



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI  
SHORT ABSTRACT OF THESIS

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The diminishing fossil fuel stock and soaring international crude oil price have renewed the interest in the alternative source of fuels. Oil from oilseed crops that are largely in the form of triacylglycerol (TAG) are the promising source of renewable supply of fuels in the form of biodiesel. *Jatropha curcas* L is an important non-edible oilseed crop which received worldwide attention as a biodiesel feedstock. Despite the significance of *Jatropha* seed oil as a potential source of biodiesel, not much research efforts have been made through breeding or transgenic approaches to improve its seed oil quality for sustainable biodiesel production. Transgenic approaches offer immense opportunities to improve oil content and quality through manipulation of oil biosynthetic pathway in both seed and leaves. Since DGAT1 is the only enzyme that is exclusively committed to TAG biosynthesis in Kennedy pathway, direct up-regulation of Kennedy pathway through overexpression of the DGAT1 enzyme responsible for the last and

only committed step in seed TAG biosynthesis is promising. In the present study, transgenic *Jatropha* plants were developed that accumulates a high level of TAGs in seeds as well as in leaves by the overexpression of *Arabidopsis DGATI*. In this work, the CaMV35S promoter was used because of its strong and constitutive nature in regulating transgene expression to enable ectopic overexpression of *AtDGATI* in leaves as well as in seeds. The enhanced TAG accumulation in transgenic *Jatropha* lines had no penalty on the growth rates, growth patterns, leaf number, and leaf size of plants. Enhanced expression of *AtDGATI*, in transgenic *Jatropha* lines, appears to have increased the total lipid content by 1.5 to 2 fold in leaves and 20-30 % in seed kernels. In the transgenic line (TR1), enhancement in DGAT1 activity resulted in an increase in total lipid content by two-fold in leaves and 30% in seed kernels. This increment was accompanied with no significant change in protein content in leaves, but a minor increase in protein content in seed kernels was found in the transgenic plants. In contrast to proteins in leaves and seeds, a significant increase in carbohydrate content in leaves and a marked decrease in carbohydrate content in seeds of transgenic lines were observed. These results suggest that increased accumulation of total sugar may have contributed to the reallocation of precursor for enhanced TAG synthesis in transgenic leaves. Carbohydrates are important osmotic solutes in leaves and seeds and are potentially involved in the carbon source transformation to lipids. Previous studies suggested that carbohydrate content in seed was correlated with seed oil contents. However, the reallocation of precursors for photosynthesis to TAG biosynthesis is more in leaves than seeds of transgenic *AtDGATI* *Jatropha* lines, and possibly the contribution to TAG biosynthesis in these transgenic lines by leaves are more than seeds. The mechanisms underlying these changes need to be addressed in future investigations.

The second phase of investigation involved comprehensive metabolite profiling by LC-FT-ICR-MS used to study the consequences of the *AtDGATI* gene transfer on *Jatropha* metabolites. The metabolite analysis allowed a complete assessment of the plants metabolic status following genetic manipulations. It was found that sucrose concentration was decreased in all transgenic lines, which indicates towards utilization of carbon source was significantly enhanced in transgenic lines. Several lipids such as fatty acyls, glycerophospholipids, glycolipids, sterol lipids, sphingolipids, PI, PE, and apart from that many flavonoids, alkaloids, and other secondary metabolites were identified. The abundance of these lipid derivatives indicated significant changes in metabolic profiles between the transgenic lines and wild-type *Jatropha curcas*. Characterization of these metabolic patterns also emphasized several interesting differences among the transgenic lines. The vast array of lipids and their derivatives, flavonoids, and other secondary metabolites tentatively identified in this study suggest an important role for the metabolite class in *Jatropha* oil biosynthesis pathways.