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Evolutionary relationship of legumes: A phylogenetic analysis approach using DNA sequence

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Abstract

Evolution is eminent for all living organisms which determines how the organism will change and adapt to the ever changing external environment. This provides divergence to different species and helps in creation of new ones. The organisms that we see at present have evolved from one or other ancestor. This study was held in *Rabi* 2021 to decipher the evolutionary relationships between different members of Fabaceae. Phylogenetic analysis of *TFL* sequence from different members of Fabaceae revealed that *Lablab purpureus* is closely related to *Phaseolus vulgaris* in terms of evolutionary history of *TFL*. The analysis pointed out that *TFL* loci of Indian bean and common bean have evolved from cowpea. While, *TFL* loci of soybean, groundnut and cowpea showed separate lineage.

Keywords: Determinate (DT), indeterminate (IDT), Terminal flowering locus 1 (TFL), Phylogenetics

Introduction

Indian bean [Lablab purpureus (L.) Sweet] (2n = 22) is native to Africa belonging to Fabaceae family. Grown for green pods, dry seeds and as fodder throughout the tropics and sub-tropics of Asia and Africa; it is hardy, drought tolerant and suitable for growing as a rainfed crop. In general, the cultivated species are annual or short-lived perennial vines, but the wild species are perennial. Genotypes are distinguished based on differences in shape, size and colour of pods, flower and leaves. It is important source of protein (25 to 30%) and can fix atmospheric nitrogen in soil with a symbiotic relationship with nitrogen fixing bacteria. TFL1 acts as a repressor for floral initiation and maintains the inflorescence meristem through suppression of the expression of APETALA1 (AP1) and LEAFY (LFY) (Boss, 2007; Bradley, 1997; Nilsson et al. 1998, Ohshima et al 1997) ^[7, 8, 9, 10] which inhibits flowering and imparts continued growth of vegetative axis that causes indeterminate growth. Indian bean includes two different types of growth habits which are Determinate (DT) and indeterminate (IDT). This change in growth habit is conferred by the changes in a specific gene which is Terminal Flowering Locus 1 (TFL). This gene in Indian bean is named as LprTFL. This study aims to undertake the phylogenetic analysis of LprTFL with other members of the Fabaceae family. Phylogenetics enables the understanding of how nucleotide sequences, genes, genomes and species evolve through time. It not only gives the idea about how the sequences came to be the way they are today, but also general principles that enable us to predict how they will change in the future. Less experimentation in Indian bean has made it an orphan crop. The present work is an attempt to understand the evolutionary relationship of *LprTFL* with other *TFL* in Fabaceae.

Materials and Methods

The *LprTFL1* sequence was used as query for BLASTn at https://blast.ncbi.nlm.nih.gov/Blast.cgi for finding homologous sequences with reference to NCBI (National Center for Biotechnology Information) nucleotide database (Altschul et al. 1997) ^[1]. Sequences showing matches with LprTFL1 from Fabaceae family were retrieved from NCBI nucleotide database. MEGA X (Molecular Evolution Genetics Analysis) software was used to align TFLI nucleotide sequences of different species from Fabaceae family using the CLUSTAL W alignment algorithm according to Tamura et al. (2021)^[5]. FASTA sequence of these legume species were downloaded from NCBI. All the alignment settings were employed at default values. The nucleotide substitutions selected with complete deletion of gaps or missing data were used to analyse sequences. The phylogenetic tree was inferred using Maximum Likelihood method based on Tamura 3 parameter model (Gamma) (Tamura and Nei, 1993)^[2].

The initial tree was inferred with default setting using Neighbour Joining method and Nearest-Neighbour Interchange was used as ML heuristic search method. The reconstructions of phylogenetic trees were conducted using Maximum Likelihood Method. Bootstraps with 1000 replicates for Poisson correction model were performed to assess node support (Felsenstein, 1985)^[6]. The best scoring ML tree was searched simultaneously to represent the evolutionary history of the genotypes tested.

Results and Discussion

MEGA X (Molecular Evolution Genetics Analysis) software was utilized to align TFL nucleotide sequence of 15 different members of Fabaceae including Indian bean using Clustal W multiple alignment. The reconstructions of phylogenetic trees were conducted using Maximum Likelihood Method (Kumar et al., 2018)^[4]. Bootstraps with 1000 replicates for Poisson correction model were performed to assess node support. TFL genes from several plant species belonging to Fabaceae family were compared to LprTFL and it showed that the LprTFL gene displayed a high level of similarity among the plant species. It showed the maximum closeness to Phaseolus *vulgaris TFL1y* suggesting evolutionary closeness of the gene among these two species. In addition, both revealed to be related to Vigna unguiculata which is staggering as Vigna unguiculata does not share evolutionary closeness with other members of the same genus viz., Vigna radiata, Vigna umbeletta and Vigna angularis. Previous studies have also shown closeness of Lablab purpureus to Vigna unguiculata and their predictable evolution from Glycine max in Phaseoleae clade (McClean et al., 2010)^[3]. Specie pairs which were found to be closely related were Glycine max and Glycine soja, Cicer arietinum and Pisum sativum, Vigna radiata and Vigna umbeletta, Lupinus angustifolius and Vigna angularis and Medicago sativa and Trifolium partense. It was astounding to discover that Arabidopsis thaliana TFL shared evolutionary closeness to Arachis hypogea. However LprTFL is least related to Medicago sativa and Trifolium partense TFL.



Fig 1: Phylogenetic tree of *TFL1* gene. The tree was constructed by Maximum Likelihood Method with a 1000-replication bootstrap value using MEGA X software with DT-1 and IDT-1 as queries. The numbers near branch nodes represent the bootstrap value at each branching.

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