# Abstract

Polyunsaturated fatty acids are the key components of cell membranes and influence membrane fluidity as well as a range of properties of membrane-bound receptors and enzymes. They are generated by the activities of fatty acid desaturases namely, omega-6 (Fad2), omega-3 (Fad3) and Δ6 desaturases. Microsomal Fad3 being methyl-ended desaturases that convert linoleic acid to α-linolenic acid, whereas the Δ6 desaturases are the front-end desaturases responsible for conversion of linoleic acid to γ-linolenic acid and α-linolenic acid to stearidonic acid. In the present study, a putative *Fad3* gene was cloned from the developing seed tissues of Indian mustard (*Brassica juncea*) by RT-PCR approach and the sequence was analyzed by bioinformatics study. The *BjFad3* gene was found to encode a methyl-end directed desaturase having three histidine-rich motifs, four transmembrane domains and an endoplasmic reticulum retrieval motif similar to other microsomal Fad3 desaturases reported from a few plants. The *BjFad3* gene was heterologously expressed in model plant tobacco and its stable integration in the genome of transgenic tobacco plants was confirmed by Southern hybridization. The analyses of fatty acid profiles from transgenic seeds by gas chromatography showed that α-linolenic acid content of the transgenic seed lipids increased significantly in comparison to the untransformed seeds. This is due to the omega-3 desaturase activity of BjFad3. Further, it was observed that the BjFad3 enzyme has broad range of substrate specificity involving in desaturation of palmitic acid (C16:0), and oleic acid (C18:1) as evident from the fatty acid profile of transgenic tobacco seeds. In an analogous study, a putative Δ6 desaturase (*D6*) gene without intron was cloned from the genomic DNA of a local cultivar of *indica* rice (*Oryza sativa*) by PCR approach. Sequence analysis of *OsD6* revealed that the gene encodes a front-end desaturase as it possesses the N-terminal cytochrome $b_5$ domain (-HPGG), three histidine rich boxes and a histidine to glutamine residue replacement in the third histidine box which are characteristics of other front-end desaturases. The *OsD6* gene product upon heterologous expression in model plant tobacco modified the seed fatty acid profile by producing γ-linolenic acid, albeit in low level. In addition to finding and characterizing two desaturase genes, search for alternative crops whose seed fatty acid profile is suitable for generation of polyunsaturated fatty acids is being carried out in this study. Sesame (*Sesamum indicum*) is considered one such important crop. However, *in vitro* successful shoot regeneration and genetic transformation strategies are yet to be established in this crop. The present research work also includes optimization of *in vitro* plantlet regeneration and a transient gene expression protocol in sesame. Of all the explants tested in this study for *in vitro* shoot regeneration, transverse thin cell layers (tTCLs) were found to be the best explants, first time reported for *S. indicum*. Several parameters like age of mother plant, position of the internode from where explants were taken, thickness of the tTCL sections and phytohormones used in media play key role in increasing the shoot regeneration efficiency of the explants. This work also leads to development of a transient protocol for gene expression which is potentially suitable for studies on gene regulation, protein localization and other molecular aspects in sesame plant.

Key words: PUFA; Fatty acid desaturase; Methyl-end desaturase; Front-end desaturase; Cytochrome $b_5$; Gas chromatography; Fatty acid profile; Shoot regeneration; Somatic embryogenesis; Sesame; *Brassica*; Rice; Tobacco; Transverse thin cell layer