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FULL-LENGTH RESEARCH ARTICLE

Nodulation in the Legume Biofuel Feedstock Tree *Pongamia pinnata*

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Abstract The legume tree *Pongamia pinnata* (also called *Millettia pinnata*) is gaining importance as a biofuel feedstock tree because of the abundant annual production of oil-rich seeds, adaptation to a wide range of geoclimatic conditions and significant resistance to abiotic stress, such as water-deficit, salinity and acidity of soils. The major defining benefit of using pongamia as a biofuel feedstock is that it is a legume, enabling biological nitrogen fixation through symbiosis with soil bacteria, collectively called rhizobia, which results in root nodulation. Here, we report preliminary data, (i) indicating the range of rhizobia that can form nodules on pongamia, (ii) demonstrating the measurement of nitrogen fixation activity of pongamia nodules via the classical acetylene reduction assay, (iii) illustrating nodule morphology and development and (iv) demonstrating aspects of nodule regulation by external nitrate as well as internal autoregulation of nodulation. We note that in pongamia most nodulation-related characteristics are similar to those found in other annual crop legumes such as soybean.

Keywords Rhizobia · Legume · Biodiesel · Autoregulation of nodulation · Nitrogen fixation

Introduction

Pongamia pinnata (also called *Millettia pinnata*; hereafter referred to as pongamia) is a medium-sized tree legume native to India, Malaysia, northern Australia and Indonesia [33]. Large diversity exists [17]. The tree is generally considered to be a fast-growing, saline- and drought-tolerant species and is able to grow in a range of suboptimal conditions, including water-logged and alkaline soils [10].

Historically used as a medicinal plant, green manure and a source of heating fuel in India [1], pongamia is a non-edible crop that has recently become of interest to the renewable energy industry for its ability to produce seeds with an oil content of approximately 30–50 % [4, 17]. Pongamia's attributes as a sustainable feedstock for biofuel production stem from its (i) high annual yield potential (up to 90 kg of seed per tree per year), (ii) high oil content per seed (up to 54 % oil comprising approximately 55 % oleic acid; C18:1), (iii) silvicultural adaptability, including growth on so-called marginal land, (iv) absence of human food value and (v) legume biology allowing symbiotic nitrogen fixation [1, 12, 18, 22].

The latter point is important in the evaluation of a feedstock species for biofuel [16], as all plants require reduced nitrogen for protein and nucleotide synthesis, and general metabolism. Most legume species, including many annual food crops of soybean, pea, lentil, bean and peanut, and trees such as *Acacia* spp. have the ability to form root nodules via nitrogen-fixing symbioses with soil bacteria, collectively called rhizobia. In contrast, the common biofuel feedstocks sugarcane, canola, sweet sorghum, maize, and woody trees (e.g. eucalypts and willows) do require nitrogen to be supplied as a reduced form, usually nitrate, urea or ammonia. The production and application of nitrogen fertilisers represents a large economic and energetic burden as costs have increased due to a dependence on fossil fuel and natural gas. Moreover, the application of

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