The Pharma Innovation Journal 2022; 11(12): 863-865

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(12): 863-865

www.thepharmajournal.com
Received: 22-10-2022
Accepted: 26-11-2022

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A brief phylogenetic analysis of TFL proteins in different plant species relative to *Lablab purpureus* (L.) sweet

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DOI: https://doi.org/10.22271/tpi.2022.v11.i12k.17338

Abstract
To know the origins of any living organism and to know the process of how they came to be as they are today requires a deep study of their evolutionary history and requires tracking of the changes conferred by such evolution. In this aspect, Indian bean has been orphaned out owing to the least efforts made in exploration, well established quality research and quality improvement. In this light, this study was held in *Rabi* 2022 to decipher the evolutionary relationships between different species of plants in terms of divergence/relatedness of TFL (Terminal Flowering Locus) protein among such individuals. Phylogenetic analysis of TFL protein sequence from different members revealed that *Arabidopsis lyrata subsp. lyrata* TFL protein is closely related to *Capsella rubella* but very distantly related to *Nicotiana tabacum* and *Mangifera indica*. The analysis gave out many such evolutionary relatedness between TFL proteins of many plant species.

Keywords: Terminal flowering locus (TFL), phylogenetics evolutionary relatedness

Introduction
Lablab purpureus (2n= 22) belongs to Fabaceae and is a native to Africa but is cultivated vastly in the tropics of Asia, Africa and America. It is used as a vegetable, pulse, lentil, fodder, green manure, green cover crop and as a natural means to fix atmospheric nitrogen in soil. Despite being a hardy and efficacious crop, it has long been neglected which owes to lack of established research, evaluation and evolution information being unavailable to the scientific community. This research is a part of obtaining such information in respect to the evolutionary relatedness of locus governing growth habit in Indian bean *viz.*, TFL. It has been found to be directly involved in control of growth habit in many crops and in Indian bean. TFL plays an important role in the inhibition of premature flower development and maintenance of inflorescence meristem identity. Suppressed expression of floral identity genes is a means by which TFL regulates developmental changes and inflorescence architecture. Accelerated flowering time and suppressed development of indeterminate inflorescences is attributed to the loss of function of TFL. TFL acts as a repressor for floral initiation and maintains the inflorescence meristem through suppression of the expression of APETALA1 (AP1) and LEAFY (LFY) (Boss, 2004; Bradley, 1997; Nilsson *et al*. 1998, Ohshima *et al*. 1997) [2, 3, 8, 9] which inhibits flowering and imparts continued growth of vegetative axis that causes indeterminate growth. Experimentation on this will open up a gateway to use this information for trait manipulation by various tools not only in Indian bean but because of the conserved nature of TFL it could be applied to any crop where such information is available. Phylogeny is a study of relationships between collection of genes, proteins or organisms that are derived from a common ancestor. Phylogenetics enables the understanding of how nucleotide sequences, genes, genomes and species evolve through time. It is undertaken to find evolutionary ties between organisms, to understand relationships between an ancestral sequence and it descendants, to estimate the probable time of divergence between a group of organisms that share a common ancestor and to determine the possibility of direction of evolution in future for any particular group of organisms. 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.

Materials and Methods

The TFL protein sequence was used as query for BLASTp at https://blast.ncbi.nlm.nih.gov/Blast.cgi for finding homologous sequences with reference to NCBI (National Center for Biotechnology Information) protein database (Altschul *et al*. 1997) [1]. Sequences showing matches with TFL were retrieved from NCBI protein database. MEGA X (Molecular Evolution Genetics Analysis) software was used to align TFL protein sequences of different species using the CLUSTAL W alignment algorithm according to Tamura *et al*. (2021) [10]. FASTA sequence of these TFL protein were downloaded from NCBI. All the alignment settings were employed at default values. The protein substitutions selected with complete deletion of gaps or missing data were used to analyse sequences. The evolutionary history was inferred by using the Maximum Likelihood method and Le-Gascuel model (2008) [6] Maximum Likelihood method based on initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+*G*, parameter = 1.6960)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. 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) [4]. The bestscoring 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 protein sequence of 15 different plant species using ClustalW multiple alignment. The reconstructions of phylogenetic trees were conducted using Maximum Likelihood Method (Kumar *et al.*, 2018) [5]. Bootstraps with 1000 replicates for Poisson correction model were performed to assess node support. TFL proteins from several plant species were compared to each other and it showed various different relationships with Indian bean. *Lablab purpureus* (Indian bean) TFL proteins was found to share the closest ancestral relatedness to *Glycine max* (Soybean). *Citrus trifoliata* and *Citrus sinensis* depicted to share common evolutionary relationship with *Lablab purpureus* and *Glycine max*. The TFL proteins of *Mangifera indica* (Mango) and *Nicotiana tabacum* (Tobacco) were found to be the most related to each other and the most ancient proteins among the species analysed. They shared similarity to TFL of *Populus tomentosa* (Poplar). The protein probably evolved after that and the evolved one was found to be similar in *Citrus trifoliate* (Trifoliate orange) and *Citrus sinensis* (Sweet orange). Moreover, *Thalictrum thalictroides* (Rue-anemone) TFL protein probably evolved after that sharing relatedness with *Gossypium barbadense* (Sea Island cotton) and *Nymphaea colorata* (Water lily). To further the analysis results, the protein shows further probable evolution in *Hibiscus syriacus* (Common hibiscus) succeeded by *Brassica napus* (Rapeseed). The model plant *Arabidopsis lyrata subsp lyrata* was found evolutionarily closest to the TFL protein of *Capsella rubella* (Shepherd's purse). Both of these were close to *Eutrema salsugineum* (Saltwater cress). But they showed the most distant evolutionary relatedness to Indian bean.

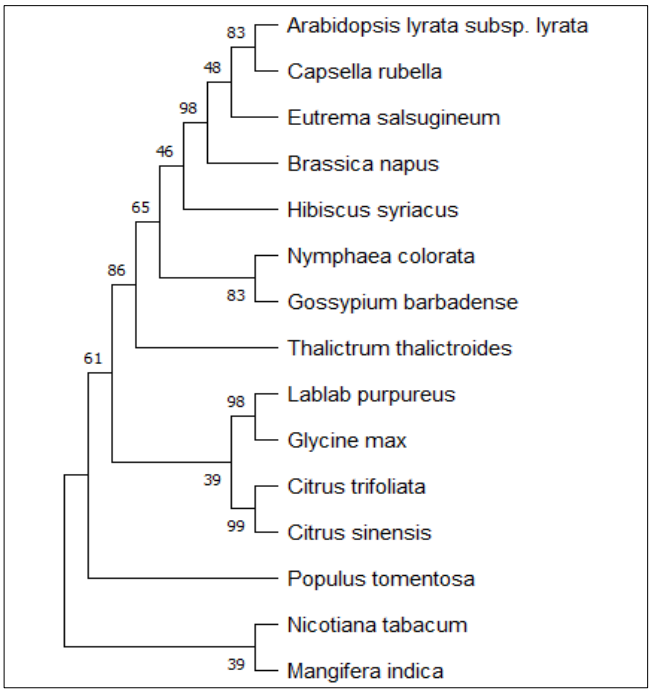

Fig 1: Phylogenetic tree of TFL protein. The tree was constructed by Maximum Likelihood Method with a 1000-replication bootstrap value using MEGA X software. The numbers near branch nodes represent the bootstrap value at each branching.

Acknowledgements

We acknowledge the Department of Genetics and Plant Breeding, N. M. College of Agriculture, Navsari Agricultural University, Navsari for providing the necessary funds and experimental materials for carrying out and completion of the trial.

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