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Isolation and Characterization of Circadian Clock Genes in the Biofuel Plant Pongamia (*Millettia pinnata*)

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Abstract The increasing human population has led to an inevitable increase in global energy demands. In the recent decades, biofuels have emerged as one of the potential solutions to the world's insatiable energy needs while reducing the high reliance on fossil fuels. Pongamia (*Millettia pinnata*), a nitrogen-fixing tree legume, has shown a great promise as an oil source for the production of biofuel with economical and environmental benefits. The generation of pongamia-derived biofuel is dependent on the success of flowering and seed development. However, molecular control of floral initiation pathways in pongamia remains largely unexplored. Photoperiod pathway has been reported to be one of the major checkpoints of plant flowering time and flower initiation. The circadian clock pathway, a part of the photoperiod pathways, is one of the key regulators of flowering time. Here, we report the identification of four pongamia circadian clock genes (*ELF4*, *LCL1*, *PRR7*, and *TOC1*) through the mapping of the pongamia transcriptome short-paired reads library, by using soybean circadian clock genes as the reference sequences. Furthermore, multiple alignments and phylogenetic analyses suggested that pongamia clock genes are conserved among legume crops such as soybean, *Medicago*, and garden pea. Gene expression studies highlight that pongamia circadian clock genes are diurnally regulated under long-day conditions. Thus, this study reports the isolation and characterization of circadian clock genes in pongamia and enhances our understanding of the molecular mechanism of flowering control in pongamia.

Keywords Circadian clock · Pongamia · Soybean · Biofuel · Flowering

Introduction

A substantial increase in the human population has led to an inevitable increase in energy requirements. For example, global energy consumption rose by 2.5 % in 2011 and 1.8 % in 2012 [1]. As fossil fuels account for 87 % of the world's energy consumption, the increased utilization resulted in the production of more harmful greenhouse gases, such as carbon dioxide (CO_2) and nitrous oxide (N_2O), which led to the observable global climate change [2]. Although the demand for crude oil rose by only 0.9 % in 2012, crude oil is still the world's primary fossil fuel, accounting for 33.1 % of global energy use [1]. Due to the increasing energy demand every year, oil reserves that took millions of years to form are being depleted much more rapidly, leading to the concern that the currently available sources of fossil fuels will be exhausted in the near future [3]. Accordingly, there is a pressing need to find alternative energy sources to fulfill the world's insatiable energy demand [4]. For the past decades, biofuels have emerged as one of the potential solutions to the world's growing energy needs.

Ideally, the sources of such biofuels will have minimal impact on the environment, global food supplies, and land and water use; tree legumes have been considered strong candidates as biofuel sources that meet the aforementioned

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