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Computational Analysis of tuberization protein linoleate 9S-lipoxygenase 3 from *Solanum tuberosum*

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Abstract: Linoleate 9S- lipoxygenase 3(POTLX-1, Accession number-Q43189) also known as plant LOX-1(lipoxygenase) are a functionally diverse class of dioxygenases involved in various physiological process such as growth, senescence and stress related responses. The present study shows the structural, functional and phylogenetic analysis of linoleate 9S-lipoxygenase 3 of *Solanum tuberosum*. Primary and secondary structure analysis of linoleate 9S-lipoxygenase 3 with their related sequences was performed using Protparam and SOPMA, followed by analysis of functional domains, families and motifs along with the phylogenetic analysis. A total of 101 BLAST hits having all the E-value 0.0 were found for the lipoxygenase query sequence. Clustalw was performed which resulted into more than 60% similarity and along with phylogenetic tree had been generated taking five different species on the basis of identity having more than 79%. Motif search resulted in finding of two motifs; first from 544th amino acid to 554th amino acid and second from 517th amino acid to 531th amino acid. Pfam analysis showed that all the five varieties share common domains. KEGG analysis enlisted all the 16 manual pathways related to LOX-1 gene. The results showed that the Linoleate 9S- lipoxygenase 3 is not the only gene responsible for tuberization in potato. There are many other factors responsible for this.
Keywords: Lipoxygenase, Amino acid sequence, Sequence alignment, Phylogeny.

1. Introduction

Plant lipoxygenases (LOXs) are a functionally diverse class of dioxygenases involved in various physiological process such as growth, senescence and stress related responses. It incorporates oxygen into their fatty acid substrates and produce hydroperoxide fatty acids that are precursors of jasmonic acid and related compounds[1].

Tuber formation in potatoes is a complex developmental process. It involves the interaction of environmental, biochemical, and genetic factors. Various important biological processes, such as carbon partitioning, signal transduction, and meristem determination are involved[2]. A transmissible signal is activated under conditions of a short-day photoperiod and cool temperature that initiates cell division and expansion and a change in the orientation of cell growth in the sub-apical region of the stolon tip[3]. In this signal transduction pathway, some environmental clues has been found in leaves which is mediated by phytochrome and gibberellins and detected at least 10 quantitative trait loci that control the ability to tuberize under long days, but none of these genes has been identified definitively.

Development of tuber at the stolon tip consists of biochemical and morphological processes[4, 5]. It has found that both processes are controlled by differential gene expression but the most of the research in this area has focused on the biochemical processes, including starch synthesis[6,7,8] and storage protein accumulation[9, 10, 11].Seven distinct BEL1-like proteins were identified that are involved in tuberization using POTH1 as bait[12]. Transgenic over expression of POTH1 produced plants that exhibited reduced expression of a key enzyme in GA biosynthesis, aberrant leaf morphology, and enhanced tuber production reported by[13]. Similar over expression mutants of *POTH1*, transgenic lines that over expressed one of the potato BEL1 partners, St BEL5, exhibited enhanced tuber formation and increased cytokinin levels[12].

Signal transduction proteins are also involved in the source-sink transition during potato tuberization. The protein profiles is examined under in vitro tuber inducing conditions using a shotgun proteomic approach involving denaturing gel electrophoresis and liquid chromatography–mass spectrometry. A total of 251 proteins were identified and classified into 9 groups according to distinctive expression patterns during the tuberization stage. Stolon stage specific proteins were primarily involved in the photosynthetic machinery. Proteins specific to the initial tuber stage included patatin. Proteins specific to the developing tuber stage included 6-fructokinase, phytoalexin-deficient 4-1, metallothionein II-like protein, and malate dehydrogenase. Novel stage specific proteins identified during in vitro tuberization were ferredoxin–NADP reductase, 34 kDa porin, aquaporin, calmodulin, ripening-regulated protein, and starch synthase. Superoxide dismutase, dehydroascorbate reductase, and catalase I were most abundantly expressed in the stolon; however, the enzyme activities of these proteins were most activated at the initial tuber[14].

From earlier finding it is clear[15,16,17] that phytohormones play a prominent role in tuberization phenomenon. Several genes expressed during early tuber formation have been identified, including tubulins[18] , S adenosylmethionine decarboxylase[19], MADS box genes[20], acyl carrier protein thioesterase [21], and lipoxygenases[22]. However there are many molecular mechanisms known till date which provides us information about the tuberization in potato.

Potato plants undergo several phases of development during the tuber life cycle, involving tuberization, dormancy and sprouting. Tuberization is a complex developmental process involving the differentiation of an underground stolon (a modified stem) into a specialised storage organ, the tuber[23]. The process of tuberisation comprises inhibition of the longitudinal growth in the tip of the stolon and followed by the initiation and growth of the tuber[24].

The final tuber size is determined by further increase in cell volume of perimedullary and cortical parenchyma tissues[18,19]. During this growing/filling stage the tuber is highly metabolically active[1] and two major biochemical changes occur; accumulation of starch and formation of storage proteins.

It has been found through RNA hybridization analysis that the accumulation of LOX 1 class transcripts was restricted to developing tubers, stolen and roots that make mRNA accumulation correlated positively with tuber initiation and growth. In situ hybridization showed that LOX 1 class transcripts is accumulated in the apical and sub apical regions of the newly formed tuber, specifically in the vascular tissue of the most active cell growth during tuber enlargement. By expressing antisense coding sequence of a specific tuber LOX, suppression mutants is produced designated POTLX-1 exhibited a significant reduction in LOX activity in stolons and tubers[6].

Lipoxygenases (EC 1.13.11.-) are a class of iron-containing dioxygenases which catalyzes the hydroperoxidation of lipids, containing a cis, cis-1, 4-pentadiene structure. In higher plants, the natural substrates for these enzymes are linolenic and linoleic acids[25,26]. The primary products are fatty acid hydroperoxides that are metabolized enzymatically into compounds like traumatin, jasmonic acid (JA), and methyl jasmonate (MJ)[27,28]. The primary products are hydroperoxy fatty acids, which usually are rapidly reduced to hydroxy derivatives. The hydroperoxy fatty acid products of the LOX reaction can be further converted to different compounds through the action of enzymes participating in at least six pathways (Fig.1)[29]. Lipoxygenases are common in plants and are found to be involved in a number of diverse aspects of plant physiology including growth and development, pest resistance, and senescence or responses to wounding.

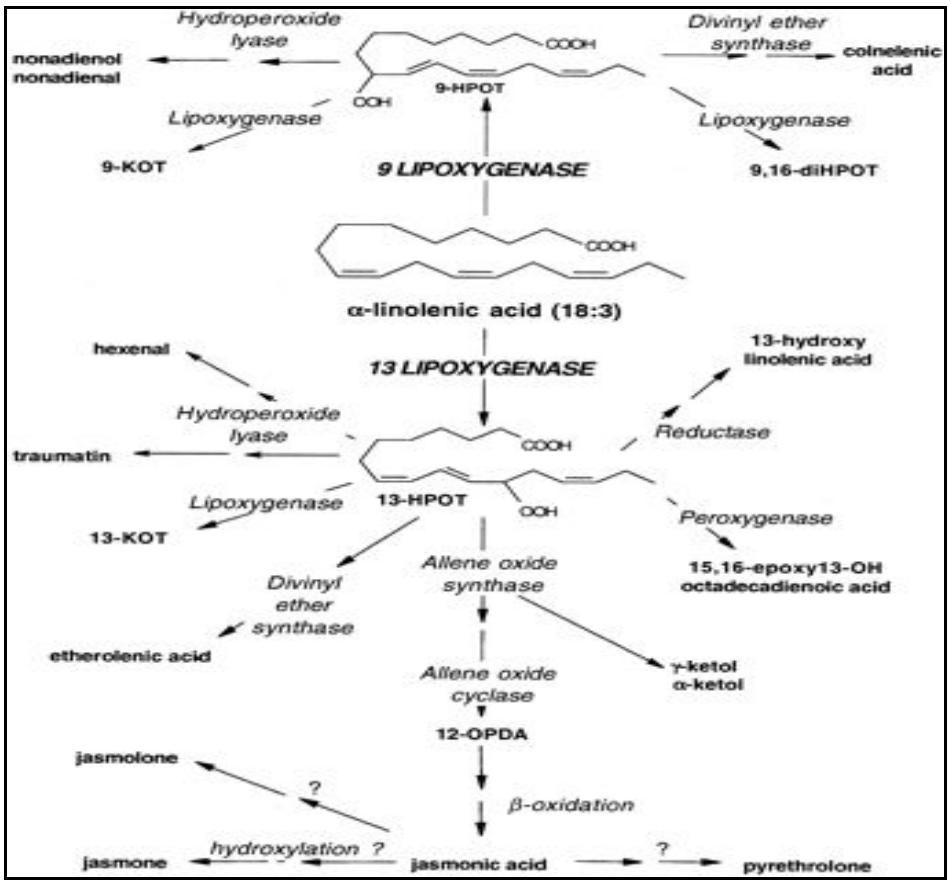

Fig.1The LOX pathway. The dioxygenation of long chain fatty acids such as α-linoleic acid (18:3) catalysed catalysed by 9- and 13- LOXs, results in derivatives with several known or proposed functions in the plant cell (Table 1). 9-HPOT,(10E,12Z)- 9 hydroperoxy-10,12,15- octadecaterieonoic acid; 9-KOT, (10E,12Z)-9-keto-10,12,15 octadecatrienoic acid; 13-HPOT, (10E,12Z)-13-keto-10,12,15-octadecatrienoic acid; 12 oxo-PDA,12-oxo-10,15- phytodienoic acid9 [29].

In plants, products of the LOX pathway have several diverse functions (Table 1)[29]. In addition, LOX has been associated with some processes in a number of developmental stages.;[25] and with the mobilization of storage lipids during germination[30]. LOX is also used as a storage protein during vegetative growth[31]; Figure 2.

Table1. Products of LOX metabolism with a known or proposed activity[29].

Products of LOX metabolism with a known or proposed activity		
Compound	Branch	Activity
(13S)- Hydroperoxy- (9Z-11E)- octadecadienoic (13-HPOD) and HPOT	-	Inhibitors of mycotoxin synthase
9- and 13-HPOD or HPOT	-	Development of hypersensitive cell death.
Jasmonic acid(JA)	Allene oxide synthase (AOS)	Signaling in several stresses and tendril coiling.
OPDA	AOS	Signaling in wounding and pathogen attack tendril coiling.
in plant defense(C6-)	Hydroperoxide lyase (HPL)	Signaling in wounding;

volatiles (aldehydes and alcohols)		attractors to enemies of herbivores; antimicrobial; odors
Dinor-oxo- phytodienoic acid	AOS	Signaling in wounding
9- and 13- ketodiene	LOX	Signaling in wounding and pathogen attack; induction of cell death.
Traumatin	HPL	Signaling in wounding
(Z)- jasmone	AOS	Herbivore repellent and attractor of enemies of herbivore; signaling in plant defense.
Colneilic and Colneilic acids	Divinyl ether synthase(DES)	Antifungal

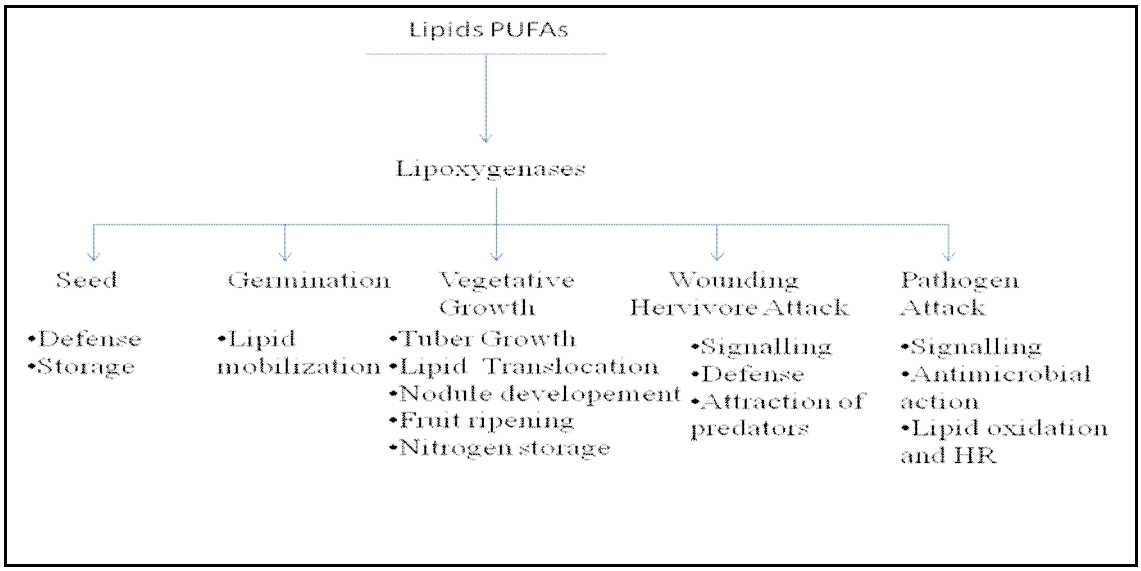

Figure 2. LOXs have active roles in several processes during plant life. PUFAs, Polyunsaturated fatty acids[31].

Several lines of correlative evidence suggest that LOXs function is involved in the regulation of plant growth and development. LOX isozyme profiles change quantitatively and qualitatively during soybean leaf development[32] and during seed germination in cucumbers[33, 34]. In addition, many LOX genes are regulated differentially during Arabidopsis seedling development[35] pea nodule formation[36], tomato fruit ripening[37, 38] potato tuber development, and pea carpel development[39]. LOX-derived products such as jasmonic acid, methyl jasmonate, and tuberonic acid can play an important role in potato tuberization. These hormone like compounds have strong tuber-inducing activity in vitro[40, 41] and they may function by regulating the reorientation of microtubules that allows radial cell expansion leading to tuber enlargement[42, 43].

PLAT domain belongs to cl00011 super family .Lipoxygenase domains belong to cl09510 super family. Two conserved sequence have been found in this particular gene. The acquisition of POTLX-1 in plants indicates one of the important events in its physiological growth. Because of the inherent role of POTLX-1 gene in the physiological growth of plant of solanaceae family we aimed to do computational analysis of this particular gene in various species of plant to determine whether this gene is responsible for tuberization in potato which makes it quite different from other species.

2. Materials and methods

2.1 Data Set, Sequence Alignment and Construction of Phylogenetic Tree

Uniprot database was for all available protein sequences of the POTLX-1. The retrieved sequences were saved in FASTA format. An initial first-pass phylogenetic tree was constructed using Neighbour Joining method (maximum sequence difference of 0.85) using Domain Enhanced Lookup Time Accelerated Basic Local Alignment Search Tool [DELTA BLAST] pairwise alignments between the query and the database sequences searched.

The list of sequences obtained after DELTA BLAST was narrowed down to five species on the basis of E-value (0.0) and identity greater than 79% and subjected to Multiple Sequence Alignment. The concerned retrieved sequences were then aligned using Clustal W server. The phylogenetic tree was constructed using the maximum likelihood method implemented in the PhyML program.

2.2 Identification of Motifs

Conserved sequence of POTLX gene is searched by Motif search database

2.3 Comparison of physiochemical properties

Expasy's ProtParam prediction server was used to compare the physiochemical properties of POTLX-1 gene among the five species being considered[44].

2.4 Secondary structure analysis

The tool SOPMA (Self-Optimized Prediction Method with Alignment), in Expasy was used for the secondary structure prediction, secondary structure class identification and for the computation of percentages of α-helical, β- strand and coiled regions of all the five species.

2.5 Functional domain and family analysis

Then analysis of functional domains and families by using Pfam server was done.

2.6 Analysis of conserved domain

A domain is also a conserved sequence pattern defined as an independent functional and structural unit. Domains are normally longer than motifs. A domain consists of more than 40 residues and up to 700 residues, with an average length of 100 residues. A domain may or may not include motifs within its boundaries. Examples of domains include transmembrane domains and ligand- binding domains[45].

2.7 Analysis of KEGG pathway

As KEGG PATHWAY mapping represents molecular datasets mapping, especially large-scale datasets in genomics, transcriptomics, proteomics, and metabolomics, for biological interpretation of higher-level systemic functions. So, hereby LOX-1 gene has been analyzed in KEGG pathway and enlisted all the 16 manual pathways related to LOX-1 gene. Then a table was prepared representing all the vital enzymes their definition and their amino acid sequence involved in the α-linoleic acid metabolism pathway.

3. Results and discussion

3.1 Identification of sequence alignment and phylogenetic analysis

The tuberization protein sequence was taken from Uniprot linoleate 9S-lipoxygenase 3 from '*Solanum tuberosum*'. Thus, the FASTA sequence of linoleate 9S-lipoxygenase 3 having I.D. Q43189 and of 861 amino acids length was downloaded and used for further structural, phylogenetic and functional analysis.

The sequence analysis of linoleate 9S-lipoxygenase 3 was carried using BLASTP which exhibited a total of 101 BLAST hits all were having E-value 0.0. Figure3.

Amongst all the sequences the FASTA format of five different species having E-value 0.0 and identity more than 79% were retrieved and submitted to the multiple sequence alignment servers CLUSTALW shown in Figure 4.

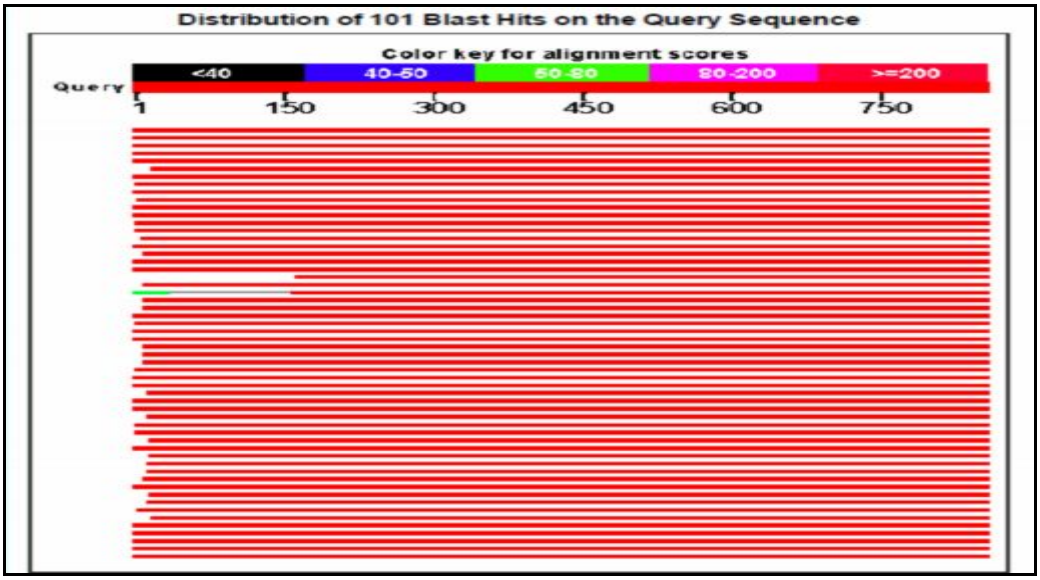

Figure 3. Blast hits shown by protein of I.D. Q43189

>gi|75282480|sp|Q43189.1|LOX13_SOLTU RecName: Full=Probable linoleate 9S-lipoxygenase 3

MIGQITSGLFGGHDDSKKVKGTVVMMNKNVLDFTDLASSLTGKIFDVLGQKVSFQLISSVQGDPTN
GLQGKHSNPAYLENSLFTLTPLTAGSETAFGVTFDWNEEFGVPGAFIIKNMHITEFFLKSLTLEDVPN
HGKVHFVCNSWVYPSLNYKSDRIFFANQPYLPSDTPELLRKYRENELLTLRGDGTGKREAWDRIYD
YDIYNDLGNPDQGKENVRTTLGGSAEYPYPRRGRTGRPPTRTDPKSESRIPLILSTDIYVPRDERFGH
LKMSDFLTYALKSIVQFILPELHALFDGTPNEFDSFEDVLRLYEGGIKLPQGPLFKALTAAIPLEMIRE
LLRTDGEGILRFPTPLVIKDSKTAWRTDEEFAREMLAGTNPVIISRLQEFPPKSKLDPEAYGNQNSTIT
AEHIEDKLDGLTVDEAMNNNKLFILNHHDLLIPYLRRINTTITKTYASRTLLFLQDNGSLKPLAIELSL
PHPDGDQFGVTSKVYTPSDQGVESSIWQLAKAYVAVNDSGVHQLISHWLNTHAVIEPFVIATNRQL
SVLHPIHKLLYPHFRDTMNINALARQILINAAGVFESTVFQSKFALEMSAVVYKDWVFPDQALPADL
VKRGVAVEDSSSPHGVRLLIEDYPYAVDGLEIWSAIKSWVTDYCSFYYGSDEEILKDNELQAWWKE
LREVGHGDKKNEPWWPEMETPQELIDSCTTIIWIASALHAAVNFGQYPYAGYLPNRATVSRRFMPE
PGTPEYEELKKNPDKAFLKTITAQLQTLLGVSLVEILSRHTTDEIYLGQRESPEWTKDKEPLAAFDRF
GKKLTDIEKQIIQRNGDNILTNRSGPVNAPYTLLFPTSEGGLTGKGIPNSVSI

>gi|350539986|ref|NP_001234856.1| linoleate 9S-lipoxygenase A [Solanum lycopersicum]

MLGQLVGGLIGGHHDSKKVKGTVVMMKKNALDFTDLAGSLTDKIFEALGQKVSFQLISSVQSDPA
NGLQGKHSNPAYLENFLLTLTPLAAGETAFGVTFDWNEEFGVPGAFVIKNMHINEFFLKSLTLEDVP
NHGKVHFVCNSWVYPSFRYKSDRIFFANQPYLPSETPELLRKYRENELVTLRGDGTGKREAWDRIY
DYDVYNDLGNPDQGKENVRTTLGGSADYPYPRRGRTGRPPTRTDPKSESRIPLILSLDIYVPRDERFG
HLKMSDFLTYALKSIVQFILPELHALFDGTPNEFDSFEDVLRLYEGGIKLPQGPLFKALTDAIPLEMIR
ELLRTDGEGILRFPTPLVIKDSKTAWRTDEEFAREMLAGVNPVIISRLEEFPPKSKLDPELYGNQNSTI
TAEHIEGKLDGLTIDEAINSNKLFILNHHDVLIPYLRRINTTTTKTYASRTLLFLQDNGSLKPLAIELSL
PHPDGDQFGVTSKVYTPSDQGVEGSIWQLAKAYVAVNDSGVHQLISHWLNTHAVIEPFVIATNRQL
SVLHPIHKLLYPHFRDTMNINALARQILINAGGVLESTVFPSKFAMEMSAVVYKDWVFPDQALPAD
LVKRGVAVEDSSSPHGVRLLIDDYPYAVDGLEIWSAIKSWVTDYCSFYYGSNEEILKDNELQAWWK
EVREVGHGDKKNEPWWAEMETPQELIDSCTTIIWIASALHAAVNFGQYPYAGYLPNRPTVSRKFMP
EPGTPEYEELKKNPDKAFLKTITAQLQTLLGVSLIEILSRHTTDEIYLGQRESPEWTKDKEPLAAFERF
GNKLTDIEKQIMQRNGNNILTNRTGPVNAPYTLLFPTSEGGLTGKGIPNSVSI

>gi|407930087|gb|AFU51542.1| lipoxygenase 3 [Capsicum annuum]

MIKNLVDGLIHHDSSKKVKGTVVMMKKNALDFTDLAGSLTDKLFEALGQKVSLQLISSVQGDPAN
GLQGKHSNPAYLENFLFTPTRLTAGESAFGVTFDWNEEFGVPGAFTIKNSHINEFFLKSLILEDVPNH
GKVHFVCNSWVYPSFRYKTDRIFFANQPYLPSETPEPLRKYRESELKTLRGDGTGKLEAWNRVYDY
DVYNDLGNPDQGPEHVRTTLGGSADYPYPRRGRTSRPPTRTDPKSESRIPLLLSLDIYVPRDERFGHL
KLSDFLTYALKSLVQFILPELHALFDGTPNEFDSFEDVLRLYEGGIKLPQGPLFKALTDAIPLEMIREL
LRTDGEGILRFPTPLVIKDSKSAWRTDEEFAREMLAGVNPVIISRLQEFPPKSKLDPNVYGNQDSTIT
AEHIQDKLDGLTIDQAINNNKLFILNHHDILTPYLRRINTTTTKTYASRTLLFLQDNGSLKPLAIELSLP
HPDGDQFGVISKVYTPSDQGVESSIWQLAKAYAAVNDSGVHQLISHWLNTHAVIEPFVIATNRQLSV
LHPIHKLLYPHFRDTMNINALARQILINAGGVLESTVFPSKYAMEMSAVVYKDWVFPDQALPADLI
KRGIAVEDSSSPHGVRLLIQDYPYAVDGLEIWSAIKSWVTEYCNVYYKSNEDILKDNELQEWWKEL
REVGHGDKKDAPWWPEMESPEDLIESCTIIIWIASALHAAVNFGQYPYAGYLPNRPTVSRRFMPEPG
TPEYEELKTNPDKAFLKTITAQFQTLLGVSLIEILSRHTSDEIYLGQRESPEWTKDKEPLAAFDRFGKK
LTEIENHIIQRNGDQILKNRSGPVNAPYTLLFPTSEGGLTGKGIPNSVS

>gi|32454706|gb|AAP83134.1| lipoxygenase [Nicotiana attenuata]

MFPIKNIVDGLIGHNDSKKVKGIVVMMKKNALDFTDIAGAVVDGVLEFVGQKVSLQLISSAHGDPA
NDLQGKHSNPAYLENWLTTITPLTAGESAYGVTFDWDEEFGLPGAFIIKNLHFTEFFLKSVTLEDVP
NHGTVHFVCNSWVYPANKYKSDRIFFANKTYLPSETPAPLLKYRENELLTLRGDGTGKLEAWDRV
YDYALYNDLGDPDQGAQHVRPILGGSSDYPYPRRGRTGRAPTRTDPESESRIPLLLSLDIYVPRDERF
GHLKLSDFLTYALKSMVQFILPELHALFDSTPNEFDSFEDVLRLYEGGIKLPQGPLFKALISSIPLEMV
KELLRTDGEGIMKFPTPLVIKEDKTAWRTDEEFGREMLAGVNPVIIRNLQEFPPKSKLDPQVYGNQD
STITIQHIEDRLDGLTIDEAIKSNRLFILNHHDTIMPYLRRINTTTTKTYASRTLLFLQDNGCLKPLAIEL
SLPHPDGDQFGAISKVYTPTDEGVEGSIWELAKAYVAVNDSGVHQLISHWLNTHAVIEPFVIATNRQ
LSVLHPIHKLLHPHFRDTMNINAMARQILINAGGVLESTVFPSKYAMEMSAVVYKNWIFPDQALPT
DLVKRGMAVEDSSSPHGIRLLIQD

YPYAVDGLEIWSAIKSWVTEYCSFYYKSDDSILKDNELQAWWKELREEGHGDLKDEPWWPKMEN
CQELIDSCTIIIWTASALHAAVNFGQYPYAGYLPNRPTVSRRFMPEPGTSEYELLKTNPDKAFLRTIT
AQLQTLLGVSLIEILSRHTSDEIYLGQRDSPKWTYDEEPLAAFDRFGNKLSDIENRIIEMNGDQIWRN
RSGPVKAPYTLLFPTSEGGLTGKGVPNSVSI

>gi|505490393|gb|AGL96414.1| 9-lipoxygenase, partial [Nicotiana benthamiana]

MSLEKIVDAISGKDDGKKVKGTVVLMKKNVLDFTDINASVLDGVLEFLGRRVSLELISSVHVDPAN
GLQGKRSKAAYLENWLTNKTPIAAGESAFRVTFDWDDEEFGVPGAFIIKNLHFSEFFLKSLTLEDVP
NHGKVHFVCNSWVYPANKYKSPRIFFANQAYLPSETPEPLRKCRENELVTLRGDGTGKLEEWDRV
YDYAYYNDLGDPDKGKELSRPVLGGSSEYPYPRRGRTGREPTKSDPNSESRIPLLMSLDIYVPRDER
FGHIKLSDFLTFALKSIVQLLLPEFQALFDSTPNEFDSFEDVLKLYEGGIKLPQGPLLKAITDNIPLEILK
ELLRSDGEGLFKYPTPQVIQEDKTAWRTDEEFGREMLAGVNPVVISRLQEFPPKSKLDPKTYGNQNS
TITREQIEDKLDGLTIDEAIKTNKLFILNHHDILMPYLRRINTSTDTKTYASRTLLFLQDNGTLKPLAIE
LSLPHPDGDQFGAVSKVYTPADQGVEGSIWQLAKAYAAVNDSGVHQLISHWLNTHAVIEPFVIATN
RQLSTLHPIYKLLHPHFRETMNINALARQILINGGGLLELTVFPAKYSMEMSAVVYKDWVFPEQALP
TDLIKRGVAVEDSSSPHGIRLLIQDYPYAVDGLKIWSAIKSWVTEYCNYYYKSDDAVQKDTELQAW
WKELREEGHGDKKDEPWWPKMQTVQELIDSCTITIWIASALHAAVNFGQYPYAGYLPNRPTLSRKF
MPEPGSPAYEELKTNPDKVFLETITPQLQTLLGISLIEILSRHSSDTLYLGQRESPEWTKDQEPLSAFG
RFGKKLSDIEDQIMQMNGDEKWKNRSGPVKVPYTLLFPTSEGGLTGKGIPNSVSI

Figure 4: Identified similar protein sequences of linoleate 9S-lipoxygenase 3 of five different species.

Clustalw involves a progressive strategy for aligning pairs of sequences. The ClustalW server was selected for sequence analysis as it exploits the fact that similar sequences are likely to be evolutionary related and it expressed the degree of similarity in a relatively concise format. As a part of its operation, the program produced information required to produce a phylogenetic tree.

The obtained FASTA format of the linoleate 9S-lipoxygenase 3 gene from five different species is aligned using the bioinformatics tool CLUSTALW. The aligned results show the similar and non similar parts in a tabular form **Figure 5**.

```
  CLUSTAL 2.1 multiple sequence alignment


gi|75282480|sp|Q43189.1|LOX13_      --MIGQITSGLFGGHDDSKKVKGTVVMMNKNVLDFTDLASSLTGKIFDVL 48
gi|350539986|ref|NP_001234856.      --MLGQLVGGLIGGHHDSKKVKGTVVMMKKNALDFTDLAGSLTDKIFEAL 48
gi|407930087|gb|AFU51542.1|         --MIKNLVDGLIH-HDSSKKVKGTVVMMKKNALDFTDLAGSLTDKLFEAL 47
gi|32454706|gb|AAP83134.1|          MFPIKNIVDGLIG-HNDSKKVKGIVVMMKKNALDFTDIAGAVVDGVLEFV 49
gi|505490393|gb|AGL96414.1|         -MSLEKIVDAISG-KDDGKKVKGTVVLMKKNVLDFTDINASVLDGVLEFL 48
                                       : ::...:   :...***** **:*:**.*****: .:: . ::: :

gi|75282480|sp|Q43189.1|LOX13_      GQKVSFQLISSVQGDPTNGLQGKHSNPAYLENSLFTLTPLTAGSETAFGV 98
gi|350539986|ref|NP_001234856.      GQKVSFQLISSVQSDPANGLQGKHSNPAYLENFLLTLTPLAAG-ETAFGV 97
gi|407930087|gb|AFU51542.1|         GQKVSLQLISSVQGDPANGLQGKHSNPAYLENFLFTPTRLTAG-ESAFGV 96
gi|32454706|gb|AAP83134.1|          GQKVSLQLISSAHGDPANDLQGKHSNPAYLENWLTTITPLTAG-ESAYGV 98
gi|505490393|gb|AGL96414.1|         GRRVSLELISSVHVDPANGLQGKRSKAAYLENWLTNKTPIAAG-ESAFRV 97
                                    *::**::****.: **:*.****:*:.***** * . * ::** *:*: *

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gi|32454706|gb|AAP83134.1|          TFDWD-EEFGLPGAFIIKNLHFTEFFLKSVTLEDVPNHGTVHFVCNSWVY 147
gi|505490393|gb|AGL96414.1|         TFDWDDEEFGVPGAFIIKNLHFSEFFLKSLTLEDVPNHGKVHFVCNSWVY 147
                                    ****: ****:**** *** *:.******: ********.**********

gi|75282480|sp|Q43189.1|LOX13_      PSLNYKSDRIFFANQPYLPSDTPELLRKYRENELLTLRGDGTGKREAWDR 197
gi|350539986|ref|NP_001234856.      PSFRYKSDRIFFANQPYLPSETPELLRKYRENELVTLRGDGTGKREAWDR 196
gi|407930087|gb|AFU51542.1|         PSFRYKTDRIFFANQPYLPSETPEPLRKYRESELKTLRGDGTGKLEAWNR 195
gi|32454706|gb|AAP83134.1|          PANKYKSDRIFFANKTYLPSETPAPLLKYRENELLTLRGDGTGKLEAWDR 197
gi|505490393|gb|AGL96414.1|         PANKYKSPRIFFANQAYLPSETPEPLRKCRENELVTLRGDGTGKLEEWDR 197
                                    *: .**: ******:.****:**  * * **.** ********* * *:*

gi|75282480|sp|Q43189.1|LOX13_      IYDYDIYNDLGNPDQGKENVRTTLGGSAEYPYPRRGRTGRPPTRTDPKSE 247
gi|350539986|ref|NP_001234856.      IYDYDVYNDLGNPDQGKENVRTTLGGSADYPYPRRGRTGRPPTRTDPKSE 246
gi|407930087|gb|AFU51542.1|         VYDYDVYNDLGNPDQGPEHVRTTLGGSADYPYPRRGRTSRPPTRTDPKSE 245
gi|32454706|gb|AAP83134.1|          VYDYALYNDLGDPDQGAQHVRPILGGSSDYPYPRRGRTGRAPTRTDPESE 247
gi|505490393|gb|AGL96414.1|         VYDYAYYNDLGDPDKGKELSRPVLGGSSEYPYPRRGRTGREPTKSDPNSE 247
                                    :***  *****:**:* :  *. ****::*********.* **::**:**

gi|75282480|sp|Q43189.1|LOX13_      SRIPLILSTDIYVPRDERFGHLKMSDFLTYALKSIVQFILPELHALFDGT 297
gi|350539986|ref|NP_001234856.      SRIPLILSLDIYVPRDERFGHLKMSDFLTYALKSIVQFILPELHALFDGT 296
gi|407930087|gb|AFU51542.1|         SRIPLLLSLDIYVPRDERFGHLKLSDFLTYALKSLVQFILPELHALFDGT 295
gi|32454706|gb|AAP83134.1|          SRIPLLLSLDIYVPRDERFGHLKLSDFLTYALKSMVQFILPELHALFDST 297
gi|505490393|gb|AGL96414.1|         SRIPLLMSLDIYVPRDERFGHIKLSDFLTFALKSIVQLLLPEFQALFDST 297
                                    *****:** ************:*:*****:****:**::***::****.*

gi|75282480|sp|Q43189.1|LOX13_      PNEFDSFEDVLRLYEGGIKLPQGPLFKALTAAIPLEMIRELLRTDGEGIL 347
gi|350539986|ref|NP_001234856.      PNEFDSFEDVLRLYEGGIKLPQGPLFKALTDAIPLEMIRELLRTDGEGIL 346
gi|407930087|gb|AFU51542.1|         PNEFDSFEDVLRLYEGGIKLPQGPLFKALTDAIPLEMIRELLRTDGEGIL 345
gi|32454706|gb|AAP83134.1|          PNEFDSFEDVLRLYEGGIKLPQGPLFKALISSIPLEMVKELLRTDGEGIM 347
gi|505490393|gb|AGL96414.1|         PNEFDSFEDVLKLYEGGIKLPQGPLLKAITDNIPLEILKELLRSDGEGLF 347
                                    **********:**************:**:   ****:::****:****::

gi|75282480|sp|Q43189.1|LOX13_      RFPTPLVIKDSKTAWRTDEEFAREMLAGTNPVIISRLQEFPPKSKLDPEA 397
gi|350539986|ref|NP_001234856.      RFPTPLVIKDSKTAWRTDEEFAREMLAGVNPVIISRLEEFPPKSKLDPEL 396
gi|407930087|gb|AFU51542.1|         RFPTPLVIKDSKSAWRTDEEFAREMLAGVNPVIISRLQEFPPKSKLDPNV 395
gi|32454706|gb|AAP83134.1|          KFPTPLVIKEDKTAWRTDEEFGREMLAGVNPVIIRNLQEFPPKSKLDPQV 397
gi|505490393|gb|AGL96414.1|         KYPTPQVIQEDKTAWRTDEEFGREMLAGVNPVVISRLQEFPPKSKLDPKT 397
                                    ::*** **::.*:********.******.***:* .*:**********:

gi|75282480|sp|Q43189.1|LOX13_      YGNQNSTITAEHIEDKLDGLTVDEAMNNNKLFILNHHDLLIPYLRRINT- 446
gi|350539986|ref|NP_001234856.      YGNQNSTITAEHIEGKLDGLTIDEAINSNKLFILNHHDVLIPYLRRINT- 445
gi|407930087|gb|AFU51542.1|         YGNQDSTITAEHIQDKLDGLTIDQAINNNKLFILNHHDILTPYLRRINT- 444
gi|32454706|gb|AAP83134.1|          YGNQDSTITIQHIEDRLDGLTIDEAIKSNRLFILNHHDTIMPYLRRINT- 446
gi|505490393|gb|AGL96414.1|         YGNQNSTITREQIEDKLDGLTIDEAIKTNKLFILNHHDILMPYLRRINTS 447
                                    ****:**** ::*:.:*****:*:*::.*:******** : ********

gi|75282480|sp|Q43189.1|LOX13_      TITKTYASRTLLFLQDNGSLKPLAIELSLPHPDGDQFGVTSKVYTPSDQG 496
gi|350539986|ref|NP_001234856.      TTTKTYASRTLLFLQDNGSLKPLAIELSLPHPDGDQFGVTSKVYTPSDQG 495
gi|407930087|gb|AFU51542.1|         TTTKTYASRTLLFLQDNGSLKPLAIELSLPHPDGDQFGVISKVYTPSDQG 494
gi|32454706|gb|AAP83134.1|          TTTKTYASRTLLFLQDNGCLKPLAIELSLPHPDGDQFGAISKVYTPTDEG 496
gi|505490393|gb|AGL96414.1|         TDTKTYASRTLLFLQDNGTLKPLAIELSLPHPDGDQFGAVSKVYTPADQG 497
                                    * ***************** ********************. ******:*:*
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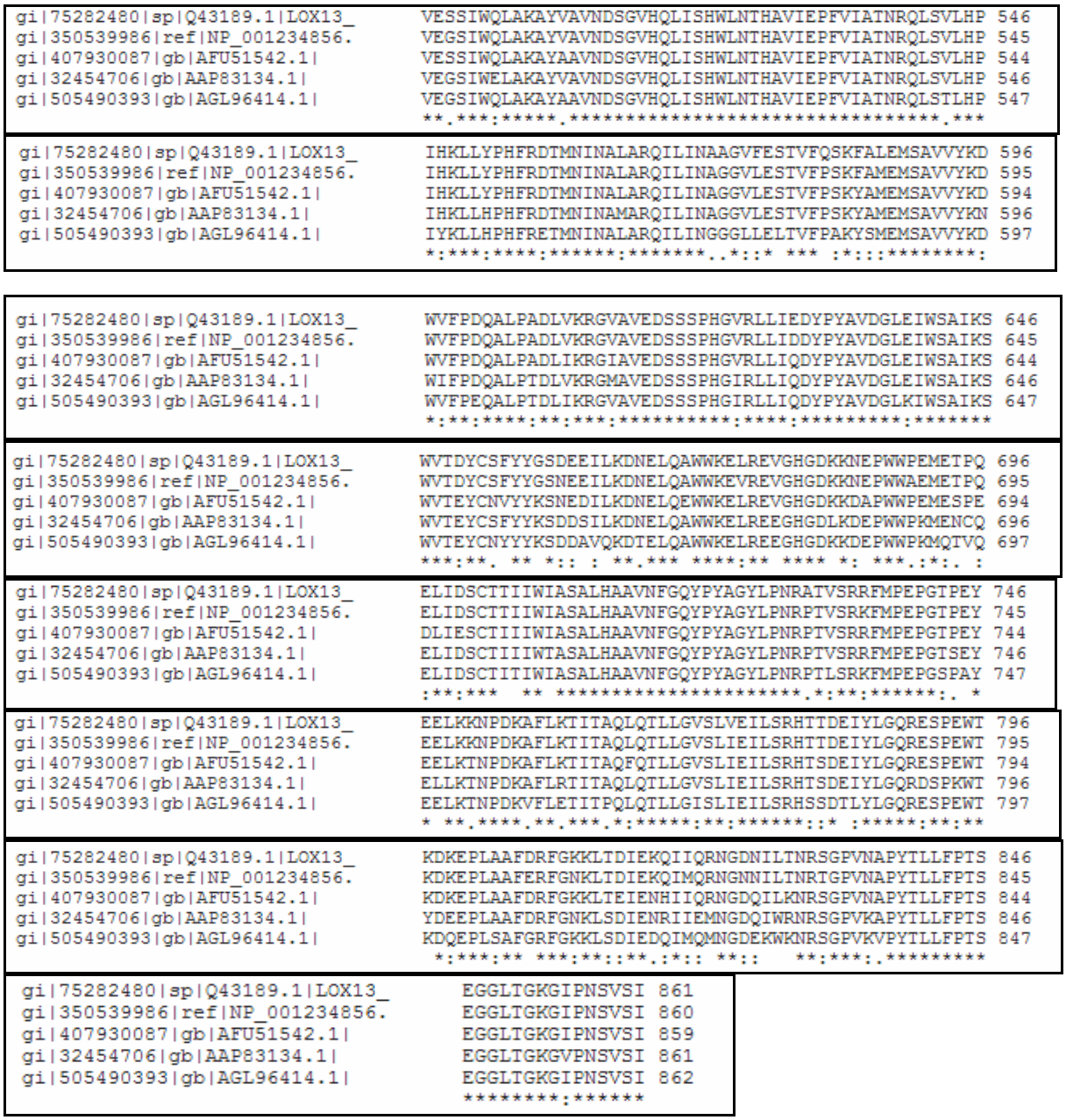

Figure 5: Multiple sequence alignment

From the alignment it's clear that there are fair number of *,**:** and**.** More than 60% similarity which depicts that the sequences are likely to share one or more functional domains. Table 2 represents pairwise alignment for each Figure 6: Phylogenetic tree of Linoleate 9S-lipoxygenase 3 with related genes.

possible pair of sequences using the Needleeman-Wunsch global alignment method and records these similarity scores for the pairwise comparisons. The maximum alignment score has been identified 93.84 between sequences 1:2 whereas minimum alignment is 78.28 between sequences 1:5. It

clearly reflects that species 1 *Solanum tuberosum* and species 2 *Solanum lycopersicum* are closely related and *Solanum tuberosum* and *Nicotina benthamiana* are diverse in nature.

Table 2: Scores of Pairwise Alignment

SeqA	Name	Length	SeqB	Name	Length	Score
1	gi|75282480|sp|Q43189.1|LOX13_SOLTU	861	2	gi|350539986|ref|NP_001234856.1|	860	93.84
1	gi|75282480|sp|Q43189.1|LOX13_SOLTU	861	3	gi|407930087|gb|AFU51542.1|	859	88.94
1	gi|75282480|sp|Q43189.1|LOX13_SOLTU	861	4	gi|32454706|gb|AAP83134.1|	861	82.11
1	gi|75282480|sp|Q43189.1|LOX13_SOLTU	861	5	gi|505490393|gb|AGL96414.1|	862	78.28
2	gi|350539986|ref|NP_001234856.1|	860	3	gi|407930087|gb|AFU51542.1|	859	90.22
2	gi|350539986|ref|NP_001234856.1|	860	4	gi|32454706|gb|AAP83134.1|	861	83.02
2	gi|350539986|ref|NP_001234856.1|	860	5	gi|505490393|gb|AGL96414.1|	862	79.65
3	gi|407930087|gb|AFU51542.1|	859	4	gi|32454706|gb|AAP83134.1|	861	84.87
3	gi|407930087|gb|AFU51542.1|	859	5	gi|505490393|gb|AGL96414.1|	862	79.74
4	gi|32454706|gb|AAP83134.1|	861	5	gi|505490393|gb|AGL96414.1|	862	81.65

3.2 Analysis of Protein Motif

A motif is a short conserved sequence pattern associated with distinct functions of a protein or DNA. In all the five species same two motif region has been identified. First motif is from 544th amino acid to 554th amino acid whose sequence is LHPIYKLLHPH. Similarly second motif is from517 amino acid to 531 amino acid (HQLISHWLNTHAVIE).Table 3 represent analysis of motif. A phylogenetic tree was constructed among these species. In the tree below, species having Accession number Q43189 and NP_001234856 are sister groups — they are closest relatives. Cladogram (Figure 6) has been constructed following UPGMA method which presents the phylogenetic relationship among five species.

Table 3: Protein motif analysis

Found Motif	Position	PROSITE	Description	Related Sequences	Related Structures
LIPOXYGENASE_2	544..554 Detail	PS00081	Lipoxygenases iron-binding region signature 2.	68	40
LIPOXYGENASE_1	517..531 Detail	PS00711	Lipoxygenases iron-binding region signature 1.	68	39

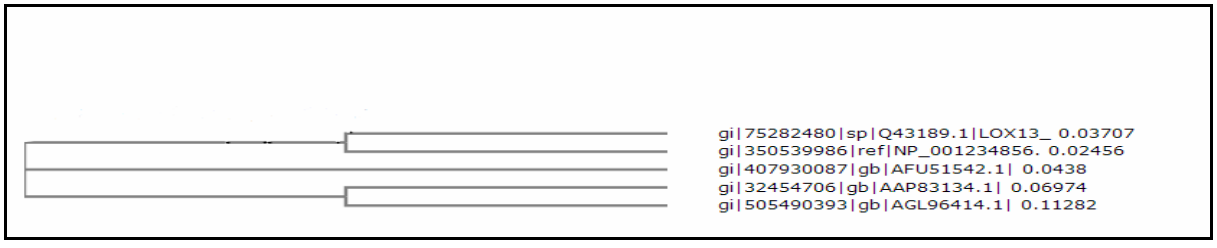

Figure 6: Phylogenetic tree of Linoleate 9S-lipoxygenase 3 with related genes.

Physiochemical analysis

Table 4 represent the physiochemical properties of Linoleate 9S-lipoxygenase 3(POTLX1) calculated using PROTPARAM.

Table 4: Comparative Physiochemical properties of linoleate 9S-lipoxygenase 3 gene of five different species of *Solanaceae* family.

S.NO.	Parameters	S. tuberosum	S. lycopesicum	N. Attenuata	C. annum	N. benthamiana
1	Number of amino acids	861	860	861	859	862
2	Molecular weight	96974.0	96765.0	97214.6	97041.2	97445.8
3	Theoretical pI	5.43	5.55	5.36	5.69	5.52
4	Negatively charged residues (Asp + Glu)	111	110	113	110	117
5	Positively charged residues (Arg + Lys)	87	88	85	90	97
6	Ala (A)	5.7%	5.8%	5.8%	5.7%	5.3%
7	Arg (R)	4.5%	4.5%	4.5%	4.7%	4.3%
8	Asn (N)	4.8%	4.9%	4.4%	4.8%	4.1%
9	Asp (D)	6.3%	5.9%	6.7%	6.3%	6.5%
10	Cys (C)	0.3%	0.3%	0.6%	0.3%	0.5%
11	Gln (Q)	3.3%	3.0%	2.9%	3.3%	3.5%
12	Glu (E)	6.6%	6.9%	6.4%	6.5%	7.1%
13	Gly (G)	6.7%	7.1%	6.6%	6.3%	6.6%
14	His (H)	2.4%	2.6%	2.8%	2.8%	2.1%
15	Ile (I)	6.3%	6.0%	6.7%	11.1%	5.9%
16	Leu (L)	10.8%	10.9%	10.8%	5.8%	11.1%
17	Lys (K)	5.6%	5.7%	5.3%	1.2%	7.0%
18	Met (M)	1.4%	1.5%	1.9%	1.2%	1.4%
19	Phe (F)	4.6%	4.5%	4.2%	4.4%	4.1%
20	Pro (P)	6.4%	6.5%	6.5%	6.9%	6.6%
21	Ser (S)	6.5%	6.0%	6.4%	6.8%	6.8%
22	Thr (T)	7.0%	6.5%	6.2%	6.1%	5.8%
23	Trp (W)	1.7%	1.7%	2.0%	1.7%	2.0%
24	Tyr (Y)	3.6%	3.6%	3.8%	3.7%	3.8%
25	Val (V)	5.5%	5.8%	5.5%	5.4%	5.6%
26	Formula	$C_{4389}H_{6788}N_{1152}O_{1300}S_{15}$	$C_{4384}H_{6785}N_{1153}O_{1288}S_{16}$	$C_{4403}H_{6796}N_{1152}O_{1292}S_{21}$	$C_{4400}H_{6805}N_{1161}O_{1291}S_{13}$	$C_{4416}H_{6853}N_{1151}O_{1304}S_{16}$
27	Extinction coefficient	128690	128815	142920	130305	13740
28	Estimated half-life	30 hours	30 hours	30 hours	30 hours	30 hours
29	Instability index	39.99	37.54	40.65	40.60	40.34
30	Aliphatic index	88.11	88.88	90.03	89.34	87.99
31	GRAVY	-0.334	-0.321	-0.295	-0.360	-0.394

At neutral pH, the fraction of negatively charged residues implies information about the location of the protein. Intracellular proteins tend to have a higher fraction of negatively charged residues than extra cellular proteins. Among the five species *Capsicum annum* and *Nicotina benthamiana* has 110 and 117 negatively charged residues. The value of positively charged residue of five varieties ranges from 85 in *Capsicum annum* to 97 in *Nicotina benthamiana*. The aliphatic index (AI) which is defined as the relative volume of a protein occupied by aliphatic side chains is regarded as a positive factor for the increase of thermal stability of globular proteins. Aliphatic index for POTLX1 is 90.03 *Capsicum annum* in 88.11, 89.34 in *Capsicum annum* and 88.88 in *Solanum lycopersicum*. The very high aliphatic index of particular gene in *Nicotina attenuata* (90.03) indicates that it may be stable for a wide temperature range. The instability index was computed to be 40.65 in *Nicotina attenuate,* 40.60 in *Capsicum annum* and 40.34 in *Nicotina benthamiana* which classify the protein as stable. The protein is hydrophilic in nature since the GRAVY value is negative (-0.295 to -0.394) in all the five varieties.

3.4 Secondary structure prediction

SOPMA computed the secondary structure of the considered protein and has been analysed in the five species of Solanaceae family. Table 5 shows the three-state description of the secondary structure (alpha-helix, beta-sheet and coil) along with their values.

Table 5: Comparative Secondary structure analysis of linoleate 9S-lipoxygenase 3

Parameters	***Solanum tuberosum***	***Solanum lycopersicum***	***Capsicum annum***	***Nicotina attenuata***	***Nicotina benthamiana***
Alpha helix(Hh)	36.59%	36.98%	37.83%	36.47%	37.24%
Extended strand	13.82%	14.88%	13.50%	14.87%	14.27%
Beta turn (Tt)	3.95%	5.23%	4.31%	4.07%	3.60%
Random coil (Cc)	45.64%	42.91%	44.35%	44.60%	44.90%

3.5 Functional domain and family analysis

Considering the analysis of Pfam Linoleate 9S-lipoxygenase 3**,** consists of PLAT domain and Lipoxygenases enzyme (EC 1.13.11). The PLAT domain is protein domain that is found in a variety of membrane or lipid associated proteins. It is called the PLAT (Polycystin-1, Lipoxygenase, Alpha-Toxin) domain or LH2 (Lipoxygenase homology) domain. The known structure of pancreatic lipase shows this domain binds to procolipase Pfam PF01114, which mediates membrane association. This domain is found in a variety of membrane or lipid associated proteins. It forms a beta-sandwich composed of two sheets of four strands each. Lipoxygenases are a class of iron-containing dioxygenases involved in the catalysis of the hydroperoxidation of lipids, containing a cis, cis-1, 4-pentadiene structure. The iron atom present in lipoxygenases is bound by four ligands, three of which are histidine residues. Six histidines are found to be conserved in all lipoxygenase sequences, five of them are found clustered in a stretch of 40 amino acids. This region contains two of the three zinc-ligands; the other histidines have been shown to be important for the activity of lipoxygenases.

Figure 7: Arrangement of Pfam domains

3.6 Conserved domain analysis

NCBI-CDD server resulted that PLAT superfamily PLAT (Polycystin-1, Lipoxygenase, Alpha-Toxin) domain or LH2 (Lipoxygenase homology 2) domain. It belongs to cl0011 superfamily. It consists of an eight stranded beta-barrel. The domain can be found in various domain architectures, in case of lipoxygenases, alpha toxin, lipases and polycystin, but also as a single domain or as repeats. The putative function of this domain is to facilitate access to sequestered membrane or micelle bound substrates.

Lipoxygenase superfamily- It belongs to cl09510. All five species has given the same result of conserved domain

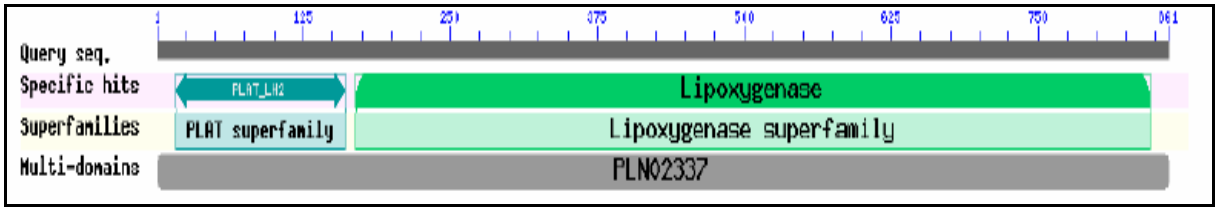

Figure 8: Conserved domain analysis

3.7 KEGG pathway analysis

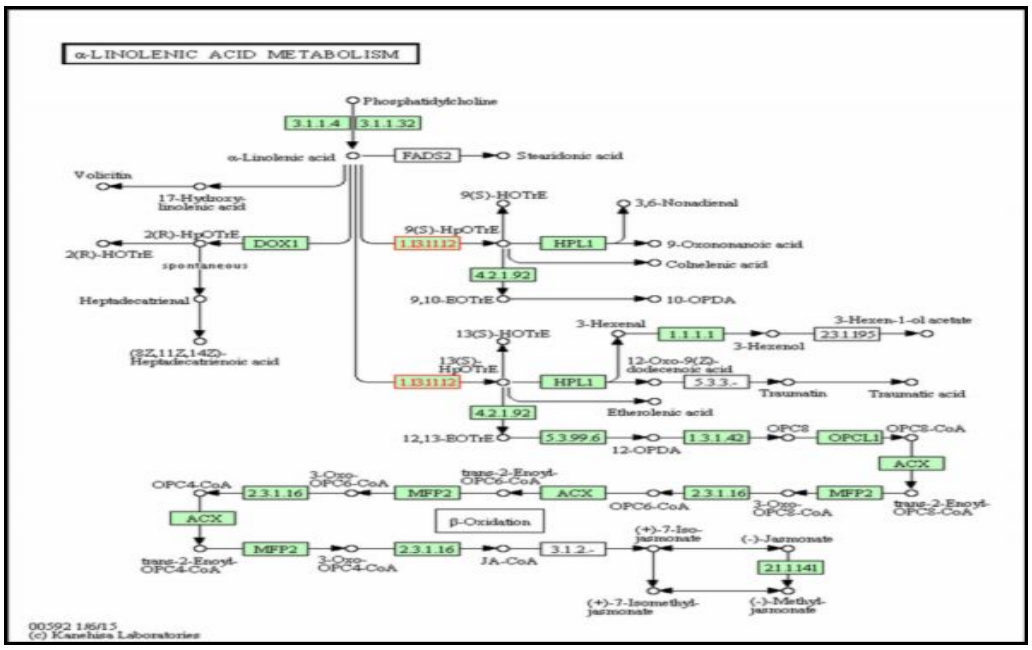

Figure 9: KEGG Pathway of linoleic acid metabolism in potato.

Table 6: List of enzymes involved in the lipoxygenase pathway.

Enzymes	Definition	Amino acid sequence
3.1.1.4	triacylglycerol lipase SDP1-like	824
3.1.1.32	uncharacterized LOC102583663	365
DOX1	alpha-dioxygenase 1-like	638
1.13.11.12	lipoxygenase 6, chloroplastic-like	168
HPL1	fatty acid hydroperoxide lyase	480
4.2.1.92	allene oxide synthase	510
1.13.11.12	lipoxygenase 6, chloroplastic-like	168
HPL1	fatty acid hydroperoxide lyase	480
5.3.99.6	allene oxide cyclase	246
1.3.1.42	12-oxophytodienoate reductase-like protein-like	349
OPCL1	4-coumarate--CoA ligase-like 5-like	551
ACX	acyl-coenzyme A oxidase 4, peroxisomal-like	428
MFP2	glyoxysomal fatty acid beta-oxidation multifunctional protein MFP-a-like	727
2.3.1.16	3-ketoacyl-CoA thiolase 2, peroxisomal-like	465
2.1.1.141	jasmonate O-methyltransferase-like	377
Enzymes	Definition	Amino acid sequence
3.1.1.4	triacylglycerol lipase SDP1-like	824
3.1.1.32	uncharacterized LOC102583663	365
DOX1	alpha-dioxygenase 1-like	638
1.13.11.12	lipoxygenase 6, chloroplastic-like	168
HPL1	fatty acid hydroperoxide lyase	480
4.2.1.92	allene oxide synthase	510
1.13.11.12	lipoxygenase 6, chloroplastic-like	168
HPL1	fatty acid hydroperoxide lyase	480
5.3.99.6	allene oxide cyclase	246
1.3.1.42	12-oxophytodienoate reductase-like protein-like	349
OPCL1	4-coumarate--CoA ligase-like 5-like	551
ACX	acyl-coenzyme A oxidase 4, peroxisomal-like	428
MFP2	glyoxysomal fatty acid beta-oxidation multifunctional protein MFP-a-like	727
2.3.1.16	3-ketoacyl-CoA thiolase 2, peroxisomal-like	465
2.1.1.141	jasmonate O-methyltransferase-like	377

LOX -1 gene is analyzed in KEGG pathway and about 16 manual pathways have been presented. They are

- Carotenoid biosynthesis
- Biosynthesis of secondary metabolites
- Linoleic acid metabolism
- Alpha Linoleic acid metabolism
- ABC transporters
- Cutine suberine and wax
- Lysine degradation
- Butanoate metabolism
- C5-Branched dibasic acid metabolism
- Monoterpenoid biosynthesis
- Ubiquinone and other terpenoid-quinone biosynthesis
- Biosynthesis of antibiotics

- Arachidonic acid metabolism
- Tropane, piperidine and pyridine
- Phagosome

Following Table 6 represents about all the vital enzymes their definition and amino acid sequence involved in the pathway.

4. Conclusion

Lipoxygenase have continued to gain importance due to its functionally diverse class of dioxygenases which is implicated in various physiological processes of growth and senescence. The main function of lipoxygenase is to catalyse the hydroperoxidation of lipids and to regulate the plant growth and development. Although advancements in research methodologies has led to increase in discovery of the role of lipoxygenases in tuber development in potato, but finding a potential gene still remain a tedious process. On the other hand, computational approach can help in analyzing the role of lipoxygenase in plant growth and checking its functionality through knowledge based database approach. This approach helps in identifying the prospective role of lipoxygenase; further comparing its characteristics with other species of same family.

This study was undertaken to identify the potential role of linoleate 9S- lipoxygenase 3 in the formation of tuber in potato, using bioinformatics tools. All the five species being considered are closely related in terms of homology as linoleate 9S- lipoxygenase 3 also known as POTLX-1 is conserved according to delta Blast result. It also shows orthology with POTLX-3 (according to KEGG analysis). The physiochemical properties of POTLX-1 gene was analyzed using Protparam and the aliphatic index of all the five species have come in the range of 88.11in *Solanum tuberosum* to 90.03 in *Nicotina attenuate.* The very high aliphatic index of particular gene in *Nicotina attenuata* indicates that it may be stable or can survive at wide temperature range. PLAT domain is an important constituent of all the five species which facilitates access to sequestered membrane or micelle bound substrate. Secondary structure analysis shows strong resemblance among all the five species being considered.

The information obtained through this study would be useful in gaining further information on lipoxygenases. This will not only help in identifying prospective role of genes involved in tuberization but will also facilitate wet lab techniques in discovering the particular genes and pathways.

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