Genetic and Genomic Analysis of the Tree Legume *Pongamia pinnata* as a Feedstock for Biofuels

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Abstract

The tree legume Pongamia {*Pongamia pinnata* (L.) Pierre [syn. *Millettia pinnata* (L.) Panigrahi]} is emerging as an important biofuels feedstock. It produces about 30 kg per tree per year of seeds, containing up to 55% oil (w/v), of which approximately 50% is oleic acid ($C_{18:1}$). The capacity for biological N fixation places Pongamia in a more sustainable position than current nonlegume biofuel feedstocks. Also due to its drought and salinity tolerance, Pongamia can grow on marginal land not destined for production of food. As part of the effort to domesticate Pongamia our research group at The University of Queensland has started to develop specific genetic and genomic tools. Much of the preliminary work to date has focused on characterizing the genetic diversity of wild populations. This diversity is reflective of the outcrossing reproductive biology of Pongamia and necessitates the requirement to develop clonal propagation protocols. Both the chloroplast and mitochondrial genomes of Pongamia have been sequenced and annotated (152,968 and 425,718 bp, respectively), with similarities to previously characterized legume organelle genomes. Many nuclear genes associated with oil biosynthesis and nodulation in Pongamia have been characterized. The continued application of genetic and genomic tools will support the deployment of Pongamia as a sustainable biofuel feedstock.

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Dedicated bioenergy crops as feedstocks for biofuel production and as alternatives to fossil fuels have been at the forefront of discussions regarding future sustainability of energy and resources for many years. The arguments for the exploitation of biomass to meet an increasing demand for energy are many, any one of which is substantive. First, it is widely accepted that the global status of peak oil, where the demand for fossil fuels is in excess of the capacity to meet this demand, has either been passed, is current, or is soon to be reached. Of particular immediate concern is the demand for liquid fuels, which makes up the predominant component (70 to 80%) of energy consumption (BP, 2012). While the currently predicted production levels of biofuels are unlikely to completely replace all sources of fossil fuels, there is certainly an expectation that biofuels will be able to meet a significant proportion of the foreseeable demand for liquid fuels. With respect to the potential of a future biofuels industry in Australia that includes Pongamia as one of the feedstock species, it is expected that seed-borne Pongamia oil will be able to meet a substantial proportion of the domestic diesel fuel demand. Based on an annual production of 3 to 5 t of oil ha^{-1} and the expectation that initial formulations of biodiesel will include 80% mineral diesel, approximately 7000 to

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Abbreviations: ACP, acyl carrier protein; CoA, coenzyme A; cpDNA, chloroplast DNA; EC, electrical conductivity; FA, fatty acid; FAME, fatty acid methyl ester; GFP, green fluorescent protein; GHG, greenhouse gas; IR, inverted repeat; ISSR, inter-simple sequence repeat; LAP, low agriculturally productive; mtDNA, mitochondrial DNA; NEB, net energy balance; PISSR, Pongamia inter-simple sequence repeat; PUFA, polyunsaturated fatty acid; RNA, ribonucleic acid; RNAi, ribonucleic acid interference; rRNA, ribosomal ribonucleic acid; RuBisCO, ribulose-1,5-bisphosphate carboxylase oxygenase; tRNA, transfer ribonucleic acid; TAG, triacylglyceride.