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In Silico Structural and Functional Insights into the Lipoxygenase Enzyme of Legume Cajanus Cajan

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Abstract—Lipoxygenases (LOX) are a family of enzymes known to play a key role in the plant response related to osmotic, drought and high-salinity stress. Plants contain multiple isoforms of the lipoxygenase (LOX) enzyme which differs in terms of its substrate preference, optimal pH, product formation, and stability. Till date, no study has been done to focus on the structural and functional aspect of lipoxygenase enzyme of *Cajanus cajan*, an important legume crop of Indian sub-continent. The present work revealed that the LOX enzyme of *Cajanus cajan*is a 96.65 kDa monomeric stable protein. It also contained an iron ion as a ligand in its predicted structure. Ramchandran's analysis showed the modeled tertiary structure to be a stable one with 91.7% residues in the favoured regions. This study will be helpful to understand the structural aspects of LOX enzyme and would help future studies to enhance the expression of this enzyme in response to major abiotic (mainly salt stress) and biotic stresses.

Keywords-*Lipoxygenase; Cajanus cajan; homology modeling; abiotic stress.*

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I. INTRODUCTION

Lipoxygenases (EC 1.13.11.) are a family of iron-containing enzymes most of which catalyze the whichcatalyze the regio- and stereo-specific dioxygenation of polyun-saturated fatty acids to conjugated unsaturated fatty acid hydroperoxides[1–3].Although these enzymes are widely distributedin the animal and plant kingdoms, they are particularly abundant ingrain legume seeds and potato tubers[4,5]. Plants furthermore contain multiple isoforms of the lipoxygenase (LOX) enzyme whichdiffers in terms of its substrate preference, optimal pH, productformation, and stability[6–8].

Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a legume crop mainly grown in the tropics and subtropics of the Indian subcontinent. More than any other legume adapted to the tropical region, Pigeonpea uniquely combines optimal nutritional profiles, high tolerance to environmental stresses, high biomass productivity and most nutrient and moisture contributions to the soil [9].Due to long duration, crop is affected by terminal drought and encounters various abiotic stresses; however, north-western and north-eastern parts of India have temperature stress because of extremes of low or high temperature [10,11]. Perception of stress signal(s) and activation of complex signaling pathway(s) bring about drastic changes in the cellular gene expression which is a prerequisite for plants to acclimatize under extreme conditions[12]. Although salt, drought and cold stresses are different in nature, they are known to activate certain non-specific sets of common genes in plants. Such non-specific gene expression explains the cross-tolerance to various stresses in a *particular* line or variety. Temperature stress, heavy metals, salt stress and water deficit lead to increase in production of reactive oxygen species (ROS) with downstream alterations of oxidative signaling [13,14].

LOX enzymes are involved in various physiological processes, including defense responses to biotic and abiotic stresses in plants [15]. The plant LOXs, classified as 9-LOX and 13-LOX, catalyze the oxygenation of linoleic acid and linolenic acid[16].Many 13-LOX-derived compounds, such as JA, 12-oxophytodienoic acid and 13-hydroxyoctadecatrienoic acid, are well-known regulators of the plant defense response[17]. Lipoxygenases in pepper has been reported to play a key role in osmotic, drought and high-salinity stress [15-18]. LOX-pathways metabolites – oxylipins interact with other signaling pathways in plant cells, including signaling pathways of the plant hormones auxin, gibberellin, ethylene, abscisic acid (ABA) and salicylic acid (SA). The role of LOX metabolites in biotic stress responses has been described in many published works [19].The role of oxylipins in plant adaptation to abiotic stress conditions is less studied; there is also obvious lack of available data compilation and analysis in this area of research [20].

Studies involving the structural analysis of lipoxygenases are scarce. In fact there is no reported 3-D structure of lipoxygenase enzyme of Pigeonpea which is an important crop for the tropical and sub-tropical regions especially for Indian subcontinent. This study aims to understand the structural and functional role of lipoxygenase enzyme. Homology modeling of lipoxygenase enzyme has been done along with its physiochemical analysis and protein-protein interaction studies. This study would help to understand the metabolic implications of lipoxygenases and would help the agriculture scientists to manage the response of pigeonpea in major stress conditions.

II. MATERIAL AND METHODS

a. Retrieval and selection of reference sequence

Protein and corresponding gene sequence oflipoxygenasefor seven different legume crops were retrieved from NCBI(http://www.ncbi.nlm.nih.gov/) database in FASTA format.This sequence was used for further computational investigationincluding the molecular modeling.

b. *Construction of phylogenetic tree of similar sequences*
The protein sequences obtained were subject to phylogenetic analysis with selected protein and phylogenetic tree was constructed using MEGA7 software to find the evolutionary distances among the proteins [21]. Whole genome sequences were omitted and partial sequences were selected from the search result.

c. Primary sequence analysis
Primary sequence analysis included the physicochemical characteristics of the K. pneumoniae protein (AEQ29825.1). Expasy protparam tool was used to determine the molecular weight and amino acid composition [22].

d. Secondary structure prediction
Prediction of secondary structure (which involved the estimation of number of helices, sheets, turns, and coils in the amino acid sequence) was done from CFSSP: Chou and Fasman secondary structure prediction server(www.biogem.org/tool/choufasman) [23,24].

e. Tertiary homology protein modeling and evaluation
SWISS-MODEL (ProMod3 version 1.0.2) workspace was used for determining the homology protein model of AEQ29825.1[25]. Evaluation of built model was done in SAVES server (http://services.mbi.ucla.edu/SAVES/). Ramachandran's plot was constructed in RAMPAGE (http://morded.bioc.cam.ac.uk/~rapper/rampage.php) using the pdb file. The energetically allowed regions for backbone dihedral angles ϕ against ψ amino acid residues was visualized in the Ramachandran plot.

f. Functional analysis
The motif search tool (http://www.genome.jp/tools/motif/) was used to identify functional motifs in the AEQ29825.1 protein sequence.

III. RESULTS AND DISCUSSION

a. Retrieval and selection of reference sequence
In silico study of lipoxygenase protein of*Cajanus cajan*has not been previously studied, that's why the present work wasundertaken. But, similar computational investigation was donein other legume crops.W. Minor et al. (1996) worked onsoybean lipoxygenase giving a detailed structuraland functional insight on the protein[27].

b. Construction of phylogenetic tree of similar sequences
Phylogenetic analysis of seven different lipoxygenase protein from legume crops were carried out (Fig. 1). The protein sequences were that of *Vigna radiata* (AGS94394.3), *Lens culinaris*(CAA50483.1), *Pisum sativum*(CAA55319.1), *Phaseolus vulgaris*(AAB18970.2), *Glycine max*(AAA96817.1), *Cajanus cajan*(KYP63640.1), and*Glycine soja*(KHN11252.1).

The evolutionary history was inferred using the Neighbor-Joining method [28]. The optimal tree with the sum of branch length = 1.13396148 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [29]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site[30].

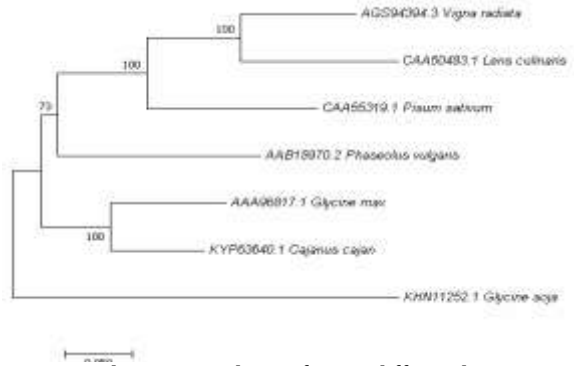

Figure 1. Evolutionary analyses of seven different lipoxygenase proteins of prominent legume crops.

c. Primary sequence analysis
Primary sequence analysis revealed a set of physicochemical characteristics of the protein of interest. The protein consisted of 865 amino acid residues having molecular weight ~96.65kDa with a theoretical P_I of 5.88. Leucine was found highest in content in terms of percentage of the total amino acids (Table 1). Physicochemical characterization is important in terms of knowing the nature of protein[31].

d. Secondary structure prediction
Prediction of secondary structure (Fig. 2) showed high percentage of alpha helix in all the protein secondary structure. The secondary structure of lipoxygenase of *Cajanus cajan*has secondary elements of alpha helix 67.6%, sheets 43.6% and turns 13.3% (Fig. 3). The high content of alpha helices indicated the stable nature of the protein[32].

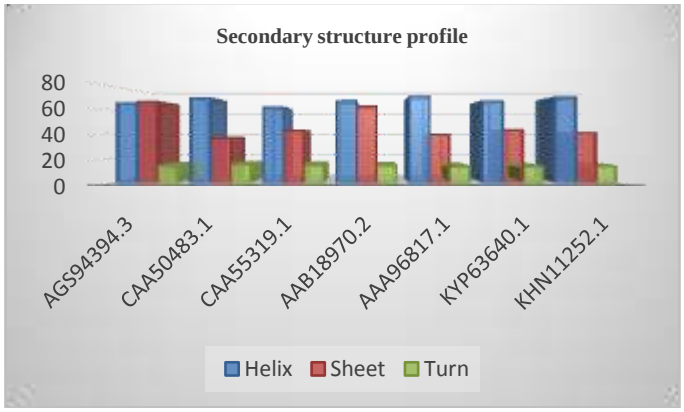

Figure 2. Secondary structure profile of all the seven legume lipoxygenases.

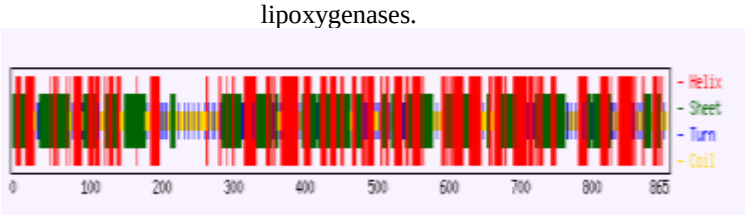

Figure 3. Secondary structure of KYP63640.1 protein.

Table 1. Amino acid composition of lipoxygenase (*Cajanus cajan*)

Amino acids	Composition of aa	Percentage of aa
Ala (A)	52	6.00%
Arg (R)	38	4.40%
Asn (N)	44	5.10%
Asp (D)	50	5.80%
Cys (C)	3	0.30%
Gln (Q)	27	3.10%
Glu (E)	49	5.70%
Gly (G)	65	7.50%
His (H)	19	2.20%
Ile (I)	57	6.60%
Leu (L)	87	10.10%
Lys (K)	48	5.50%
Met (M)	11	1.30%
Phe (F)	38	4.40%
Pro (P)	55	6.40%
Ser (S)	60	6.90%
Thr (T)	56	6.50%
Trp (W)	12	1.40%
Tyr (Y)	40	4.60%
Val (V)	54	6.20%
Pyl (O)	0	0.00%
Sec (U)	0	0.00%

e. *Tertiary homology protein modeling and evaluation*

After target-template alignment (Fig. 4), homology protein model was built (Fig. 5) using the best match template (1iuk.1.A) obtained from SWISS MODEL sever. Ramachandran's plot (Fig. 6) revealed that 91.2% residues resided in favored region, 6.0% in allowed region and rest 2.8% were in outlier region. A good qualitymodel is expected to have more than 90% in the favored region[31].Moreover, the overall quality factor for the pdb model was 97.007% for each chain as found from SAVES server. It implied the characteristics of a good quality as well as a high resolution model.

Figure 4.Target-template sequence alignment.

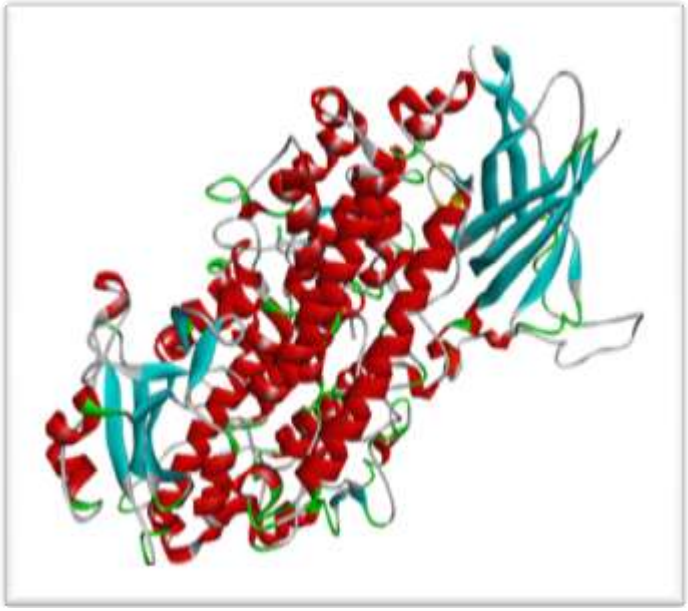

Figure 5. Predicted 3-D Model of lipoxygenase enzyme.

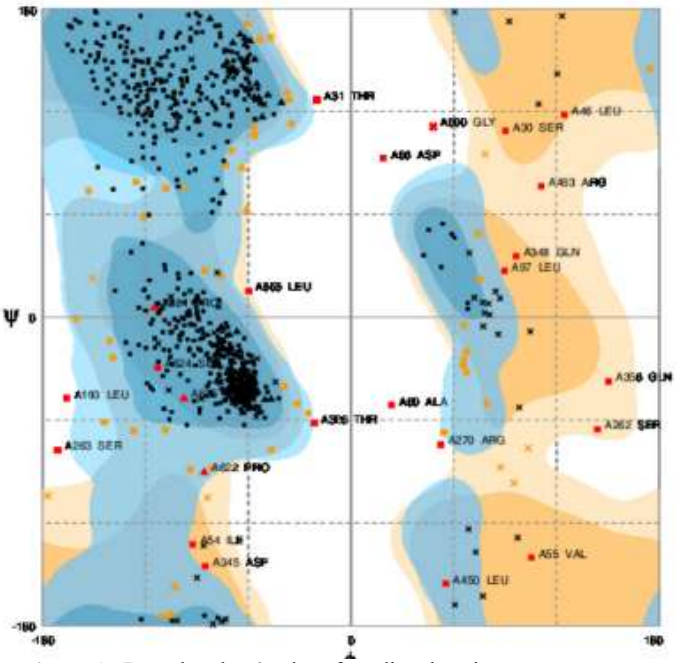

Figure 6. Ramchandran's plot of predicted tertiary structure.

f. Functional analysis

Two functionalmotifs were found from motif search (Fig. 10) which includeslipoxygenase enzyme (PF00305) andPLAT/LH2 domain conserved region (PF01477).

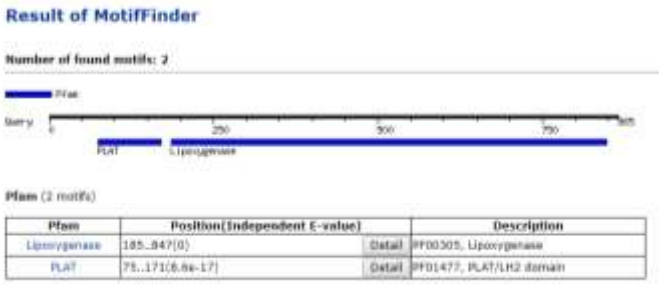

Figure 7. Predicted conserved motifs detected in KYP63640.1 protein.

IV. CONCLUSIONS

This study focus on the Homology Modeling of lipoxygenase enzyme of *Cajanus cajan* which has been predicted for the first time.*Cajanus cajan* crop represents about 5% of world legume production, with more than 70% being produced in India (although there is also substantial Pigeon Pea production in East Africa, the Americas, and Burma). Lipoxygenase is a 96.65 kDa protein with an instability index of 35.97 which indicates the stable nature of the protein.The role of LOX metabolites in biotic stress responses has been proven significant in the survival of important crops/cereals. This study will help the agriculture scientists to focus on the stress response elements in plants and study the structural and functional aspects of such elements which may be critical in future keeping in mind the significant reduction of agricultural lands and continuous depletion in the production quality of the agricultural lands and soil.

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