

Evaluation of Lipid Transfer Protein (Ltp) 1 Gene in Sesame and Other Plants Using Bioinformatics Approach

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Abstract

This study was aimed at using bioinformatics tools to characterize the Lipid Transfer Protein 1 gene in some selected accessions with special reference to Benny seed (*Sesamum indicum*) Lipid Transfer Protein 1 sequence as a query sequence. Nucleotide and amino acid sequences of 30 accessions were retrieved from NCBI database and analyzed for homology, physicochemical properties, motifs, GC content as well as phylogenetic relationships. Results showed that nucleotide and amino acid sequence lengths of this gene among the selected accessions differs. Its nucleotide length varied between 599–8461bp, while the amino acids sequence varied between 96–355 residues, Molecular weight range from 10008.77–35532.61daltons. With *Sesamum indicum* having the lowest molecular weight and *Physcomitrium patens* having the highest molecular weight. Result on the Theoretical PI was above 4.61 for all the amino acid sequences of Lipid Transfer Protein 1 gene in the selected accessions. It was observed that the total number of negatively charged residues ranged from 1-20. The instability index and aliphatic index ranged from 20.23–69.39, 73.48–102.24 respectively. Some of the proteins are stable, while twelve were considered unstable following the results for instability index. Extinction coefficient was highest for *Sesamum indicum* (14480). *Daucus carota subsp. Sativus* (-0213) is the only accessions with a negative GRAVY. The motifs N-glycosylation site, Plant lipid transfer proteins signature, N-myristoylation site, Casein kinase II phosphorylation site, Protein kinase C phosphorylation site were the most common across the selected accessions. GC content analysis revealed that it ranged from 29.73–54.55%. Analysis of the secondary structure of the amino acid sequences of the Lipid Transfer Protein 1 gene showed that the region covered by random coil was the highest in the sequences compared to alpha helix and extended strand. Alpha helix ranges from 33.11–54.31%, the extended strands ranged from 9.17–15.13%, while the random coil ranges from 32.77–51.16% across the accessions. Following the results of the present study, it can be concluded that Lipid Transfer Protein 1 gene sequence of *Sesamum indicum* is closely related to Lipid Transfer Protein 1 gene in *Brachypodium distachyon* and distant to that in *Glycine max*, *Vigna unguiculata*, *Capsicum annum*.

Keywords: lipid transfer protein 1; sesamum indicum; bioinformatics

Introduction

Sesamum indicum is an annual herb of the family Pedaliaceae. The plant is commonly known as benniseed, benniseed in English and it is found in tropical and subtropical areas of Asia, Africa and South America. Compared to similar crops, such as peanuts, soybean, and rapeseed, the seeds of sesame are believed to have the most oil. Sesame seed is one of the oldest oilseed crops known, domesticated well over 3000 years ago. *S. indicum* has many other species, most being wild and native to sub-Saharan Africa. *S. indicum*, the cultivated type, originated in India (Ogasawara, et al., 1988) and is tolerant to drought-like conditions, growing where other crops fail (Raghav et al., 1990). Sesame has been widely known for its oil seeds production which has also finds wide applications in the food and

pharmaceutical industries due to their significance. The genes responsible for this oil production trait is the lipid transfer protein 1 gene whose properties, functions and structures, this research tries to analyze using in silico approach. The expression in silico was first used in public in 1989 in the workshop “Cellular Automata”: Theory and Application in Los Alamos New Mexico by Pedro Miramontes a Mathematician from National Autonomous University of Mexico (UNAM) who presented the report “DNA and RNA physiochemical constraints, Cellular Automata and Molecular Evolution. Plants genome shows a wide array of architectures i.e. (genetic make-up) varying immensely in size, structures and content. Some organelle DNA’s have even developed elaborate peculiarities such as scrambled coding regions, non-standard genetic codes and convoluted modes of post-

transcriptional modification and editing all of which has been deciphered using bioinformatics tools.

Bioinformatics is an interdisciplinary field that develops methods and software tools for understanding biological data. As an interdisciplinary field of science, bioinformatics combines Computer Science, Biology, Mathematics and Engineering to analyze and interpret biological data. Bioinformatics has been used for *in silico* analyses of biological data and genes using mathematical and statistical techniques. Bioinformatics has become an important part of many areas of biology. In experimental molecular biology, Bioinformatics techniques such as image and signal processing allow extraction of useful results from large amount of raw data. In the field of genetics and genomics, it aids in sequencing and annotating genomes and their observed mutations. It plays a role in the text mining of biological literature and the development of biological and gene ontologies to organize and query biological data. It also plays a role in the analysis of gene and protein expression and regulation. Bioinformatics tools aid in the comparison of genetic and genomic data and more generally in the understanding of evolutionary aspects of molecular biology. At a more integrative level, it helps to analyze and catalogue the biological pathways and networks that are an important part of systems biology. In structural biology, it aids in the simulation and modeling of DNA, RNA, proteins as well as bimolecular interactions.

In Genetics and Biochemistry, *in silico* studies can be used to examine the molecular modeling of gene, gene expression, gene sequence analysis and 3D structure of proteins, identification of diseases and prediction of lipid metabolic pathways. *In silico* studies/drugs designing software plays an important role to design innovative proteins.

With the increasing concern on the side effects caused by modern synthetic on chemical drugs, oil seeds and medicinal plants remain the main source of a large range of basic healthcare and pharmaceutical products. Successful attempts, to produce some of the valuable in relatively large quantities by cell cultures have been reported.

Oil rich plants such as *Sesame indicum* have been used as a source of food and medicine since historic times. In the era of high volume, high throughput data generation across the biosciences, bioinformatics plays a crucial role in food, drug design, drug discovery and metabolism. Availability of the functional and active components of lipid transfer

protein genes in plant species is an indication of increase yield and high output for medicinal relevance and therapeutic security. The yield and potency of active ingredients in the oil rich plants will depend largely on the expressions in functional and structural of the LTP gene that controls photosynthesis, nutrition and lipid metabolism in general. There is therefore, need to study lipid transfer protein genes underlying this potential to unveil the physiochemical characteristics and other parameters which makes the usefulness of these genes unique to the plant breeders and biotechnologists. The study is aimed at using *in silico* approach to evaluate lipid transfer protein (LTP) gene variations in sesame and other plants. The objectives of the study are to identify the percentage identity and similarity in the Lipid Transfer Protein (LTP) gene in sesame and other plants, to determine the variations in the physiochemical properties of the Lipid transfer protein (LTP) gene in sesame and other plants, to determine the Lipid transfer protein (LTP) gene stability in terms of their Guanine–cytosine contents.

Materials and Methods

Experimental site

The *in-silico* study was carried out in the bioinformatics laboratory of the Department of Genetics and Biotechnology, University of Calabar, Calabar.

Retrieval of Nucleotides and Amino Acid Sequences

The nucleotides and amino acid sequences of the lipid transfer protein genes in low and high oil seeds producing varieties of sesame was retrieved using the FASTA format from the National Centre for Biotechnology information (NCBI) database. The accession numbers, sequence lengths and E-values was recorded.

Determination of Percent Identity and Similarity (Homology)

Percentage identity and similarity among the nucleotides and amino acid sequences of the retrieved sequences for lipid transfer protein genes in low and high oil seeds producing varieties of sesame plants was determined using similarity homology comparison tool for more than two sequences option of the basic alignment search tool.

Determination of Physico Chemical Properties of the lipid transfer protein genes in low and

high oil seeds producing varieties of sesame plant species

The physico-chemical properties of the lipid transfer protein genes in low and high oil seeds producing varieties of sesame were determined using the Expert Protein Analysis System (EXPASY) which is a proteomic server of the Swiss Institute of Bioinformatics (SIB) using the online program of the Expasy site. The proteomic server of the expasy.org was used to access the protparam site which displays the physiochemical properties of the query amino acid sequences.

Determination of secondary and tertiary protein structures of lipid transfer protein genes in low and high oil seeds producing varieties of sesame

Prediction of motif for secondary structure was done using NSOPMA software. The motif for the prediction tertiary structure (3D protein structure) for the lipid transfer protein genes was done by pasting the protein sequences on the interactive online platform of Phyre and phyre or phyre 2 (Protein Homolog Y Analysis Recognition Engine) based on translated amino acid sequence retrieved from the NCBI databases as modified by Kelley and Stemberg, 2009. The Rasmol (Raswin) software was used to fine tune the 3D protein structure to ribbons or cartoons with desired colours and magnitudes.

Phylogenetics analysis of lipid transfer protein genes in low and high oil seeds producing varieties of sesame

The MEGA X software was used to align the retrieved DNA and protein sequences and subject to phylogenetic analysis for phylogenetic tree, substitution model selection, evolutionary distance estimation, phylogeny inference, substitution rate and pattern estimation, test of natural selection and ancestral sequence inference.

Determination of Guanine-Cytosine contents and N-terminal amino acids side chains

The Genscan online interaction programme of Expasy.org. and SWISS institute of Bioinformatic (SIB) suite was used to determine the guanine - cytosine contents of the nucleotide sequences for the lipid transfer protein genes for the low and high oil seeds yielding varieties of sesame as well as the N-terminal amino acid side chains.

Results

Determination of nucleotide and amino acid sequences length in selected accessions

Results obtained for sequence lengths of nucleotide and amino acids sequences of Lipid Transfer Protein 1 gene showed that the nucleotide sequence lengths ranged from 599–8461bp. While amino acid sequence lengths ranged from 96 – 355 residues. It was observed that nucleotide sequence of Lipid Transfer Protein 1 gene in *Sorghum bicolor* (8461bp) was the longest while that in *Sesamum indicum* had the shortest sequence (599bp) as shown in table 1.

Table 1: Nucleotide and amino acid sequences of Lipid Transfer Protein 1 gene in selected plants.

Name of Plants	Accession number	AA	DNA sequencing
<i>Sesamum indicum</i>	NC_026157	96	599
<i>Ricinus communis</i>	NW_002994932	115	848
<i>Daucus carota subsp. sativus</i>	NC_030386	123	1349
<i>Medicago truncatula</i>	NC_053042	151	967
<i>Helianthus annuus</i>	NC_035433	119	1026
<i>Nicotiana tabacum</i>	NW_015904831	117	1217
<i>Gossypium hirsutum</i>	NC_053433	120	761
<i>Vigna unguiculata</i>	NC_040279	183	1856
<i>Nicotiana attenuata</i>	NC_031995	117	1372
<i>Dendrobium catenatum</i>	NW_021319309	118	1321
<i>Musa acuminata</i>	NC_025202	117	1264
<i>Brassica rapa</i>	NC_024803	147	794
<i>Oryza sativa Japonica Group</i>	NC_029256	120	924
<i>Physcomitrium patens</i>	NC_037253	355	1856
<i>Vigna radiata</i>	NW_014543115	117	1670
<i>Prunus persica</i>	NC_034014	117	821
<i>Arachis hypogaea</i>	NC_037619	116	1528
<i>Solanum tuberosum</i>	NW_006239682	114	968
<i>Sorghum bicolor</i>	NC_012877	119	8461

<i>Glycine max</i> (2)	NC_016088	119	4094
<i>Brachypodium distachyon</i>	NC_016131	172	2871
<i>Capsicum annum</i>	NC_029977	142	769
<i>Arabidopsis thaliana</i>	NC_003071	118	951
<i>Solanum pennellii</i>	NC_028646	114	930
<i>Vitis vinifera</i>	NC_012007	201	2620
<i>Juglans regia</i>	NC_049903	119	1151
<i>Pistacia vera</i>	NW_022196275	117	613
<i>Brassica napus</i>	NC_027765	147	773
<i>Olea europaea</i> var. <i>sylvestris</i>	NC_036249	118	736
<i>Glycine max</i>	NC_016088	119	4094

Determination of Percentage Identity and Similarity of the Nucleotide Sequence of Lipid Transfer Protein Gene in the Selected Accessions

Result of the percentage identity of amino acid sequence of Lipid Transfer Protein 1 gene in the selected accessions showed that the highest identity to the LTP1 sequence in the query.

Physicochemical Properties of Lipid Transfer Protein 1 gene in the selected sequences

Result of the analysis of the physicochemical properties of amino acid of Lipid Transfer Protein 1 gene in the selected accessions showed that the number of amino acid residues ranged from 96–355 residues. Molecular weight ranged from 10008.77–35532.61daltons. With *Sesamum indicum* having the lowest molecular weight and *Physcomitrium patens*.

having the highest molecular weight, as shown in Table 3. None of the other accessions had amino acid residues and molecular weight equal to that of Lipid Transfer Protein 1 gene in the query. Result of the theoretical PI was above 4.61 for all the amino acid sequence of Lipid Transfer Protein 1 gene in the selected accessions. It was observed that the total number of negatively charged residues ranged from 1–20. The total number of positively charged residues ranged from 6–19. The instability index and the aliphatic index ranged from 20.23–69.39, 73.48–102.24 respectively. Some of the proteins are stable, while twelve were considered unstable following the results for instability index. Extinction coefficient was highest for *Sesamum indicum* (14480). *Daucus carota* subsp. *Sativus* (-0213) is the only accessions with a negative GRAVY. (Table 3).

Table 2: Percentage identity, similarity of Lipid Transfer Protein 1 gene in selected accessions.

Name of Plants	Accession number	Percentage identity (%)	Percentage Similarity (%)
<i>Sesamum indicum</i>	NC_026157	96	97
<i>Ricinus communis</i>	NW_002994932	93	95
<i>Daucus carota</i> subsp. <i>sativus</i>	NC_030386	88	92
<i>Medicago truncatula</i>	NC_053042	97	100
<i>Helianthus annuus</i>	NC_035433	91	94
<i>Nicotiana tabacum</i>	NW_015904831	94	97
<i>Gossypium hirsutum</i>	NC_053433	92	96
<i>Vigna unguiculata</i>	NC_040279	98	100
<i>Nicotiana attenuata</i>	NC_031995	88	93
<i>Dendrobium catenatum</i>	NW_021319309	96	99
<i>Musa acuminata</i>	NC_025202	87	95
<i>Brassica rapa</i>	NC_024803	97	100
<i>Oryza sativa Japonica Group</i>	NC_029256	40	70
<i>Physcomitrium patens</i>	NC_037253	87	93
<i>Vigna radiata</i>	NW_014543115	85	92
<i>Prunus persica</i>	NC_034014	83	91
<i>Arachis hypogaea</i>	NC_037619	88	92
<i>Solanum tuberosum</i>	NW_006239682	96	98
<i>Sorghum bicolor</i>	NC_012877	93	97
<i>Glycine max</i> (2)	NC_016088	97	100
<i>Brachypodium distachyon</i>	NC_016131	94	98
<i>Capsicum annum</i>	NC_029977	92	100

<i>Arabidopsis thaliana</i>	NC_003071	91	96
<i>Solanum pennellii</i>	NC_028646	87	92
<i>Vitis vinifera</i>	NC_012007	84	92
<i>Juglans regia</i>	NC_049903	86	91
<i>Pistacia vera</i>	NW_022196275	92	95
<i>Brassica napus</i>	NC_027765	95	100
<i>Olea europaea</i> var. <i>sylvestris</i>	NC_036249	95	96
<i>Glycine max</i>	NC_016088	97	100

Table 3: Physico-chemical properties of amino acid sequence of lipid transfer protein 1 gene in selected accessions.

S/N	Access. No	Name Of Plants	Aa	Mol. Wt	The . Pi	Ext. Coeff .	Ii	A Index	Gravy	-Ve (Asp+ Glu)	+Ve (Arg+ Lys)	Seq. Length (Bp)	No. Of Atoms
1.	NC_02 6157	<i>Sesamum indicum</i>	96	10008.77	7.50	14480	33.79	85.31	0.408	7	8	599	1405
2.	NW_00 299493 2	<i>Ricinus communis</i>	115	11766.75	8.05	3605	23.04	83.91	0.449	5	7	848	1633
3.	NC_03 0386	<i>Daucus carota</i> subsp. <i>Sativus</i>	123	13429.78	9.55	1490	20.23	75.45	-0.213	13	19	1349	1919
4.	NC_05 3042	<i>Medicago truncatula</i>	151	15344.13	8.81	2115	52.24	94.97	0.321	5	11	967	2189
5.	NC_03 5433	<i>Helianthus annuus</i>	119	12418.56	9.37	4970	31.86	89.24	0.161	4	13	1026	1748
6.	NW_01 590483 1	<i>Nicotiana tabacum</i>	117	11757.69	8.10	3480	30.91	97.61	0.534	4	6	1217	1645
7.	NC_05 3433	<i>Gossypium hirsutum</i>	120	11834.88	9.04	3605	27.55	89.50	0.463	2	9	761	1660
8.	NC_04 0279	<i>Vigna unguiculata</i>	183	19377.62	8.06	8980	50.51	88.42	0.190	13	15	1856	2732
9.	NC_03 1995	<i>Nicotiana attenuate</i>	117	11793.81	8.63	4970	31.64	99.32	0.539	3	7	1372	1656
10.	NW_02 131930 9	<i>Dendrobium catenatum</i>	118	12095.22	8.89	8980	69.39	86.95	0.417	4	9	1321	1696
11.	NC_02 5202	<i>Musa acuminata</i>	117	11758.80	9.32	3480	40.34	97.52	0.446	4	12	1264	1675
12.	NC_02 4803	<i>Brassica rapa</i>	147	15420.64	9.59	2115	47.37	102.24	0.266	3	17	794	2229
13.	NC_02 9256	<i>Oryza sativa Japonica Group</i>	120	12092.24	9.28	6460	38.34	100.08	0.558	1	9	924	1709
14.	NC_03 7253	<i>Physcomitrium patens</i>	355	35532.61	4.61	4105	51.69	78.45	0.315	20	11	1856	4914
15.	NW_01 454311 5	<i>Vigna radiata</i>	117	11704.85	9.08	2115	30.54	94.36	0.598	2	10	1670	1655
16.	NC_03 4014	<i>Prunus persica</i>	117	11806.90	9.25	4970	32.61	94.27	0.434	1	9	821	1664
17.	NC_03 7619	<i>Arachis hypogaea</i>	116	11539.70	9.28	1990	20.31	86.81	0.585	1	9	1528	1619
18.	NW_00 623968 2	<i>Solanum tuberosum</i>	114	11471.64	8.73	3605	39.04	95.88	0.510	5	10	968	1622

19.	NC_01 2877	<i>Sorghum bicolor</i>	119	11564. 19	9.30	3480	36.69	88.82	0.446	3	10	8461	1614
20.	NC_01 6088	<i>Glycine max(2)</i>	119	12583. 66	9.95	3605	48.54	86.13	0.150	4	16	4094	1760
21.	NC_01 6131	<i>Brachypod ium distachyon</i>	172	16678. 42	7.50	4970	60.91	93.31	0.592	7	8	2871	2355
22.	NC_02 9977	<i>Capsicum annuum</i>	142	14654. 83	9.55	625	28.53	109.8 6	0.387	3	16	769	2136
23.	NC_00 3071	<i>Arabidopsi s thaliana</i>	118	11754. 89	9.30	3605	46.19	97.71	0.475	1	10	951	1658
24.	NC_02 8646	<i>Solanum pennellii</i>	114	11542. 76	8.87	3605	38.30	95.88	0.473	5	11	930	1636
25.	NC_01 2007	<i>Vitis vinifera</i>	201	21011. 92	5.84	1047 0	40.33	73.48	0.042	17	16	2620	2922
26.	NC_04 9903	<i>Juglans regia</i>	119	11741. 95	9.20	3480	35.83	95.88	0.524	3	11	1151	1671
27.	NW_02 219627 5	<i>Pistacia vera</i>	117	12090. 92	4.86	5095	22.39	78.55	0.241	5	4	613	1658
28.	NC_02 7765	<i>Brassica napus</i>	147	15420. 64	9.59	2115	47.37	102.2 4	0.266	3	17	773	2229
29.	NC_03 6249	<i>Olea europaea var. sylvestris</i>	118	11980. 28	9.20	9440	30.17	90.00	0.398	4	13	736	1704
30.	NC_01 6088	<i>Glycine max</i>	119	12583. 66	9.95	3605	48.54	86.13	0.150	4	16	4094	1760

Table 4: Motifs in Amino acid sequence of Lipid Transfer Protein 1 gene in selected accessions.

Plants	Accessions	Motif
<i>Sesamum indicum</i>	NC_026157	N-myristoylation site, Protease inhibitor/seed storage/ <i>LTP</i> family, Extensin_1 Extensin-like protein repeat
<i>Ricinus communis</i>	NW_002994932	N-glycosylation site, Casein kinase II phosphorylation site, Protein kinase C phosphorylation site, N-myristoylation site, Protease inhibitor/seed storage/ <i>LTP</i> family, Big-1 (bacterial Ig-like domain 1) domain profile
<i>Daucus carota subsp. sativus</i>	NC_030386	Casein kinase II phosphorylation site, N-myristoylation site, Protein kinase C phosphorylation site, SCP-2 sterol transfer family, Protein kinase C phosphorylation site, Microbodies C-terminal targeting signal
<i>Medicago truncatula</i>	NC_053042	Protein kinase C phosphorylation site, N-myristoylation site, Casein kinase II phosphorylation site, Lysosome-associated membrane glycoprotein family, Proline-rich region profile, Tryp_alpha_amyl Protease inhibitor family, MYRISTYL N-myristoylation site, DEC-1 protein, N-terminal region
<i>Helianthus annuus</i>	NC_035433	Casein kinase II phosphorylation site, Plant lipid transfer proteins signature, N-myristoylation site, Protein kinase C phosphorylation site, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Nicotiana tabacum</i>	NW_015904831	N-myristoylation site, Plant lipid transfer proteins signature, Casein kinase II phosphorylation site, N-glycosylation site, Agouti protein, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Gossypium hirsutum</i>	NC_053433	Protein kinase C phosphorylation site, Plant lipid transfer proteins signature, Casein kinase II phosphorylation site, N-myristoylation site, Amastin surface glycoprotein, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Vigna unguiculata</i>	NC_040279	cAMP- and cGMP-dependent protein kinase phosphorylation site, Protein kinase C phosphorylation site, Casein kinase II phosphorylation site, N-glycosylation site N-myristoylation site, Big-1 (bacterial Ig-like domain 1) domain profile, Protease inhibitor/seed storage/ <i>LTP</i> family, NPR nonapeptide repeat
<i>Nicotiana attenuata</i>	NC_031995	N-glycosylation site, Casein kinase II phosphorylation site, N-myristoylation site, Plant lipid transfer proteins signature, Agouti protein, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Dendrobium catenatum</i>	NW_021319309	N-myristoylation site, N-glycosylation site, Plant lipid transfer proteins signature, Ankyrin repeat, Sialic-acid binding microneal adhesive repeat, Protease inhibitor/seed storage/ <i>LTP</i> family

<i>Musa acuminata</i>	NC_025202	Casein kinase II phosphorylation site, Protease inhibitor/seed storage/ <i>LTP</i> family, N-myristoylation site, Plant lipid transfer proteins signature
<i>Brassica rapa</i>	NC_024803	Proline-rich region profile, N-glycosylation site, Protease inhibitor/seed storage/ <i>LTP</i> family, Protein kinase C phosphorylation site, N-myristoylation site, Procylic acidic repetitive protein (PARP)
<i>Oryza sativa Japonica Group</i>	NC_029256	N-glycosylation site, Protease inhibitor/seed storage/ <i>LTP</i> family, Plant lipid transfer proteins signature, N-myristoylation site
<i>Physcomitrium patens</i>	NC_037253	N-myristoylation site, Protein kinase C phosphorylation site, Casein kinase II phosphorylation site, N-glycosylation site, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Vigna radiata</i>	NW_014543115	Plant lipid transfer proteins signature, Protein kinase C phosphorylation site, N-myristoylation site, Casein kinase II phosphorylation site, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Prunus persica</i>	NC_034014	Plant lipid transfer proteins signature, N-myristoylation site, Casein kinase II phosphorylation site, Protein kinase C phosphorylation site, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Arachis hypogaea</i>	NC_037619	Plant lipid transfer proteins signature, N-myristoylation site, Casein kinase II phosphorylation site, Protein kinase C phosphorylation site, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Solanum tuberosum</i>	NW_006239682	Plant lipid transfer proteins signature, N-myristoylation site, Protein kinase C phosphorylation site, Casein kinase II phosphorylation site, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Sorghum bicolor</i>	NC_012877	Plant lipid transfer proteins signature, Casein kinase II phosphorylation site, N-myristoylation site, Alanine-rich region profile, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Glycine max</i> (2)	NC_016088	Plant lipid transfer proteins signature, Protein kinase C phosphorylation site, Casein kinase II phosphorylation site, N-myristoylation site, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Brachypodium distachyon</i>	NC_016131	N-myristoylation site, Protein kinase C phosphorylation site, N-glycosylation site, Alanine-rich region profile, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Capsicum annum</i>	NC_029977	Prokaryotic membrane lipoprotein lipid attachment site profile, Proline-rich region profile, Protein kinase C phosphorylation site, cAMP- and cGMP-dependent protein kinase phosphorylation site, N-myristoylation site, Amidation site, Protease inhibitor/seed storage/ <i>LTP</i> family, Penaeidin, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Arabidopsis thaliana</i>	NC_003071	Prokaryotic membrane lipoprotein lipid attachment site profile, Plant lipid transfer proteins signature, N-myristoylation site, Casein kinase II phosphorylation site, Protein kinase C phosphorylation site, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Solanum pennellii</i>	NC_028646	Plant lipid transfer proteins signature, N-myristoylation site, Protein kinase C phosphorylation site, Casein kinase II phosphorylation site, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Vitis vinifera</i>	NC_012007	N-myristoylation site, Casein kinase II phosphorylation site, Protein kinase C phosphorylation site, N-glycosylation site, CHCH domain, Protease inhibitor/seed storage/ <i>LTP</i> family
<i>Juglans regia</i>	NC_049903	Plant lipid transfer proteins signature, N-myristoylation site, Casein kinase II phosphorylation site, Protease inhibitor/seed storage/ <i>LTP</i> family, cAMP- and cGMP-dependent protein kinase phosphorylation site
<i>Pistacia vera</i>	NW_022196275	Casein kinase II phosphorylation site, Plant lipid transfer proteins signature, N-glycosylation site, Big-1 (bacterial Ig-like domain 1) domain profile, Protein kinase C phosphorylation site, N-myristoylation site
<i>Brassica napus</i>	NC_027765	Proline-rich region profile, N-glycosylation site, Protein kinase C phosphorylation site, N-myristoylation site
<i>Olea europaea var. sylvestris</i>	NC_036249	N-myristoylation site, Plant lipid transfer proteins signature, Protein kinase C phosphorylation site, Protease inhibitor/seed storage/ <i>LTP</i> family, Casein kinase II phosphorylation site
<i>Glycine max</i>	NC_016088	Plant lipid transfer proteins signature, Protein kinase C phosphorylation site, Casein kinase II phosphorylation site, N-myristoylation site, Protease inhibitor/seed storage/ <i>LTP</i> family

Table 5: G-C contents and other parameters of nucleotide sequence of Lipid Transfer Protein 1 gene in selected accessions.

Accessions	Accession numbers	GC Contents (%)	Sequence Length (bp)	Start Codon	End Codon
<i>Sesamum indicum</i>	NC_026157	48.75	599	127	417
<i>Ricinus communis</i>	NW_002994932	39.86	848	82	419
<i>Daucus carota subsp. sativus</i>	NC_030386	34.91	1349	175	300
<i>Medicago truncatula</i>	NC_053042	34.75	967	127	582
<i>Helianthus annuus</i>	NC_035433	35.87	1026	58	407
<i>Nicotiana tabacum</i>	NW_015904831	36.57	1217	96	439
<i>Gossypium hirsutum</i>	NC_053433	45.07	761	330	519
<i>Vigna unguiculata</i>	NC_040279	39.82	1856	86	425
<i>Nicotiana attenuata</i>	NC_031995	36.44	1372	216	559

<i>Dendrobium catenatum</i>	NW_021319309	35.43	1321	72	422
<i>Musa acuminata</i>	NC_025202	48.18	1264	76	419
<i>Brassica rapa</i>	NC_024803	40.68	794	102	545
<i>Oryza sativa Japonica Group</i>	NC_029256	54.55	924	121	489
<i>Physcomitrium patens</i>	NC_037253	52.86	1856	474	1445
<i>Vigna radiata</i>	NW_014543115	31.80	1670	96	436
<i>Prunus persica</i>	NC_034014	43.48	821	97	440
<i>Arachis hypogaea</i>	NC_037619	31.48	1528	267	475
<i>Solanum tuberosum</i>	NW_006239682	35.85	968	96	430
<i>Sorghum bicolor</i>	NC_012877	41.73	8461	247	342
<i>Glycine max(2)</i>	NC_016088	29.73	4094	216	565
<i>Brachypodium distachyon</i>	NC_016131	48.69	2871	396	735
<i>Capsicum annum</i>	NC_029977	36.15	769	150	271
<i>Arabidopsis thaliana</i>	NC_003071	40.06	951	129	475
<i>Solanum pennellii</i>	NC_028646	34.30	930	104	438
<i>Vitis vinifera</i>	NC_012007	35.84	2620	102	465
<i>Juglans regia</i>	NC_049903	39.88	1151	83	436
<i>Pistacia vera</i>	NW_022196275	43.07	613	102	234
<i>Brassica napus</i>	NC_027765	40.49	773	81	524
<i>Olea europaea var. sylvestris</i>	NC_036249	43.07	736	113	436
<i>Glycine max</i>	NC_016088	29.73	4094	216	565

Motifs in Amino Acid Sequences of Lipid Transfer

Result of the analysis of Motifs in amino acid sequence of Lipid Transfer Protein 1 gene in the selected accessions showed that the motifs were within 3 - 9 across accessions. The motifs N-glycosylation site, Plant lipid transfer proteins signature, N-myristoylation site, Casein kinase II phosphorylation site, Protein kinase C phosphorylation site were the most common motifs found in the amino acid sequences of Lipid transfer Protein 1 gene in the selected accessions.

GC content and start and end codons of Lipid Transfer Protein 1 gene in the Selected Accessions

Result of GC analysis revealed that GC content of Lipid Transfer Protein 1 gene in the selected accessions ranged from 29.73 - 54.55% with *Glycine*

max having the lowest GC content and *Oryza sativa Japonica Group* having the highest GC content (54.55%). The start and end codons for the Lipid Transfer Protein 1 gene in the accessions selected varied generally although some accessions had the same start codons but not the same end codons. (Table 5).

Secondary Structure of Amino Acid and Sequences of Lipid Transfer Protein 1 gene in the Selected Accessions

Results of the analysis of secondary structures of the amino acid sequences of Lipid Transfer Protein 1 gene showed that the region covered by random coil was the highest in the sequences compared to alpha helix and extended strand. Alpha helix ranges from 33.11 - 54.31%, the extended strands ranged from 9.17 - 15.13%, while the random coil ranges from 32.77 - 51.16% across the accessions. (Table 6).

Table 6: Secondary structure of amino acids sequence of Lipid Transfer Protein 1 gene in selected accessions.

Plants	Accession Numbers	Sequence Length	Alpha helix (%)	Extended strand (%)	Random coil (%)
<i>Sesamum indicum</i>	NC_026157	96	35.42	12.50	42.71
<i>Ricinus communis</i>	NW_002994932	115	46.96	13.04	38.26
<i>Daucus carota subsp. sativus</i>	NC_030386	123	38.21	15.45	38.21
<i>Medicago truncatula</i>	NC_053042	151	33.11	13.91	50.33
<i>Helianthus annuus</i>	NC_035433	119	47.90	10.92	37.82
<i>Nicotiana tabacum</i>	NW_015904831	117	46.15	9.40	42.74
<i>Gossypium hirsutum</i>	NC_053433	120	45.83	9.17	44.17
<i>Vigna unguiculata</i>	NC_040279	183	36.07	14.21	46.99
<i>Nicotiana attenuata</i>	NC_031995	117	44.44	9.40	41.03
<i>Dendrobium catenatum</i>	NW_021319309	118	46.61	11.02	40.68
<i>Musa acuminata</i>	NC_025202	117	43.59	11.11	41.88

<i>Brassica rapa</i>	NC_024803	147	34.01	17.01	45.58
<i>Oryza sativa Japonica Group</i>	NC_029256	120	36.67	15.00	45.00
<i>Physcomitrium patens</i>	NC_037253	355	35.49	9.58	49.86
<i>Vigna radiata</i>	NW_014543115	117	46.15	13.68	38.46
<i>Prunus persica</i>	NC_034014	117	47.01	10.26	42.74
<i>Arachis hypogaea</i>	NC_037619	116	54.31	7.76	36.21
<i>Solanum tuberosum</i>	NW_006239682	114	41.23	9.65	45.61
<i>Sorghum bicolor</i>	NC_012877	119	51.26	12.61	32.77
<i>Glycine max</i> (2)	NC_016088	119	39.50	15.13	42.86
<i>Brachypodium distachyon</i>	NC_016131	172	40.12	6.98	51.16
<i>Capsicum annuum</i>	NC_029977	142	38.73	10.56	47.18
<i>Arabidopsis thaliana</i>	NC_003071	118	48.31	10.17	39.83
<i>Solanum pennellii</i>	NC_028646	114	42.11	10.53	44.74
<i>Vitis vinifera</i>	NC_012007	201	36.32	13.93	43.78
<i>Juglans regia</i>	NC_049903	119	43.70	15.13	36.13
<i>Pistacia vera</i>	NW_022196275	117	46.15	11.11	38.46
<i>Brassica napus</i>	NC_027765	147	34.01	17.01	45.58
<i>Olea europaea var. sylvestris</i>	NC_036249	118	47.46	7.63	43.22
<i>Glycine max</i>	NC_016088	119	39.50	15.13	42.86

Phylogenetic relationship of Lipid Transfer Protein 1 gene sequence in the selected accessions

The Lipid Transfer Protein 1 gene sequence from the selected accessions analyzed showed that there was

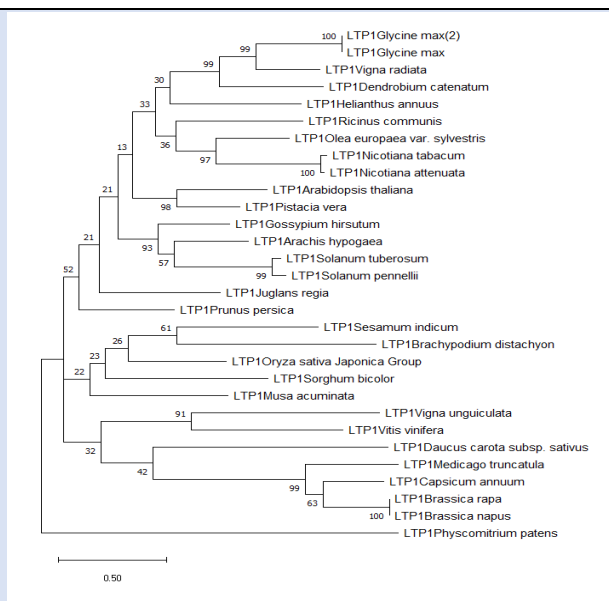


Figure 1: Phylogenetic tree of Lipid Transfer Protein 1 gene in the selected accessions.

Tertiary structure of amino acid sequences of Lipid Transfer Protein 1 gene in the selected accessions

Results of the analysis of the tertiary protein structure of Lipid Transfer Protein 1 gene in the selected

accessions are shown in Figure 2-31. In each figure, the pink portion represents the alpha-helix, the blue portion of the ribbon structure represents the random coil, the green represents the extended strand.



Figure 2: The Tertiary structure of Lipid Transfer Protein gene of *Sesamum indicum*.

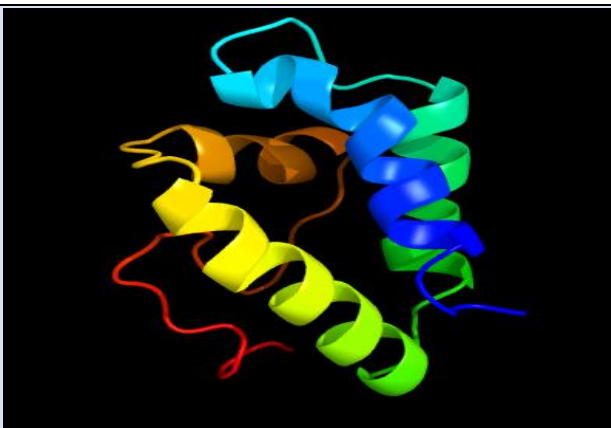


Figure 3: The Tertiary structure of Lipid Transfer Protein gene of *Ricinus communis*.

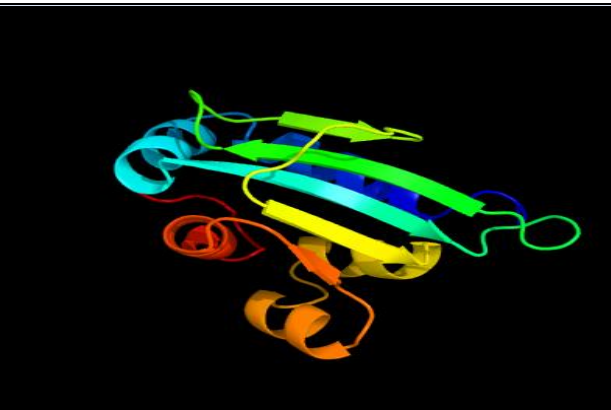


Figure 4: The Tertiary structure of Lipid Transfer Protein gene of *Daucus carota* subsp. *Sativus*.

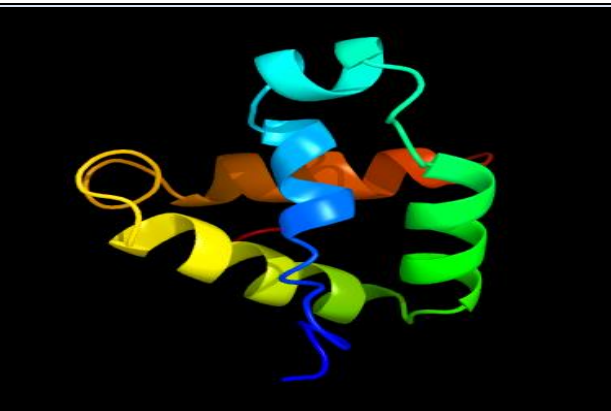


Figure 5: The Tertiary structure of Lipid Transfer Protein gene of *Medicago truncatula*.

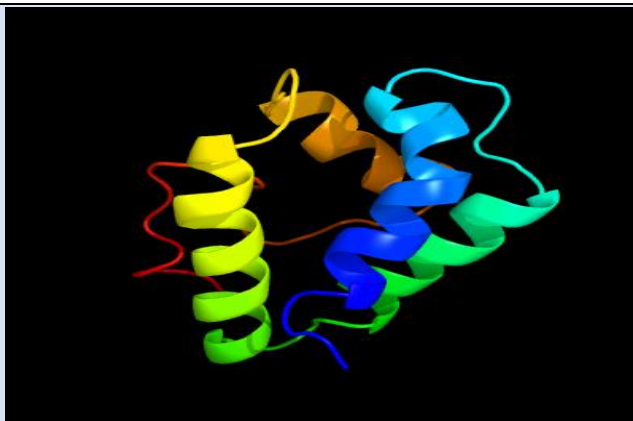


Figure 6: The Tertiary structure of Lipid Transfer Protein gene of *Helianthus annuus*.

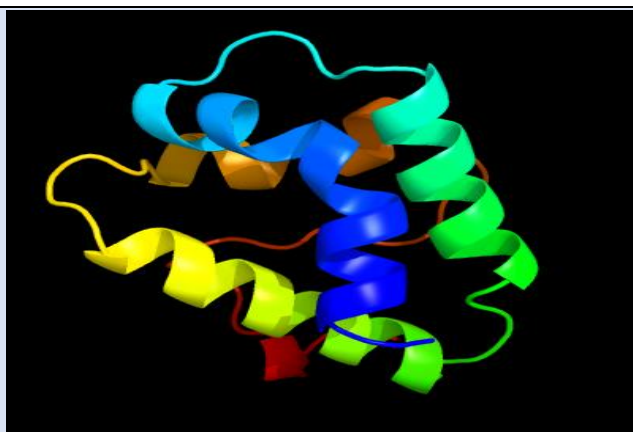


Figure 7: The Tertiary structure of Lipid Transfer Protein gene of *Nicotiana tabacum*.

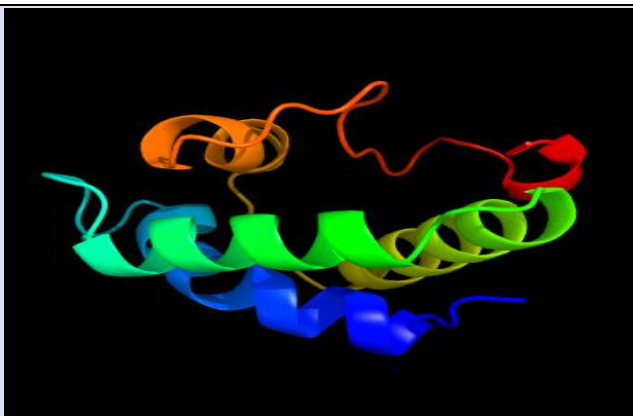


Figure 8: The Tertiary structure of Lipid Transfer Protein gene of *Gossypium hirsutum*.

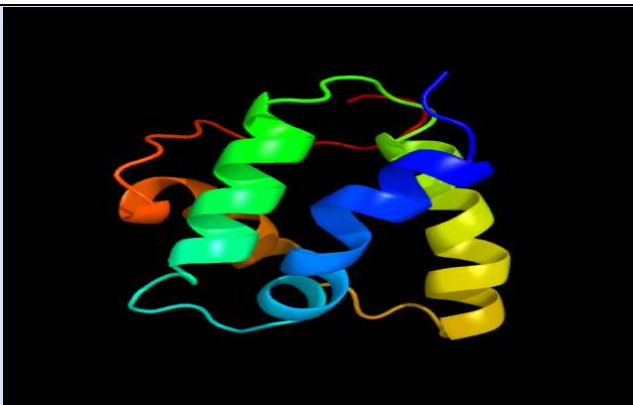


Figure 9: The Tertiary structure of Lipid Transfer Protein gene of *Vigna unguiculata*.

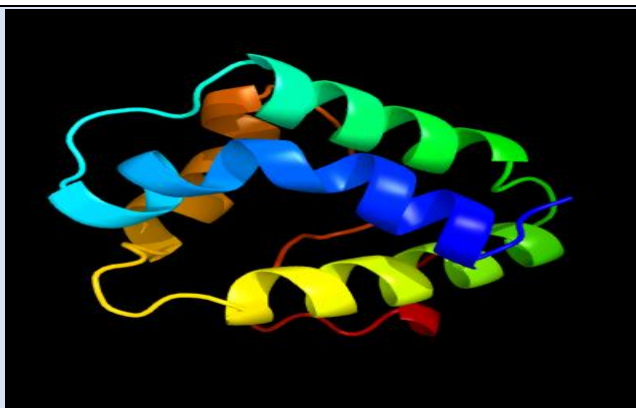


Figure 10: The Tertiary structure of Lipid Transfer Protein gene of *Nicotiana attenuata*.

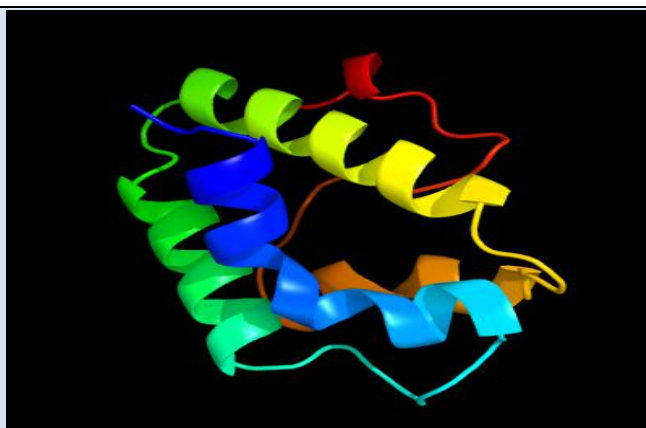


Figure 11: The Tertiary structure of Lipid Transfer Protein gene of *Dendrobium catenatum*

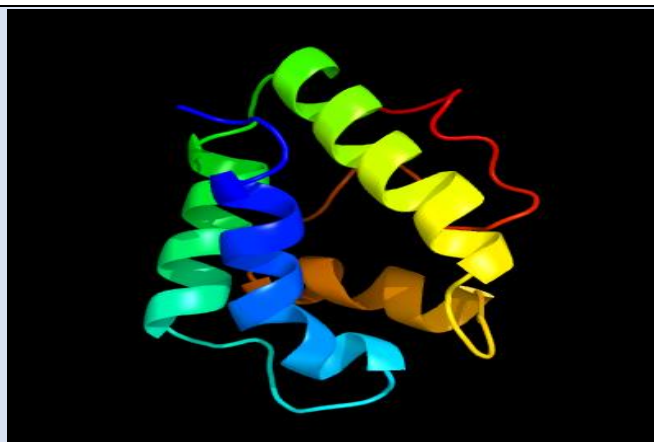


Figure 12: The Tertiary structure of Lipid Transfer Protein gene of *Musa acuminata*

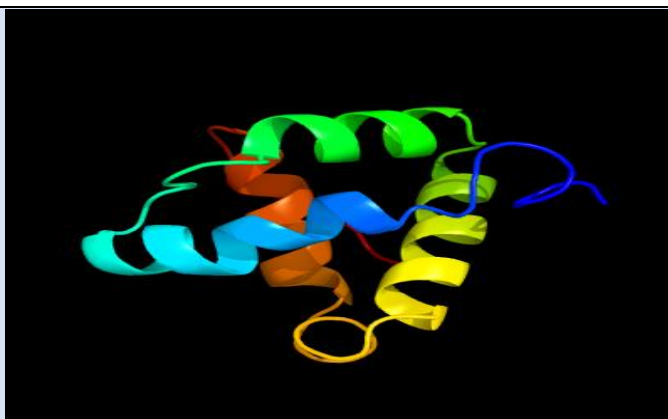


Figure 13: The Tertiary structure of Lipid Transfer Protein gene of *Brassica rapa*



Figure 14: The Tertiary structure of Lipid Transfer Protein gene of *Oryza sativa Japonica Group*

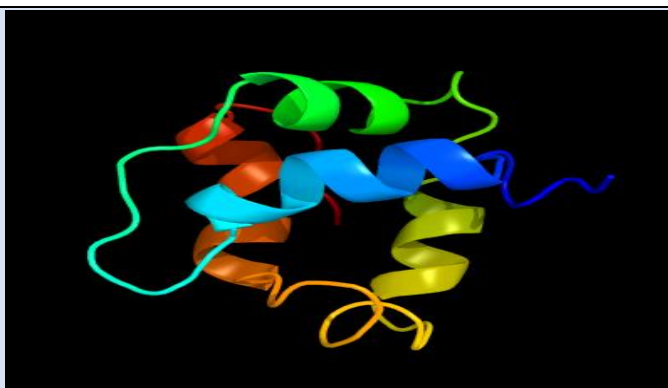


Figure 15: The Tertiary structure of Lipid Transfer Protein gene of *Physcomitrium patens*

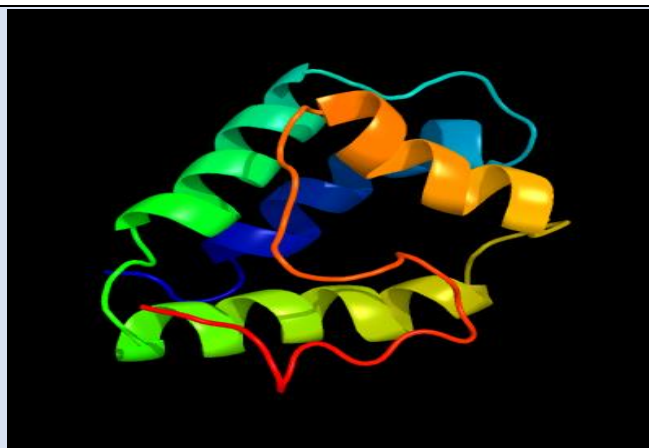


Figure 16: The Tertiary structure of Lipid Transfer Protein gene of *Vigna radiate*

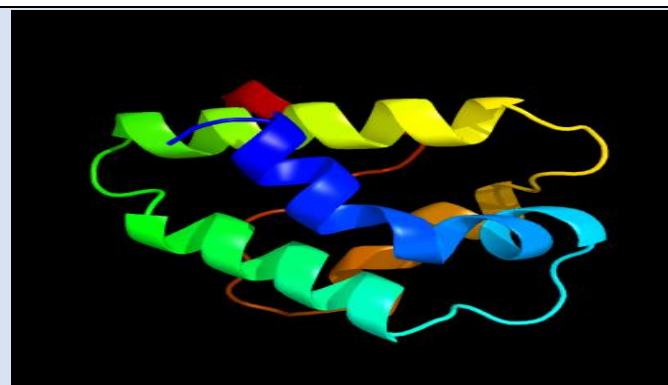


Figure 17: The Tertiary structure of Lipid Transfer Protein gene of *Prunus persica*

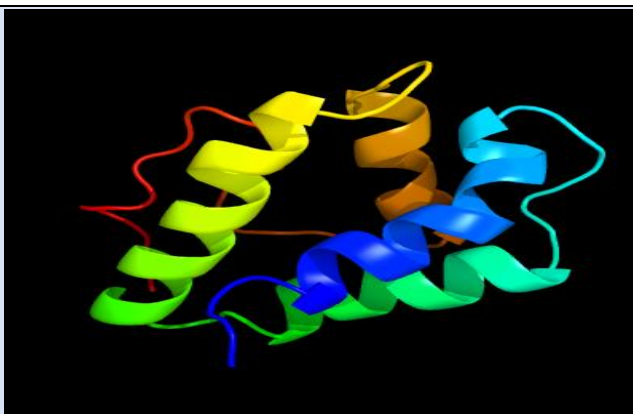


Figure 18: The Tertiary structure of Lipid Transfer Protein gene of *Arachis hypogaea*.

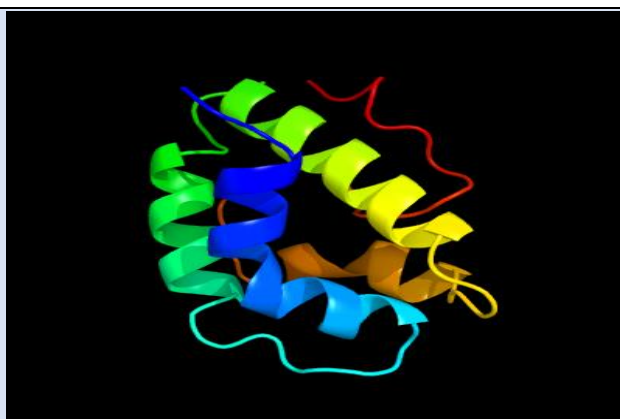


Figure 19: The Tertiary structure of Lipid Transfer Protein gene of *Solanum tuberosum*

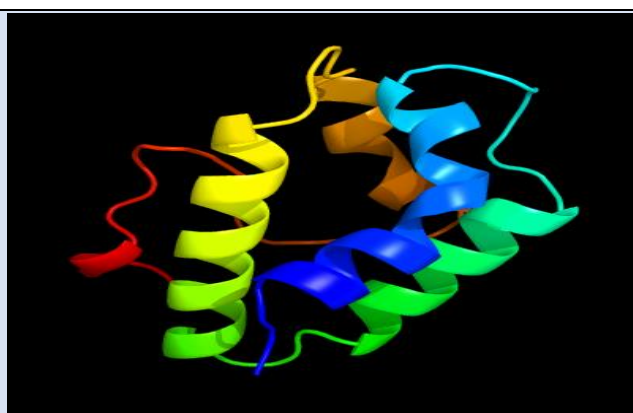


Figure 20: The Tertiary structure of Lipid Transfer Protein gene of *Sorghum bicolor*.

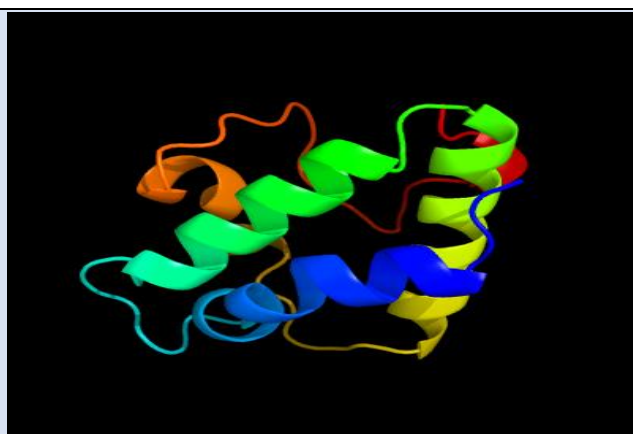


Figure 21: The Tertiary structure of Lipid Transfer Protein gene of *Glycine max* (2).

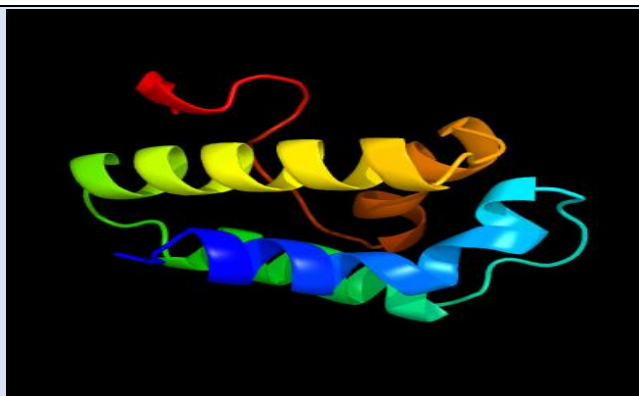


Figure 22: The Tertiary structure of Lipid Transfer Protein gene of *Brachypodium distachyon*

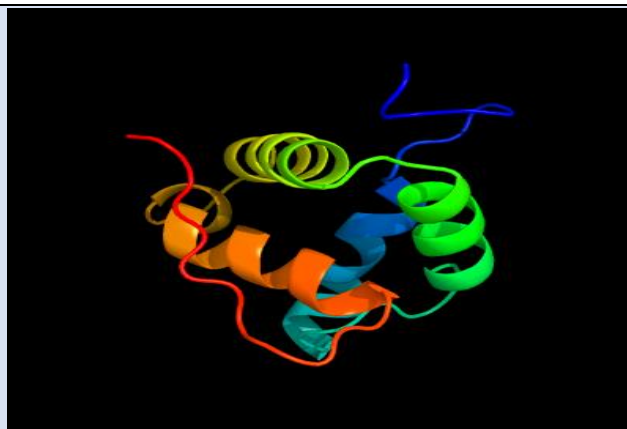


Figure 23: The Tertiary structure of Lipid Transfer Protein gene of *Capsicum annum*

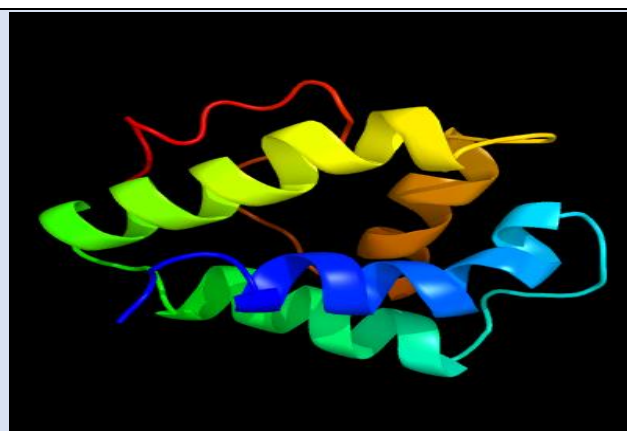


Figure 24: The Tertiary structure of Lipid Transfer Protein gene of *Arabidopsis thaliana*

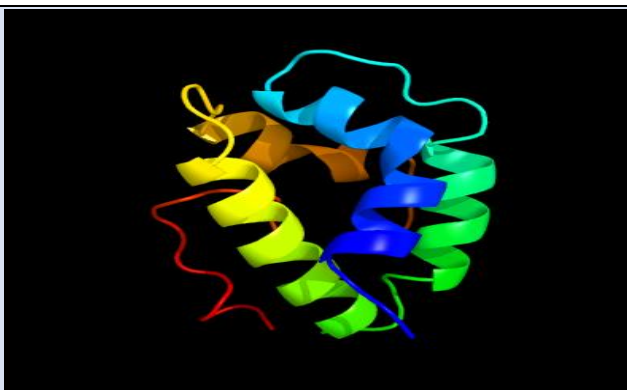


Figure 25: The Tertiary structure of Lipid Transfer Protein gene of *Solanum pennellii*

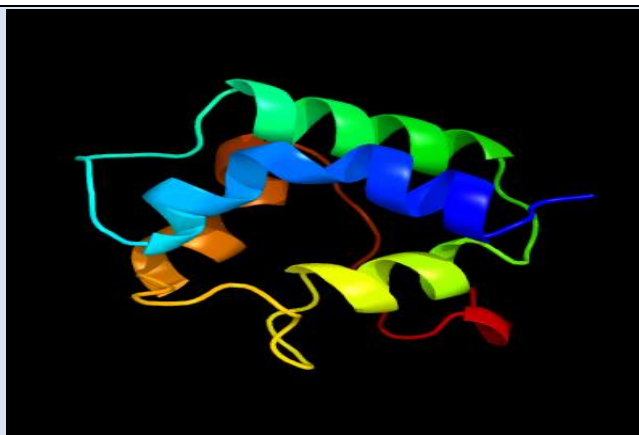


Figure 26: The Tertiary structure of Lipid Transfer Protein gene of *Vitis vinifera*

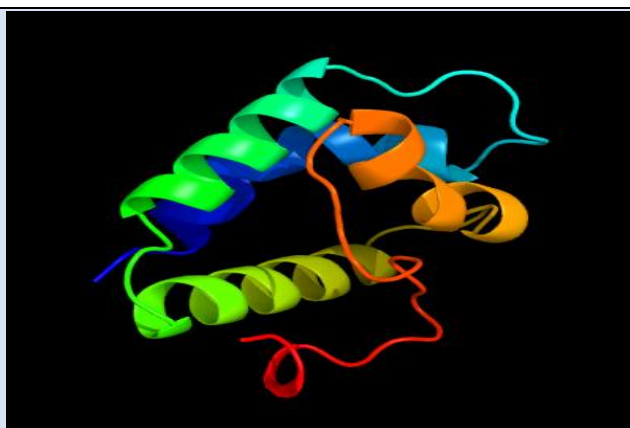


Figure 27: The Tertiary structure of Lipid Transfer Protein gene of *Juglans regia*

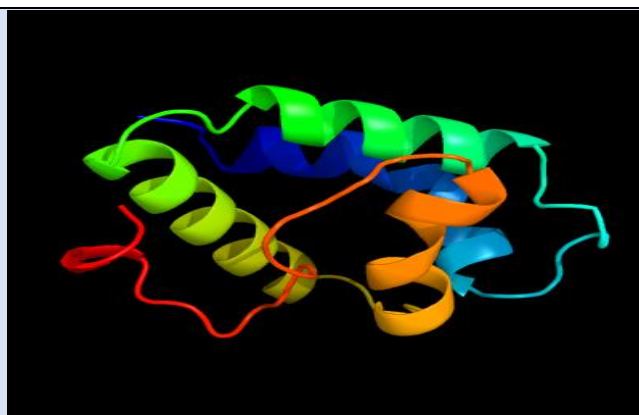


Figure 28: The Tertiary structure of Lipid Transfer Protein gene of *Pistacia vera*

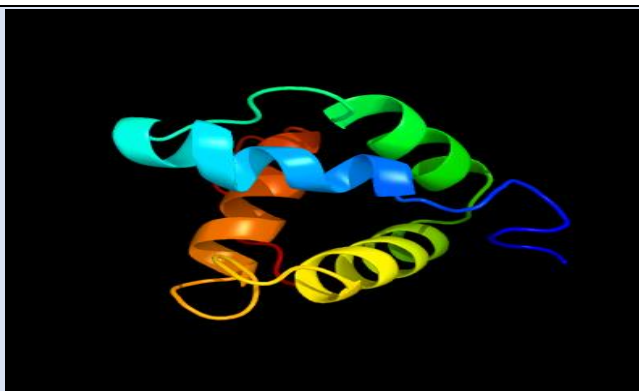


Figure 29: The Tertiary structure of Lipid Transfer Protein gene of *Brassica napus*

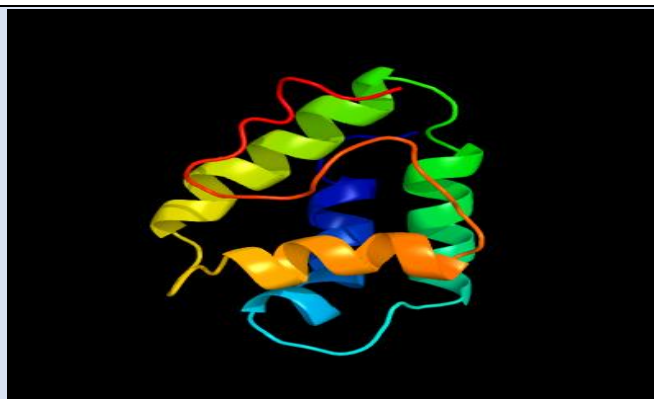


Figure 30: The Tertiary structure of Lipid Transfer Protein gene of *Olea europaea* var. *sylvestris*

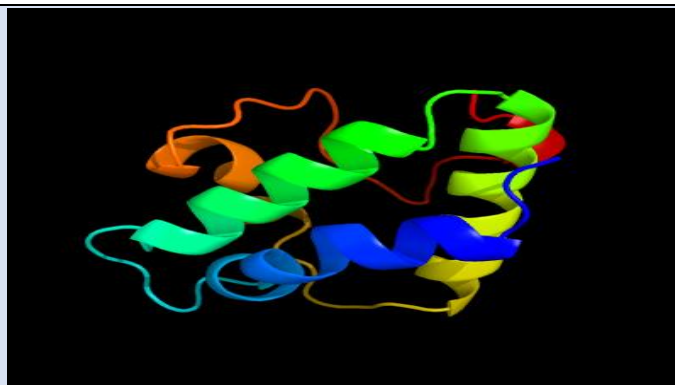


Figure 31: The Tertiary structure of Lipid Transfer Protein gene of *Glycine max*

Discussion

At the end of the study, it is expected that lipid transfer protein genes in the low and high oil seed yielding varieties of sesame will vary in their percent identity and similarity, show variable physiochemical properties and show variable genetic stability indices which is responsible for the differences in the functional and structural capacities of the *LTP* gene for oil production of the crop.

Results on the physicochemical properties of Lipid Transfer Protein 1 gene in the respective accessions showed that the higher the number of amino acids, the higher the value for the other properties; molecular weight, theoretical PI, number of negatively and positively charged residues and the extinction coefficient. Some of the accessions had instability indexes classifying the amino acids as unstable, while some were classified as stable.

Result of GC analysis revealed that GC content of Lipid Transfer Protein 1 gene in the selected accessions ranged from 29.73 – 54.55% with *Glycine max* having the lowest GC content and *Oryza sativa* Japonica Group having the highest GC content (54.55%). The start and end codons for the Lipid Transfer Protein 1 gene in the accessions selected varied generally although some accessions had the

same start codons but not the same end codons. *Solanum tuberosum*, *Capsicum annum* and *Solanum pennellii* did not show any start codon and end codon in Suboptimal exon cutoff option of (1.00). Suboptimal exon cutoff of (0.50) was used to find their start codons and end codons. In *Juglans regia*, *Brassica napus* and *Olea europaea* var. *sylvestris*, Sngl [Single-exon gene (ATG to stop)] was used to determine the start codon and end codon instead of the Init [Initial exon (ATG to 5' splice site)].

The extinction coefficient of an amino acid indicates how much light a protein absorbs at a certain wavelength (Gasteiger *et al.*, 2005). Results of the present study showed that extinction coefficient value of the amino acid sequences of Lipid Transfer Protein 1 gene in the selected accessions were higher than that in the query sequence, the implication therefore, is that there will be a variation in the amount of light these amino acids will absorb.

According to (Guruprasad *et al.* 1990), instability index greater than 40 indicates that the protein will be unstable in a test tube. Therefore, since the instability index of Lipid Transfer Protein 1 gene in twelve of the accessions were recorded to be above 40, it stands to argue that these amino acids will be unstable in a test tube. While the other eighteen are

considered stable. Aliphatic index plays an important role in a protein's thermal stability, the relative volume of a protein occupied by its aliphatic side chains is termed as Aliphatic index (AI).

Sequence motifs are short recurring patterns in the DNA that are thought to have biological function are usually specific binding sites for proteins. In the present study, the accessions selected share almost the same motifs, their position difference not withstanding indicating that the sequence-specific binding sites for proteins differ and might lead to functionality difference. The biological significant of GC content diversity in plants remains unclear due to lack of sufficiently robust genomic data (Smarda *et al.*, 2014).

Summary

The study was conducted to analyze the sequence of Lipid Transfer Protein 1 gene in 30 selected accessions. The *LTP 1* gene were retrieved from the NCBI database. Mega 10.0 software was used to align the sequences and to produce a phylogenetic tree of the accessions. The physicochemical properties, G-C content, secondary protein structure, motifs, percentage identity and similarity of the amino acid and nucleotide sequences were determined.

The physicochemical properties of Lipid Transfer Protein 1 gene proteins revealed that some of the proteins are unstable, while about twelve were considered stable following the results for instability index. The accessions contained similar motifs, the most common of which were N-glycosylation site, Plant lipid transfer proteins signature, N-myristoylation site, Casein kinase II phosphorylation site, Protein kinase C phosphorylation.

GC content analysis revealed that it ranges from 29.73–54.55% with *Glycine max* having the lowest GC content and *Oryza sativa Japonica Group* having the highest GC content (54.55%). The secondary amino acid of *LTP 1* gene in the selected accessions revealed three main structures; alpha, helix, random coil and extended strand. Alpha helix ranges from 33.11–54.31%, the extended strands ranged from 9.17–15.13%, while the random coil ranges from 32.77–51.16% across the accessions.

Results of the analysis of secondary structures of the amino acid sequences of Lipid Transfer Protein 1 gene showed that the region covered by random coil was the highest in the sequences compared to alpha helix and extended strand. Alpha helix ranges from 33.11–54.31%, the extended strands ranged from

9.17–15.13%, while the random coil ranges from 32.77–51.16% across the accessions. (Table 6).

Conclusion

In conclusion, results of the present study revealed difference in the Lipid Transfer Protein gene 1 sequences in the selected accessions following the parameters analyzed. However, results revealed that there is a high degree of sequence identity and similarity in the sequences of the selected accession, implying similar functions in these accessions.

Recommendation

Following the results of the present study, we recommend that Lipid Transfer Protein 1 gene sequences of other economically important crops, and underutilized plant species be analyzed and documented to further enable easy assessment of variability and diversity.

Also, we recommend that larger population size be explored since this makes accurate judgement possible

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Cite this article: Edu N.E, Udensi O.U, Agada F.N, Ubi G.M, Agbor R. (2023). Evaluation of Lipid Transfer Protein (Ltp) 1 Gene in Sesame and Other Plants Using Bioinformatics Approach. *Journal of BioMed Research and Reports*, BioRes Scientia Publishers. 2(6):1-20. DOI: 10.59657/2837-4681.brs.23.037

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Article History: Received: September 05, 2023 | Accepted: September 20, 2023 | Published: September 27, 2023