

## Genetic, Biochemical, and Morphological Diversity of the Legume Biofuel Tree *Pongamia pinnata*

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### Abstract

*Pongamia pinnata* is regarded as a sustainable biofuel feedstock of the future because of its abundant production of oil-rich seeds, tolerance to abiotic stress, and ability to undergo biological nitrogen fixation (minimizing nitrogen inputs). However, it needs extensive domestication through selection and genetic improvement. Owing to its outcrossing nature, *Pongamia* displays large phenotypic diversity, which is advantageous for selection of desirable phenotypes but problematic for plantation management. In this study, variation was evaluated for seed mass, oil content, and oil composition. To evaluate genetic diversity and to lay the basis for a molecular breeding approach we developed second generation sequencing (2GS)-derived ISSR markers (*Pongamia* Inter-Simple Sequence Repeats; PISSR). The special feature of PISSRs is that the number of nucleotide repeats and the 5' and 3' nucleotide extensions were not arbitrarily chosen, but were based on *Pongamia* genomic sequences obtained from a NGS (Illumina®) database. Amplification products were resolved by polyacrylamide gel electrophoresis and silver staining or automated capillary electrophoresis to yield distinct and reproducible profiles. Polymorphic bands were excised from polyacrylamide gels and sequenced to reveal similarity to DNA sequences from other legumes. We demonstrated: 1) an abundance of nucleotide core repeats in the *Pongamia* genome, 2) large genetic and phenotypic diversity among randomly sampled *Pongamia* trees, 3) restricted diversity in progeny derived from a single mature tree; 4) sta

bility of PISSR markers in *Pongamia* clones; and 5) genomic DNA sequences within PISSR markers. PISSRs provide a valuable biotechnology tool for assessment of genetic diversity, gene tagging and molecular breeding in *Pongamia pinnata*.

**Keywords:** Biofuel, *Millettia*, molecular marker, PISSR, plant oil, *Pongamia*.

**Abbreviations:** CE: Capillary electrophoresis; PAGE: polyacrylamide gel electrophoresis; SS: silver staining, PISSR: *Pongamia* Inter-Simple Sequence Repeat; 2GS: Second generation sequencing.

### Introduction

*Pongamia pinnata* (L.) Pierre (also known as *Millettia pinnata* or Indian Beech, and hereafter referred simply referred to as *Pongamia*) is a perennial, fast-growing, leguminous tree, widely distributed on the Indian subcontinent, south-east Asia, Oceania, northern Australia, the East-African coast and southern China (Murphy et al., 2012). In addition *Pongamia* has been introduced to other parts of the world, including the United States (Hawaii, Florida; Scott et al., 2008; Biswas et al., 2011; Kazakoff et al., 2011). As a nodulating and nitrogen-fixing legume the nitrogen (N) fertilizer requirements of *Pongamia* are potentially minimal or eliminated. This is of advantage for a biofuel

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# The last two authors made equal contribution to the research.