ABSTRACT

Based on the nutritional aspects, the oleaginous crop peanut (*Arachis hypogaea L.*) has higher commercial potential than other traditional oil crops of India. Depends up on the climatic and geographic features, the country has been practicing the cultivation of more than one hundred varieties of this crop. Out of the hundred varieties the most prominent twelve cultivars of the spanish group peanuts were identified for the present investigation: K134, DRG12, CO4, AK159, DRG17, VRI2, VRI3, VRI4, GG7, JL220, TMV7 and TMV13.

Peanut oil has got a high percentage of unsaturated lipid with predominant amount of oleic acid (O) 46.74% and linoleic acid (L) 28.91%. Hence the OL ratio of peanut has considered as an indicator for determining the nutritional status of the crop and the stability of the oil. The focus of the present investigation was to elucidate the functional features of the omega 6 fatty acid desaturase gene (FAD2), of *Arachis hypogaea L*. Fatty acid desaturase (FAD2) is the enzyme responsible for the formation of polyunsaturated fatty acid linoleic acid from the monounsaturated fatty acid oleic acid. In the initial phase of the study, the oil was extracted from the twelve cultivars and subjected to GC analysis for determining the fatty acid profile and lipid composition. From the GC data a comparative level of OL ratio was determined. Out of the twelve varieties subjected to GC analysis, K134 exhibited an OL ratio of 1:0.88 and the
variety VRI4 showed 1:0.5. In the entire analysis, soybean - the most popular oil crop with similar lipid composition was used for a comparison with the fatty acid profile of peanut. The soybean oil was subjected to GC analysis and its OL ratio was found as 1:1.57 indicating the highest content of polyunsaturated fatty acid linoleic acid.

Based on this analytical data a molecular comparison was done with respect to FAD2 - the marker gene responsible for the synthesis of linoleic acid from oleic acid. RNA of the twelve cultivars of peanut was isolated and the full coding FAD2 gene of size 1255 bp was amplified using the primers designed from the accession of EF192432. The amplicon of the FAD2 gene was cloned and sequenced. All the sequences were analysed for structural analysis by bioinformatic approach for determining the SNPs and associated amino acid substitutions. An effort was taken for comparing the SNPs of the FAD2 gene and associated amino acid variations of varieties with the fatty acid profile preferably the OL ratio, in order to understand the interference of the nucleotide polymorphisms and peptide sequence variations in the formation of linoleic acid. No correlation was observed in the linoleic acid content of the twelve varieties with that of the SNPs and amino acid variations of the gene, indicating the non interference. Subsequently the FAD2 gene of peanut varieties was subjected to genetic comparison with the already reported two alleles of the gene, FAD2A and FAD2B. Out of the twelve varieties, ten varieties were showed close genetic relatedness with that of FAD2A and the other two varieties stands similar
with FAD2B. Phylogenetic analysis of the varieties further supports the grouping of the ten varieties into FAD2A and the other two into FAD2B. The observation of the tripartite histidine box in all the sequences of FAD2 gene strongly support the functional stability of the gene in synthesizing linoleic acid. Based on the structural variations of the gene sequences observed in the twelve varieties, the gene sequences were deposited and registered in NCBI.

Subsequent to the bioinformatic data, the expression level of FAD2 gene of the twelve varieties was subjected to relative quantitation assay using realtime PCR. The housekeeping gene - actin depolymerizing factor (actin DF) was used as the reference gene. Prior to the quantitation study, the FAD2 amplicon of size 158 bp and actin DF of size 106 bp was amplified using the specific primers. From the realtime data it was observed that the variety K134 showed highest expression value of 2.01 and the lowest expression was found in TMV7 as 1.06. A positive correlation was observed between the quantitative gene expression of FAD2 with linoleic acid content of the cultivars. Thus the functional involvement of FAD2 enzyme in the synthesis of linoleic acid was confirmed by the molecular data.

Based on the relative quantitation of FAD2 gene, the gene was expressed in a eukaryotic system using pOREE2 vector with CaMV35S as constitutive promoter in the host plant peanut. The focus of the study was to over express FAD2 gene in the plant insitu using a constitutive viral promoter. Agrobacterium
(EHA105), mediated transformation was done and the recombinant plasmid was allowed to grow in the cultured peanut cells. The efficiency of the expression of FAD2 gene using the constitutive promoter was compared with the normal FAD2 gene of peanut by absolute quantitation using qPCR. The attempt of over expressing FAD2 gene of peanut (FAD2Pn) by using a constitutive viral promoter has no direct effect in inducing the expression. Further studies were needed to substantiate this incompatible nature of the gene using external promoter for over expression. An attempt was made to transfer the FAD2 gene of soybean (FAD2Sb) to peanut for enhancing the linoleic acid content. The FAD2Sb was transformed into cell suspension cultures of peanut using agrobacterium mediated transformation. The effective transformation of the gene into peanut cells was confirmed by PCR assay. The expression level of recombinant FAD2Sb in peanut was measured by absolute quantitation comparing with FAD2 gene in normal peanut cells. The absolute quantitation of FAD2Sb in peanut cell suspension cultures indicate a higher level of expression with a copy number of 1.5x10^{-02} ng, than that of the copy number of normal peanut - 2.12x10^{-04} ng. The sound difference in gene expression realizes the fact that the FAD2Sb in peanut can exhibit more expression than the normal peanut. Thus the data positively highlights the possibility of developing a transgenic peanut with higher amount of polyunsaturated fatty acids (PUFA) linoleic acid that could be a boon in the utilization of peanut in the nutritional point of view.