Centre of Excellence for Integrative Legume Research we have recently initiated a research program to address most of these issues. We are adopting a molecular biology and genetics approach to characterize the genome of *P. pinnata*, with particular emphasis on population diversity and the genes associated with oil biosynthesis.

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