Abstract

The seed fat of Mahua (*Madhuca latifolia*), a tropical non-conventional oil seed plant, has been found to be essentially composed of oleate, palmitate and stearate at all developmental stages. While the level of palmitate at any developmental stages was more than the stearate, the developmental profile of these two fatty acids during seed maturation showed that palmitate content diminishes where as stearate content and oleate content increase all through these stages. To investigate the possible role of any fatty acyl-ACP thioesterase (FAT) that could be responsible for the accumulation of fat (commonly known as *mowrah* fat) in its seed, FAT activities in the seed extracts were analyzed. The FAT activities in the seed extracts were found to be 100:31:8 towards three thioester substrates, *viz.*, oleoyl-, stearoyl- and palmitoyl-ACP, respectively. The accumulation of palmitate in the triacyl glycerol of *M. latifolia* has been documented to be due to the activity of house keeping LC-FAT. In addition to the house keeping oleoyl- preferring LC-FAT, presence of a stearoyl/oleoyl preferring thioesterase (SO-FAT) conscripted for the accumulation of stearate in *M. latifolia* could be identified. PCR based cloning of the acyl-ACP thioesterase from *Madhuca latifolia* (*Ml*) resulted in the identification of a thioesterase locus in the genome and isolation of a cDNA clone (which would encode for the SO-FAT) of the FAT gene (*Ml Fat*). The gene with the help of a plant promoter could be transgenically expressed in a heterologous plant system, *Brassica juncea*. Transgenic expression of *MlFat* gene into *Brassica juncea* leaves showed that gene *MlFat* is capable of altering the fatty acid profile of *Brassica* sp towards both stearate and oleate to a significant extent. The capacity to increase the stearate and oleate content in transgenic *Brassica* leaves matches well with the thioesterase specificities of SO-FAT in the partially purified *M. latifolia* seed extract, as observed. This in turn indicated that the gene (*MlFatB*) is not only functioning for FatB type but can also function for

FatA type LC-FAT. The phylogenetic lineage analysis revealed that the gene is of *FatB* type. Southern hybridization revealed further that the *FatB* gene is present as a single copy in *Madhuca latifolia* genome. The presence of both *FatB* and *FatA* trait of *MlFatB* gene (considering the enzymatic specificity of SO-FAT and the efficacy of *MlFatB* gene, as evident from the fatty acid profile of transgenic *Brassica* leaves) has been considered to be most significant on the basis of "subfunctionalization" hypothesis. This in turn is in agreement with the hypothesis that duplicated genes are redundant in function. Studying the synonynomous and non-synonymous substitution rate of the different thioesterases (both FatA and FatB type) revealed that the duplication of *FatB* and *FatA* paralogs has been subjected to purifying selection. The same study further revealed that *FatB-FatA* paralogous pairs is distinguishable from the *FatA-FatA* (having Ka<0.5) or *FatB-FatB* (having Ka>0.5) orhologous pairs, on the basis of their 'Ka' values.