Characterizing Glutamate Receptor Genes of Rat having vertebrate nervous system and Arabidopsis thaliana a plant having equivalent nervous system based on chemical properties of amino acids and investigating evolutionary relationships between them.

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Abstract

iGluR gene family of a vertebrate, Rat and AtGLR gene family of a plant, Arabidopsis thaliana [4] perform some common functionalities in neuro-transmission, which have been compared quantitatively. Our attempt is based on the chemical properties of amino acids [6,7,8] comprising the primary protein sequences of the aforesaid genes. 19 AtGLR genes of length varying from 808 amino acid (aa) to 1039 aa and 16 iGluR genes length varying from 902aa to 1482 aa have been taken as data sets. Thus, we detected the commonalities (conserved elements) during the long evolution of plants and animals from a common ancestor [4]. Eight different conserved regions have been found based on individual amino acids. Two different conserved regions are also found, which are based on chemical groups of amino acids. We have tried too to find different possible patterns which are common throughout the data set taken. 9 such patterns have been found with size varying from 2 to 5 amino acids at different regions in each primary protein sequences. Phylogenetic trees of AtGLR and iGluR families have also been constructed. This approach is likely to shed light on the long course of evolution.

Introduction

Plants grow in silence but react to wounding. Specific genes are there in plants which make control over such functionalities in neuro-transmission. On the other hand, animals have well developed nervous systems. Mammals for example can relay electrical signals at a high speed of 100 m/sec approximately [1]. Earlier it was believed that plants do not have anything like a nervous system, they do not respond to stimuli in the way animals do. But according to the great pioneer Scientist Sir Acharya J.C.Bose,[3] plant also has

an alternative sort of sensitive nervous system, not like that of animals but can responds to various external stimuli. Numerous works since Darwin [5] have proved the response of plants to various signals like light, touch, wounding, stimulating but now it has been reported that glutamate receptors of vertebrate nervous system and similar receptors in the plant equivalent of nerve have so close similarities that they must have evolved from a common ancestor [4]. Arabidopsis genome sequencing has discovered a large family of Glutamate Receptor like genes (AtGLR) in them. It has been reported that like ionotropic glutamate receptor (iGluR) gene family of animals, AtGLR gene family in Arabidopsis have some functions in neurotransmission. (GLRs) in Arabidopsis thaliana are organism those contribute in neurotransmission in it. Ionotropic glutamate receptors are essential for mammalian Central Nervous System. They intervene in chemical synaptic transmission at the clear majority of excitatory synapses, underlie well-characterized cellular models of learning and memory, modulate excitability of neuronal networks. KA1 mRNA (also known as GRIK4) has functionality in only CA3 of hippocampus, whereas, KA2 mRNA (also known as GRIK4) can be detected almost all region of rat brain [39]. Mutation in both genes can lead to neurological and psychiatric disorder. Ionotropic Glutamate receptor, delta-1 and delta-2 are the subunits, which extend the excitatory amino acid receptor family of species rat. Delta-1 is expressed in the caudate putamen and delta-2 is expressed predominantly in Purkinje cells of the cerebellum [40].

Amino acids are organic compounds containing specific biochemical compositions that instruct primary protein sequences to have specific structure, to perform specific functions and to get stability. Quantitative understanding of genes at molecular level requires some Mathematical parameters to evaluate some data, to derive some values and to verify some facts. Similarity/Dissimilarity analysis is one of the fundamental methods used to investigate evolutionary relationship between various species [6]. Analysis of DNA as well as amino acid sequences using underlying biological information hidden in them is an old approach, whereas Graphical representation of primary protein sequence give alignment free method which provides visual inspection of the sequence and instant provision of analyzing similarities with others. Numerous authors [8-37] proposed several 2D and 3D graphical representations of DNA as well as amino acid sequences. Some of them have stressed on deriving substitution matrices from several protein blocks of aligned sequence segments with the characterization of related proteins. Some research papers are reported, where multiplet structure of amino acid sequences are the theme to investigate evolutionary relationship among the species [39]. Attempt has also been made to find out invariant conservation of certain amino acids of various organism using biochemical properties of amino acid side chains [6,7,8].

In this paper, the section of materials and methods has several sub sections. In first subsection firstly, the classifications of the 20 amino acids have been taken place on the basis of their chemical nature. As our primary objective is to make quantitative comparison between iGluR gene family of mammal (Rat) and AtGLR of plant (Arabidopsis thaliana) based on their chemical features and investigate evolutionary

relationships among them, so in this context, the data sets on which the experiments are carried out has been specified in the second sub section. In third sub section the methodologies have been described thoroughly. In third section, the procedures stated are also applied on two data sets taken and experimental results are discussed clearly. Finally, total work has been concluded in the last section. Thus, the novelty lies in the mathematical modeling to establish the fact that like Glutamate Receptor gene family in mammal, plant also has Glutamate Receptors like genes which have been conserved during the long process of their evolution from a common ancestry.

Materials and Methods

Classification of Amino Acids

The chemical features of 20 amino acids are basically depended on eight chemical properties of them. A protein structure is dependent upon the chemical properties of amino acid by which it is formed. Hence it determines the biological activity of proteins. It has previously been established that amino acid properties are not only decisive in determining protein structure and functions performed by them, but also play a role in molecular evolution [6,7]. To understand the structural and chemical property of Amino Acid one need to understand the structural and chemical property of protein. It has been reported in a paper that molecular evolutionary analysis of conserved region based on chemical properties of the side chain of amino acids can determine the essential amino acids in the core catalytic region [8]. So here in this manuscript it is aimed to understand how the biochemical nature of each amino acid sequences figure out evolutionary conserved regions among glutamate genes of mammal and plant during the long process of evolution. Analyze molecular evolutionary carry out the further experiments based on 8 distinct chemical properties of amino acids. So, 20 amino acids are clustered or classified into 8 groups accordingly as shown in Table 1. Eight different numeric values are assigned to each group.

Table 1: Classification of amino acids as per their Chemical Properties and numeric representation of each Class.

Group Name	Amino Acids included	Group/Class No.
Acidic	Aspartate (D), Glutamate (E)	1
Basic	Arginine (R), Histidine (H), Lysine (K)	2
Aromatic side chain	Tyrosine (Y), Phenylalanine (F), Tryptophan (W)	3
Aliphatic side chain	Isoleucine (I), Leucine (L), Valine (V), Alanine (A), Glycine (G)	4
Cyclic	Proline (P)	5
Sulfur containing	Methionine (M), Cysteine (C)	6
Hydroxyl containing	Serine (S), Threonine (T)	7
Acidic amide	Glutamine (Q), Asparagine (N)	8

Data Set Specification

To carry out the entire experiment, AtGLR gene family of Arabidopsis thaliana and Glutamate receptor genes such as iGluR genes of Rat have been taken in account. A large family of GLR genes has been uncovered and reported in NCBI. 19 of such sequences have been selected to carry out the investigations (Table 2). 16 amino acid sequences of iGluR family of Rat have also been taken for studies which are shown in Tables 3.

Table 2. Data set specification of AtGLR gene family in Arabidopsis.

Gene Name	Accession No.	Length of Amino Acid	Gene Name	Accession No.	Length of Amino Acid
AtGLR1.1	AAF26802.1	938 aa	AtGLR2.6	CAB96653.1	925 aa
AtGLR1.2	BAA96960.1	920 aa	AtGLR2.7	AAC33239.1	925 aa
AtGLR1.3	BAA96961.2	895 aa	AtGLR2.8	AAC33237.1	962 aa
AtGLR1.4	AAF02156.1	898 aa	AtGLR2.9	AAC33236.1	895 aa
AtGLR2.1	AAB61068.1	829 aa	AtGLR3.2	CAA18740.1	1039 aa
AtGLR2.2	AAD26895.1	906 aa	AtGLR3.3	AAG51316.1	921 aa
AtGLR2.3	AAD26894.1	934 aa	AtGLR3.4	AAB71458.1	808 aa
AtGLR2.4	CAA19752.1	958 aa	AtGLR3.5	AAC69939.1	867 aa
AtGLR2.5	CAB96656.1	940 aa	AtGLR3.6	CAB63012.1	860 aa
			AtGLR3.7	AAC69938.1	858 aa

Table 3. Data set specification of iGluR gene family in Rat.

Gene Name	Accession No.	Length of Amino Acid	Gene Name	Accession No.	Length of Amino Acid
GluRA	P19490	1009 aa	KA-2	Q63273	902 aa
GluRB	P19491	1007 aa	Delta 1	Q62640	956 aa
GluRC	AAA41245.1	920 aa	Delta 2	Q63226	979 aa
GluRD	AAA41246.1	908 aa	NMDA Zeta1	P35439	1237 aa
GluR5	AAA02873.1	888 aa	NMDA2A	AAC03565.1	938 aa
GluR6	P42260	907 aa	NMDA2B	AAA41714.1	1464 aa
GluR7	AAC80577.1	883 aa	NMDA2C	AAA41713.1	1482 aa
KA-1	AAA17830.1	888 aa	NMDA(NR2D)	AAC37647.1	1323 aa

Numerical Representation of Data Set taken as per Classification

To characterize a primary protein sequence, it is necessary to replace each amino acid of that primary protein sequence by the corresponding numerical value of the class or group from which it belongs to.

Let $S = S_1, S_2, \ldots\ldots S_k,$ be an arbitrary primary protein sequence, where for any $i \in \{1, 2, \ldots, k\}$, S_i represents a single amino acid from the set S, such that $S_i \in \{A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W, Y\}$.

Let 20 amino acids are classified into a set of classes C. Then, it is possible to read each amino acid of set S by its corresponding class C, where $C \in \{1,2,3,4,5,6,7,8\}$.

As an example, according to Table1, a chunk of amino acid sequence 'MEALT' will be represented as '61447' after the encoding into the numeric representation.

Calculate Percent Identity matrix of every pair of Amino Acid sequences.

In biology the percentage identity 'pi' is defined to be the number of identical bases between two sequences in an alignment. ClustalW is used to build pairwise alignments among all the amino acid sequences. Thus, for each pair of amino acid sequences, we get a score of percent identity. A distance matrix called percent identity matrix is constructed to show percent identity of every pair of amino acid sequences.

Calculate Percent wise presence of each Amino Acid present in each sequence and each Group of Amino Acid in each sequence.

Presence of all 20 amino acid sequences are not same for all species. Even this rate of frequency vary sequence to sequence. In this paper rate of occurrence i.e., frequency of every individual amino acid. The chemical properties of the amino acids determine the biological activity of the protein. Proteins not only act as catalyst in most of the reactions in living cells, but they control virtually all cellular processes too. In addition, amino acid sequence of a protein contains the necessary information to determine how that protein will fold into a three-dimensional structure, and the stability of the resulting structure. Sometimes the amino acid encodes some information which are inherited from the previous stage (before translation). Basic and acidic Amino Acids are essential in the formation of Beta sheets, which are secondary protein structures that are stabilized by the creation of hydrogen bonds between acidic and basic amino acids [40]. Whereas, aromatic amino acids provide a negative electrostatic potential surface that leads to cation-pi interaction [41]. Cation -p interactions have significant contribution in overall stability of proteins. So, it is important

to calculate the presence of each group of amino acid which contains specific chemical properties. In this part of the paper percentage of each group of amino acid present in each sequence has been calculated to get a quantitative understanding about the presence of each chemical group in each amino acid sequence. [41-42].

Common patterns/blocks finding and identifying conserved regions

Conservation of DNA or protein sequences across species indicates that a sequence has been maintained by evolution; hence conserved protein sequence regions are extremely useful to investigate, identify and study functional and structural importance of those regions. Here in this part of the manuscript firstly conserved regions are detected.

Algorithm 1: Common Pattern finding.

Input: Set of amino acid sequences in terms of their numeric representation.

Output: Common pattern with locations and corresponding amino acid sequence.

Given an input of pattern length L ($2<L<8$), all possible combination of patterns of length L using the numerical values 1-8 are generated. Every possible pattern is investigated among the sequences, and if the pattern is found for all the sequences, the pattern is selected and stored along with the locations, otherwise the pattern is discarded. Thus, by varying pattern length L, it is possible to find patterns of different lengths along with their corresponding location.

Investigating evolutionary relationships between two species

Let $S= \{S_1, S_2,\ldots S_n\}$ be a set of Amino acid sequences, where each sequence S_i for $i=\{1,2,\ldots n\}$ can be represented through weighted directed multi graph G_m. $G_m=\{A,V\}$ is say a multigraph, where set of vertices $V=\{V_1, V_2,V_3,V_4,V_5,V_6,V_7,V_8\}$ represent the elements of class C, where $C=\{1,2,3,4,5,6,7,8\}$ and arcs A represent the possible parallel arcs present between any two vertices. For any Amino acid sequence, say V_i and V_j are any two vertices for which directed multi graphs may have parallel arcs from V_i to V_j. now define the weight of each arc from V_i to V_j as 1, so that for every arc from V_i to V_j, total weight of the arcs of graph G_m is,

$$\mathrm{w_m}(V_i, V_j) = \sum \mathrm{w}(V_i, V_j) \qquad (1)$$

In this way the graphical representation of Amino Acid (aa) sequence can give us an idea about the distribution of chemical properties in them.

Now the directed multi graph can be drawn through adjacency matrix. We can get its corresponding (8X8) adjacent matrix M for each pair of vertices as mentioned below in Table 10, which gives us 64 dimensional vectors in row order say R, where R=(w(1,1),w(1,2),.....w(1,8),....,w(8,1),…w(8,8)).

Table 4. Representing weighted directed multigraph through adjacency matrix.

Weight	1	2	3	4	5	6	7	8
1	w(1,1)	w(1,2)	w(1,3)	w(1,4)	w(1,6)	w(1,6)	w(1,7)	w(1,8)
2	w(2,1)	w(2,2)	w(2,3)	w(2,4)	w(2,6)	w(2,6)	w(2,7)	w(2,8)
3	w(3,1)	w(3,2)	w(3,3)	w(3,4)	w(3,6)	w(3,6)	w(3,7)	w(3,8)
4	w(4,1)	w(4,2)	w(4,3)	w(4,4)	w(4,6)	w(4,6)	w(4,7)	w(4,8)
5	w(5,1)	w(5,2)	w(5,3)	w(5,4)	w(5,5)	w(5,6)	w(5,7)	w(5,8)
6	w(6,1)	w(6,2)	w(6,3)	w(6,4)	w(6,6)	w(6,6)	w(6,7)	w(6,8)
7	w(7,1)	w(7,2)	w(7,3)	w(7,4)	w(7,6)	w(7,6)	w(7,7)	w(7,8)
8	w(8,1)	w(8,2)	w(8,3)	w(8,4)	w(8,5)	w(8,6)	w(8,7)	w(8,8)

Algorithm 2: Derive adjacent matrix and a weighted multi directed graph constructed from it.

Input: Set of amino acid sequences in terms of their numeric representation.

Output: An adjacent matrix and a weighted multi directed graph constructed from this matrix.

Define the sequence S_i of length L

Define a null matrix D of size 8 by 8,

for i=1: length (S) **do**

Read Si;

Find the chemical group number C

M(i)=C;

end

for i=1: length (M)-1 **do**

```
Read: (M(i), M(i+1));
If D(M(i), M(i+1)) ==0
then
D(M(i), M(i+1)) = D(M(i), M(i+1)) +1;
end
        end
t=1;
for i=1: length (M) do
      for j=1: length (M) do
if D (i , j) ≠0
then
   u(t)=i;
    v(t)=j;
    t=t+1;
end
     end
          end
G=digraph(u,v);
 plot(G);
```

Now let for any two amino acids sequences say S_1 and S_2 the corresponding set of vectors are $P=\{P_1, P_2,\ldots,P_{64}\}$ and $Q=\{Q_1, Q_2, \ldots.Q_{64}\}$ respectively, then weight deviation (WD) between the two sequences S_1 and S_2 can be represented as stated in equation 2.

$$WD(S_1, S_2) = \frac{\sum_{i=1}^{64} |P_i - Q_i|}{64} \qquad (2)$$

Thus we will get dissimilarity matrix D for a set of amino acid sequences S_1, S_2, ..S_n . According to the equation (2), the weight deviation between any two DNA sequences say S_1 and S_2, is inversely proportional to the degree of similarity of those sequences.

Result and Discussion

Derive percent identity matrix of every pair of Amino Acid sequences taken.

Percentage identity between each pair of aa sequences has been deduced. Thus, matrices have been constructed (tables 5, 6 and 7) which are able to make comparative analysis between the gene families of two species.

Table5. Percent identity matrix constructed between the Amino Acid sequences taken from Arabidopsis and RAT.

	Species	RAT															
	Sequences	GluRA	GluRB	GluRC	GluRD	GluR5	GluR6	GluR7	KA1	KA2	Delta1	Delta2	NMDAZ1	NMDA2A	NMDA2B	NMDA2D	NMDA2C
Arabidopsis	AtGLR1.1	11.88	12.87	12.38	12.87	12.25	11.88	12.75	12.38	11.39	10.40	10.89	11.63	11.39	11.01	11.63	10.89
	AtGLR1.2	11.53	10.73	12.00	11.53	10.84	11.76	11.53	11.30	12.23	11.53	11.07	12.23	10.73	10.73	11.76	11.07
	AtGLR1.3	11.86	11.28	12.56	11.63	10.00	11.63	12.09	11.40	10.70	11.28	12.67	12.33	11.51	11.86	11.51	12.67
	AtGLR1.4	9.91	12.59	10.84	13.17	11.31	10.96	13.05	13.29	10.96	10.72	9.91	10.61	10.26	11.66	11.31	9.91
	AtGLR2.1	11.62	10.98	12.93	14.43	12.27	12.02	12.57	12.39	12.31	10.13	10.77	11.83	10.55	10.66	10.34	10.77
	AtGLR2.2	12.61	13.26	11.74	12.00	14.64	12.46	12.68	13.63	12.64	11.30	12.28	12.72	11.74	13.26	14.13	12.28
	AtGLR2.3	11.73	13.30	12.63	12.63	12.05	12.18	13.48	14.08	13.18	13.74	12.07	12.51	13.07	12.51	12.07	12.07
	AtGLR2.4	11.36	12.25	12.03	12.03	11.15	11.36	11.78	12.27	10.91	10.13	11.69	10.25	10.91	10.58	11.80	11.69
	AtGLR2.5	12.55	12.67	14.84	13.03	13.03	12.18	13.15	12.30	12.06	13.51	13.75	13.39	13.87	13.15	13.63	13.75
	AtGLR2.6	11.37	10.71	12.58	11.81	11.82	9.49	11.33	9.80	9.65	13.58	12.91	11.04	11.48	12.58	13.25	12.91
	AtGLR2.7	12.53	12.21	14.02	14.21	13.63	12.79	13.25	12.27	11.42	9.64	14.13	11.24	10.60	11.35	11.35	14.13
	AtGLR2.8	11.59	13.78	13.15	14.98	14.19	12.90	13.59	11.71	14.86	10.65	11.80	12.58	10.54	11.80	12.21	11.80
	AtGLR2.9	11.60	12.23	14.02	14.43	14.19	14.00	12.57	11.82	12.97	13.19	10.96	12.79	12.02	13.09	13.19	10.96
	AtGLR3.2	10.05	11.57	13.70	14.43	12.50	12.79	13.36	12.84	12.97	11.35	10.16	11.35	10.27	10.16	11.03	10.16
	AtGLR3.3	14.15	13.08	16.30	14.76	14.19	13.23	14.27	13.96	14.30	12.75	10.61	12.54	10.93	11.47	10.72	10.61
	AtGLR3.4	11.33	12.47	13.37	13.44	10.81	13.78	13.25	13.06	13.64	10.40	10.91	13.65	10.29	10.29	11.43	10.91
	AtGLR3.5	14.75	14.41	15.20	14.97	15.77	14.64	15.52	13.63	14.64	14.30	11.62	14.97	12.96	13.52	12.18	11.62
	AtGLR3.6	10.51	10.82	10.43	10.57	10.59	13.01	12.23	11.15	12.86	10.73	10.88	10.55	10.49	9.43	10.30	10.88
	AtGLR3.7	13.14	12.27	13.37	11.78	13.63	10.92	12.34	10.47	11.64	11.07	11.62	10.86	12.81	10.86	11.84	11.62

Table6. Percent identity matrix for the Amino Acid sequences taken of Arabidopsis.

Species	ARABIDOPSIS																		
Sequences	AtGLR1.1	AtGLR1.2	AtGLR1.3	AtGLR1.4	AtGLR2.1	AtGLR2.2	AtGLR2.3	AtGLR2.4	AtGLR2.5	AtGLR2.6	AtGLR2.7	AtGLR2.8	AtGLR2.9	AtGLR3.2	AtGLR3.3	AtGLR3.4	AtGLR3.5	AtGLR3.6	AtGLR3.7
AtGLR1.1	100	44.0594	44.5545	40.4703	23.2673	23.6386	24.1337	22.2772	21.0396	19.802	25.8663	25.8663	25.7426	17.3267	18.9356	16.7079	19.4307	16.8317	18.1931
AtGLR1.2		100	78.9535	51.2821	21.9146	23.1834	23.4141	21.3379	22.0748	19.0311	25.4902	30.1038	29.2964	16.0323	19.2618	19.9539	18.9158	18.6851	19.0311
AtGLR1.3			100	49.7669	23.0233	24.5349	24.7674	23.4884	22.4367	19.0698	26.5116	26.2791	29.6512	15.814	19.5349	20	20.814	18.6047	21.7442
AtGLR1.4				100	23.31	24.5921	25.7576	23.4266	24.0048	25.2914	26.8065	27.0396	30.1865	18.1818	21.4452	19.9301	22.8438	17.2494	21.6783
AtGLR2.1					100	61.087	56.5363	55.7906	41.8577	38.7417	39.1863	38.4861	37.42	21.8378	25.5091	23.9872	25.1397	19.403	25.19
AtGLR2.2						100	76.5363	57.2383	43.0639	40.0662	40.8696	41.087	41.6304	20.4348	28.6957	27.1739	26.5922	20.7609	25.6522
AtGLR2.3							100	55.9777	41.4958	39.3296	41.4525	40.2235	41.4525	19.3296	27.486	22.905	25.2514	24.1341	26.7039
AtGLR2.4								100	38.7214	37.7506	38.9755	32.8508	38.8641	20.3786	22.4944	22.3831	21.7877	23.608	23.0512
AtGLR2.5									100	72.7382	44.029	42.9433	40.2895	18.3353	26.0555	24.2461	29.1918	24.3667	27.6236
AtGLR2.6										100	38.7417	38.521	30.6843	17.1082	22.4062	18.7638	23.352	22.6269	24.9448
AtGLR2.7											100	67.773	63.0621	23.4595	27.8671	25.1606	28.7151	18.8437	25.7329
AtGLR2.8												100	69.8936	23.6757	26.2594	24.5303	27.1508	20.3549	25.19
AtGLR2.9													100	18.8108	26.045	22.1277	27.8212	18.7234	24.5385
AtGLR3.2														100	45.0811	39.027	38.5475	37.0811	31.4875
AtGLR3.3															100	44.2658	46.1453	46.5166	40.8252
AtGLR3.4																100	70.6145	37.1102	37.1336
AtGLR3.5																	100	40	38.7709
AtGLR3.6																		100	35.9392
AtGLR3.7																			100

In Table 5 percentage identities between all the primary protein sequences of the two species has been obtained. It is observed that sequences are at least 9.49% similar and it is between AtGLR6.6 and GluRB. The maximum similarity is 16.30% which is found between AtGLR3.3 and GluRC. In the Table 6 AtGLR1.1 is most identical to AtGLR1.3 and in table 7, highest similarity takes place between Delta2 and NMDA2C. The observation shows the fact that Arabidopsis family genes are less identical with each other than that of Rat.

Table7. Percent identity matrix constructed for the AA sequences taken of Rat.

Species	RAT															
Sequences	GluRA	GluRB	GluRC	GluRD	GluR5	GluR6	GluR7	KA1	KA2	Delta1	Delta2	NMDAZ1	NMDA2A	NMDA2B	NMDA2D	NMDA2C
GluRA	100	53.7239	23.0435	21.3656	21.8468	21.8302	22.4236	21.1712	21.3969	20.3269	15.4609	16.7377	14.0733	14.1724	14.4698	15.4609
GluRB		100	22.2826	20.815	23.1982	18.4123	19.1393	20.2703	18.2927	18.8968	15.0943	16.0981	14.7964	14.7964	13.7041	15.0943
GluRC			100	76.3216	71.8468	32.6351	32.9558	33.5586	32.1508	38.6957	15	17.6087	15.8696	15.6522	16.5217	15
GluRD				100	78.8288	32.6351	34.4281	33.8964	33.7029	39.8678	15.4185	18.0617	15.7489	15.9692	15.3084	15.4185
GluR5					100	32.6577	32.1631	31.4189	32.545	39.527	15.7658	18.2432	15.991	16.3288	16.3288	15.7658
GluR6						100	68.0634	64.3018	66.1863	29.7685	16.6483	17.1996	15.5458	14.9945	15.7663	16.6483
GluR7							100	71.2344	71.2344	30.0113	15.2888	17.5538	15.402	16.1948	15.6285	15.2888
KA1								100	71.2838	31.0811	16.3288	15.991	15.7658	16.2162	15.0901	16.3288
KA2									100	28.714	15.9645	17.184	14.4124	15.8537	15.7428	15.9645
Delta1										100	16.4454	18.4435	15.8325	15.3218	16.2411	16.4454
Delta2											100	17.9104	42.0372	42.2797	46.6451	100
NMDAZ1												100	17.2708	17.6972	17.5906	17.9104
NMDA2A													100	47.7459	39.0023	42.0372
NMDA2B														100	37.037	42.2797
NMDA2D															100	46.6451
NMDA2C																100

Calculate Percentage of each Group of Amino Acid present in each sequence.

It has been discussed before that the chemical properties of primary protein sequences have significant role in protein folding and protein structure making. It can be observed that in both the Tables 8 and 9 group 4, i.e. amino acids of aliphatic group has remarkably major contributions to make primary protein sequences in both species, whose hydropathy profile is hydrophobic.

Table8. Percentage of each Group of Amino Acid present in each sequence of Rat.

RAT		GluRA	GluRB	GluRC	GluRD	GluR5	GluR6	GluR7	KA1	KA2	Delta1	Delta2	NMDAZ1	NMDA2A	NMDA2B	NMDA2C	NMDA2D
Group	1	10.5	11.1	10.9	10.7	11	10.8	11.2	10.5	10.8	10.4	10.1	8.01	10.7	11.5	11.8	9.15
	2	12	11.4	13	12.6	13.2	12	12.3	12.6	12.6	11.5	12.8	12.2	14.6	13.6	13.8	12.6
	3	8.13	9.73	10.4	10.5	9.91	11.1	11.1	11.8	10.8	9.1	8.17	8.49	8.96	9.7	9.78	8.92
	4	36.2	35.7	35.2	35.7	36.2	36.4	36.9	35.3	37.3	37.1	37.4	38.3	35.3	31.1	31.8	39.5
	5	4.56	4.07	4.02	4.07	4.28	3.64	2.94	3.6	3.88	4.5	5.92	8.41	4.16	4.92	4.99	11.5
	6	4.26	4.07	4.46	4.74	5.18	4.3	3.74	4.17	4.32	5.65	5.11	4.2	4.48	4.51	4.79	3.48
	7	15.7	14.4	14	14	12.7	12.9	13.8	13.1	13.2	13.1	13.5	13.8	13.4	15.1	14.8	10.9
	8	1.72	9.53	7.93	8.72	7.55	8.82	7.92	9.01	7.2	8.68	7.05	6.55	8.42	9.63	8.3	4.08

Table9. Percentage of each Group of Amino Acid present in each sequence of Arabidopsis.

Arabidopsis		AtGLR2.1	AtGLR2.2	AtGLR2.3	AtGLR2.4	AtGLR2.5	AtGLR2.6	AtGLR2.7	AtGLR2.8	AtGLR2.9	AtGLR3.2	AtGLR3.3	AtGLR3.4	AtGLR3.5	AtGLR3.6	AtGLR3.7	AtGLR1.1	AtGLR1.2	AtGLR1.3	AtGLR1.4
Amino Acid Group	1	8.85	9.67	10.2	9.69	12.2	11.3	10.5	10.5	10.9	9.73	9.73	9.46	10.6	9.33	10.7	10.4	10.4	10.2	10.1
	2	9.49	11	11.2	11.3	12.3	12.7	11.8	12.5	12.2	13.1	13.1	11.3	12.1	11.7	13.3	13.1	12.7	12.4	13.3
	3	11.5	11.6	12.1	10.6	10.9	9.6	10.6	11.7	11.5	10.5	10.5	10.5	11.4	9.82	10.1	9.65	10	10.4	11.4
	4	37.7	36.4	36.5	37.1	36.2	35.8	35.1	34.5	34	36.1	36.1	35.8	36.2	40	38.2	38	36.8	37.2	36.3
	5	4.8	5.11	5.25	5.46	4.22	4.86	4.28	4.38	4.68	4.54	4.54	4.78	5.03	4.62	4.45	2.23	2.88	2.79	3.26
	6	3.3	3.7	3.8	3.56	3.38	3.64	3.64	3.24	3.51	3.24	3.24	3.43	2.23	3.37	3.15	3.09	3.58	2.91	3.03
	7	14.6	14.4	14.1	15	14.1	14.6	15.2	14.7	14.8	15	15	15.8	14.3	12.1	13.5	15.7	15.9	15.9	14.6
	8	8.7	8.7	6.92	7.35	6.75	7.62	8.89	8.46	8.41	7.79	7.79	8.94	8.16	8.95	6.63	7.8	7.78	8.14	8.04

Calculate Percentage of each Amino Acid present in each sequence.

Rate of occurrence or frequency of every individual amino acid of the respective sequences which are shown in Table 10. It can be easily observed that rate of presence of Leucine (L) and Serine (S) are maximum in all the primary protein sequences. Moreover, rate of occurrence of 20 amino acids are similar in case of both the species.

Table 10. Calculate Percentage of each Amino Acid present in each sequence

Species Name	Presence of Amino Acids in percentage																				
	AA	**A**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**K**	**L**	**M**	**N**	**P**	**Q**	**R**	**S**	**T**	**V**	**W**	**Y**
RAT	**GluRA**	7	2	5	5	4	6	2	6	4	11	3	4	5	5	5	10	6	6	2	3
	GluRB	5	1	6	5	5	7	2	7	4	10	3	5	4	4	6	8	7	7	2	3
	GluRC	6	1	5	6	4	6	2	7	6	11	3	5	4	3	5	8	6	6	2	4
	GluRD	6	1	5	6	5	6	2	6	6	10	3	4	4	4	5	8	6	6	2	4
	GluR5	7	2	6	6	5	6	2	7	5	10	4	4	4	3	5	7	6	6	2	3
	GluR6	7	2	5	6	6	8	1	6	6	8	3	4	4	4	5	8	5	7	2	4
	GluR7	6	1	5	6	5	8	1	7	7	8	2	5	3	3	4	8	6	7	2	4
	KA1	7	1	4	6	6	7	2	6	6	8	3	5	4	4	5	8	5	8	2	5
	KA2	7	2	5	6	5	8	2	6	6	9	3	4	4	3	5	8	6	7	2	4
	Delta1	7	3	4	7	5	7	2	6	3	11	3	4	4	4	6	8	5	6	2	3
	Delta2	8	2	3	7	4	7	2	5	3	11	3	4	6	3	8	8	5	6	1	3
	NMDAZ1	9	2	4	4	4	8	3	3	3	10	2	3	8	4	7	8	5	9	2	3
	NMDA2A	8	1	4	6	5	6	2	6	6	8	3	5	4	4	7	8	6	7	2	3
	NMDA2B	5	2	6	5	5	6	3	5	6	9	3	6	5	4	5	10	5	7	2	3
	NMDA2C	6	2	6	6	5	7	3	6	6	7	3	5	5	3	5	10	5	7	1	4
	NMDA2D	11	2	4	5	4	10	3	3	2	10	2	2	11	2	8	7	4	6	2	3
ARABIDOPSIS	**AtGLR2.1**	6	1	4	4	6	6	1	7	4	10	2	5	5	5	4	8	6	9	2	4
	AtGLR2.2	5	1	5	5	7	7	1	5	6	9	3	5	5	4	4	8	7	9	2	3
	AtGLR2.3	5	1	5	5	7	6	1	6	6	10	2	3	5	3	4	8	6	9	2	3
	AtGLR2.4	6	1	5	5	7	7	1	6	5	9	3	4	5	3	5	8	7	9	1	3
	AtGLR2.5	6	1	5	7	6	6	2	7	6	10	3	4	4	3	5	8	6	8	1	3
	AtGLR2.6	6	1	6	6	5	6	2	6	5	10	3	5	5	3	6	8	7	7	1	3
	AtGLR2.7	5	1	5	6	6	6	2	7	6	10	3	5	4	3	4	9	6	8	2	3
	AtGLR2.8	6	1	6	5	7	5	2	6	6	9	2	5	4	3	4	8	6	8	2	3
	AtGLR2.9	6	1	5	5	7	6	2	6	6	9	3	5	5	3	4	8	7	8	2	2
	AtGLR3.2	6	1	5	5	6	7	2	7	5	10	2	5	5	3	6	9	6	6	2	3
	AtGLR3.3	6	1	5	5	6	7	2	7	5	10	2	5	5	3	6	9	6	6	2	3
	AtGLR3.4	6	1	4	5	6	6	2	6	4	9	2	5	5	4	6	11	5	8	2	3
	AtGLR3.5	6	1	5	6	6	6	1	7	6	10	2	5	5	3	5	9	5	7	2	3
	AtGLR3.6	7	1	5	5	6	8	2	7	4	9	2	6	5	3	6	7	5	9	2	3
	AtGLR3.7	7	1	5	6	6	6	2	6	5	11	2	4	4	2	6	8	5	9	2	2
	AtGLR1.1	6	1	5	6	6	6	2	7	6	10	2	4	2	3	5	10	6	9	1	2
	AtGLR1.2	6	0	4	6	6	6	3	6	4	12	3	5	3	3	6	10	6	8	2	2
	AtGLR1.3	6	0	5	6	6	6	2	6	5	12	2	5	3	3	5	9	7	8	2	2
	AtGLR1.4	7	1	4	6	6	5	2	7	5	9	2	5	3	3	6	8	7	8	2	3

Common pattern finding in all primary protein sequences

As 20 amino acids are grouped according to their chemical properties, and each of them is identified with specific numeric value, so when common pattern finding procedure has been made for all the primary protein sequences, some numerical patterns are found which are common in all the primary protein sequences taken. The patterns are shown with regions in table 11in supplementary file. In this section it is tried to make microscopic view of those patterns. Table 12 contains patterns of length of 3 amino acids (aa) for which same amino acids are contributed in same order, whereas, Table 13 and 14 contain patterns of length of 4 aa and 5 aa respectively. It is remarkable that in all cases amino acids from group 4 (aliphatic) has major contribution in pattern forming. As it is known that Aliphatic R groups are hydrophobic and nonpolar, those are one of the major driving forces for protein folding, give stability to globular or binding structures of protein [40].

Table 12. Common pattern finding (length of 3 aa) for which same amino acids are contributed in same order as per classification

Pattern - '444'					
Gene Name	**Position**	**AA Behind**	**Gene Name**	**Position**	**AA Behind**
ATGLR2.1	564	VVA	ATGLR3.5	55	VVA
ATGLR2.5	28	VVA		125	VVA
ATGLR2.6	101	VVA		238	VVA
ATGLR2.7	148	VVA		529	VVA
ATGLR2.8	169	VVA	ATGLR3.7	96	VVA
ATGLR2.9	165	VVA		414	VVA
ATGLR3.2	584	VVA	ATGLR1.1	17	VVA
ATGLR3.3	584	VVA	GluR5	75	VVA
ATGLR3.4	115	VVA	Delta1	91	VVA
	298	VVA	NMDAZ1	566	VVA
	548	VVA	NMDA2A	287	VVA
	609	VVA		572	VVA
ATGLR3.6	283	VVA	NMDA2B	69	VVA
	339	VVA	NMDA2D	323	VVA
	731	VVA		593	VVA

It has been observed in Table 12 that ‘VVA’ is a block of length 3 aa which is found in majority of primary protein sequences of Arabidopsis and 6 of Rat.

Table 13. Common pattern finding (length of 4 aa) for which same amino acids are contributed in same order as per classification.

Pattern - '3444'									Pattern - '1442'					
Gene Name	Position	AA Behind	Gene Name	Position	AA Behind	Gene Name	Position	AA Behind	Gene Name	Position	AA Behind	Gene Name	Position	AA Behind
ATGLR2.9	11	FIVL	ATGLR2.2	650	FLVL	ATGLR3.4	698	FVVL	ATGLR1.1	719	EIAK	ATGLR1.1	88	DLLK
ATGLR3.2	11	FIVL	ATGLR2.5	22	FLVL	ATGLR3.5	618	FVVL	ATGLR1.2	752	EIAK	ATGLR1.3	100	DLLK
ATGLR3.3	11	FIVL	KA1	11	FLVL		658	FVVL	GluR6	445	EIAK	ATGLR2.4	236	DLLK
GluRC	839	FIVL	NMDA2A	654	FLVL	Delta2	808	FVVL	GluR57	452	EIAK	GluRC	481	DLLK
GluRD	824	FIVL							KA1	455	EIAK	GluR5	437	DLLK
GluR5	794	FIVL							KA2	453	EIAK	GluR57	246	DLLK

Table 13 shows blocks having lengths of 4 aa. ‘FIVL’, ‘FLVL’, ‘FVVL’, ‘EIAK’ and ‘DLLK’ are the 5 blocks which are found in some primary protein sequences of both the species.

Table 14. Common pattern finding (length of 5 aa) for which same amino acids are contributed in same order as per classification

Pattern - '44344'		
Gene Name	**Position**	**AA Behind**
ATGLR2.1	863	VLFLV
GluR6	553	VLFLV
GluR7	560	VLFLV
KA1	563	VLFLV
KA2	561	VLFLV
Delta1	561	VLFLV

Table 14 shows common block ‘VLFLV’ of 5 aa length, which is found in AtGLR2.1 of Arabidopsis and in GluR6, GluR7, KA1, KA2, Delta1 of Rat.

Table 15 shows regions in each primary protein sequences, where the regions specified are conserved based on chemical groups of amino acids. The regions are varying from 1aa to 2 aa long. The It can be easily observed that the conserved regions are majorly belong to aliphatic group of chemical properties.

Table 15. Various conserved regions of all primary protein sequences taken. The regions specified are conserved based on chemical groups of amino acids.

Gene Names	Group wise Conservation of Amino Acids																							
Positions	347	359	428	483	510	596	641	661	675	773	834	836-837	922	932	934	937	942	945	960	967	1234	1246	1269	1277-1278
ATGLR37	I	V	L	I	W	I	L	F	I	I	F	ID	A	D	S	Y	L	V	L	L	I	I	L	LY
ATGLR32	L	L	L	I	W	I	F	F	I	I	Y	ID	A	D	T	Y	L	V	L	M	I	I	L	LF
ATGLR34	I	L	L	V	W	V	F	F	V	I	Y	ID	T	D	T	F	L	V	L	M	I	I	L	LF
ATGLR35	I	L	L	V	W	V	F	F	I	I	F	ID	T	D	T	F	L	V	L	M	I	I	I	LF
ATGLR33	I	L	L	V	W	L	L	F	I	I	F	ID	A	D	T	Y	L	V	L	M	I	I	L	LF
ATGLR36	V	L	L	V	W	L	F	F	V	I	F	VD	T	D	T	Y	L	V	L	M	I	I	L	LF
ATGLR11	L	I	L	V	W	L	L	I	I	V	F	VE	T	D	T	Y	I	L	F	L	I	M	F	LF
ATGLR14	L	I	L	V	W	L	L	F	I	V	Y	ID	T	D	T	F	L	V	F	L	I	I	L	LF
ATGLR12	L	I	M	V	W	L	L	L	I	V	F	IE	T	D	T	Y	L	V	F	L	I	M	I	LF
ATGLR13	L	I	L	V	W	L	L	L	I	V	F	IE	T	D	T	F	L	V	F	L	I	M	I	LF
ATGLR24	V	I	L	V	W	L	L	F	I	I	F	ID	T	D	T	Y	V	V	F	L	I	L	L	LF
ATGLR21	M	V	L	V	W	L	L	F	I	I	F	ID	T	D	S	Y	V	V	L	L	I	L	L	LF
ATGLR22	L	V	L	I	W	L	L	F	I	I	F	ID	T	D	T	F	V	I	L	L	I	L	L	LF
ATGLR23	L	V	L	L	W	L	L	F	I	I	F	ID	T	D	T	F	V	I	M	L	I	L	L	LF
ATGLR25	L	I	L	I	W	L	L	L	I	I	F	ID	T	D	A	Y	I	L	L	L	I	I	L	LF
ATGLR26	L	I	L	I	W	L	L	L	I	I	F	ID	T	D	A	Y	I	V	L	L	I	I	L	LF
ATGLR27	M	I	L	V	W	L	L	L	V	V	Y	IE	T	D	T	Y	V	M	L	L	I	I	L	LF
ATGLR28	M	I	L	V	W	L	L	L	I	V	Y	ID	T	D	T	Y	V	M	L	L	I	I	L	LF
ATGLR29	M	I	L	V	W	L	L	L	I	V	Y	IE	T	D	T	F	V	M	L	L	I	I	L	LF
NMDA2A	L	L	I	L	W	L	L	F	V	I	F	ID	T	D	S	F	I	M	L	V	L	L	L	VF
NMDA2B	L	L	I	L	W	I	L	F	I	I	F	ID	T	D	S	F	I	M	L	V	I	L	L	VF
DELTA2	L	L	V	V	W	L	F	F	V	V	F	ID	T	D	S	F	I	M	L	V	L	L	L	VF
NMDA2C	L	L	V	V	W	L	F	F	V	V	F	ID	T	D	S	F	I	M	L	V	L	L	L	VF
NMDA2D	L	V	I	L	W	V	L	F	V	V	F	ID	T	D	S	F	I	M	L	V	L	L	L	VF
NMDAZ1	V	L	L	I	W	V	F	F	I	I	F	ID	T	E	S	F	L	L	M	L	I	L	L	VF
GLURA	L	L	L	I	W	L	M	F	I	V	F	ID	T	D	S	Y	V	L	F	V	I	L	L	VF
GLURB	L	L	L	I	W	L	M	F	I	V	F	ID	T	D	T	Y	V	L	L	L	I	L	L	VF
GLUR6	L	I	L	V	Y	M	I	F	V	V	Y	VE	T	D	S	F	I	M	L	I	V	L	L	VF
GLUR7	I	I	L	I	Y	M	I	F	I	V	Y	VD	T	D	S	F	I	M	L	I	V	L	L	VF
KA1	L	V	M	L	Y	I	I	F	V	V	Y	VD	T	D	S	F	I	M	L	I	V	L	L	VF
KA2	L	I	M	V	Y	M	M	F	V	V	Y	VD	T	D	S	F	I	M	L	I	V	L	L	VF
DELTA1	V	I	V	I	Y	V	L	F	I	V	F	VD	T	D	S	F	I	L	L	V	I	L	L	IF
GLURC	V	I	I	I	Y	V	F	F	I	V	Y	LD	T	D	S	F	I	L	L	I	I	M	L	IF
GLURD	V	I	I	I	Y	V	F	F	V	V	Y	ID	A	D	S	F	I	L	L	I	I	M	L	IF
GLUR5	V	I	I	I	F	V	F	F	I	V	Y	ID	T	D	S	F	V	L	L	I	I	M	L	IF

The regions which are conserved based on individual amino acid are shown in the table 16 along with the regions of conservations. G, I, R, F, P, W, G and W are those amino acids. These types of conservations depict regional importance of those patterns from functional and structural point of view to make 3D protein structures of each primary protein sequence.

Table 16. Various conserved regions of all primary protein sequences taken. The regions are conserved based on individual amino acid.

Sr. No.	Position	Conserved Region Based on individual Amino Acid
1	821	G
2	913	I
3	917	R
4	923	F
5	952	P
6	958	W
7	1205	G
8	1240	W

Investigating evolutionary relationships between two species

To investigate and identify evolutionary relationships between the data sets taken, firstly equation 1 has been used to derive 8X8 weight matrix for each AA sequences. As examples weight matrices for AtGLR1.1 of Arabidopsis has been stated below in Tables 17.

Table 17. Weighted directed multigraph through adjacency matrix of AtGLR1.1.

Vertex Vs. Vertex	1	2	3	4	5	6	7	8
1	11	17	9	31	2	3	9	8
2	20	10	16	41	1	1	13	7
3	7	10	8	32	4	4	13	9
4	30	37	33	122	8	8	59	22
5	0	2	4	11	0	3	2	3
6	3	4	4	12	1	2	4	1
7	15	19	8	48	5	6	25	12
8	4	11	5	22	4	3	13	5

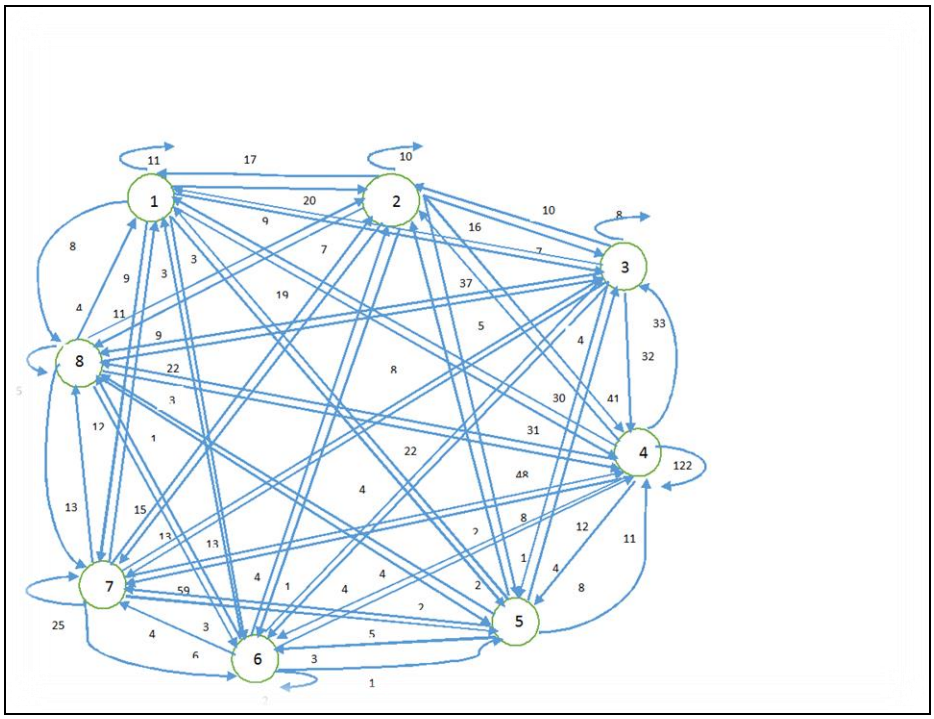

Figure1: Corresponding weighted directed multi graphical representation of AtGLR1.1 has been shown as an example.

Equation 2 mentioned in previous section is used to get weight deviation (WD) between each pair of AA sequences taken. Thus, a distance matrix say dissimilarity matrix has been formed to analyze evolutionary relationships existing between them. The matrix has been shown in Table 18 as a supplementary file. The distance matrix stated in Table 18 reflects evolutionary relationship between vertebrate Rat and plant Arabidopsis among some specific glutamate and glutamate like gene families respectively.

Fig 2 is a phylogenetic tree, which is being constructed from the dissimilarity matrix specified in table 16. So, all the 16 primary protein sequences of Rat and 19 primary sequences of Arabidopsis are participated in this figure. According to fig 2, AtGLR2.1 to AtGLR 2.9 and AtGLR3.2 to AtGLR 3.7 genes of Arabidopsis Thaliana except AtGLR2.5 and AtGLR3.6 have close evolutionary relationships with GluRA and GluRB of Rat, whereas, AtGLR3.6 are close to NMDAZ1, NMDA2B, NMDA2C and NMDA2D of Rat.

In fig 3 phylogenetic tree has been constructed with all the 19 AtGLR genes of Arabidopsis thaliana and 8 iGluR genes (GluRA, GluRB, GluRC, GluRD, GluR5, GluR6, GluR7, GluR8) of Rat to get more finer view about evolutionary relationships between glutamate gene families of Rat and Arabidopsis. According to the phylogenetic AtGLR1.1, AtGLR1.2, AtGLR1.3, AtGLR1.4 and AtGLR2.5 genes have similarities in chemical properties with 8 iGluR genes GluRA, GluRB, GluRC, GluRD, GluR5, GluR6, GluR7 and GluR8

of vertebrate Rat. The observation thus depicts the evolutionary relationships of ionotropic glutamate receptor (iGluR) genes of vertebrates with AtGLR genes in Arabidopsis.

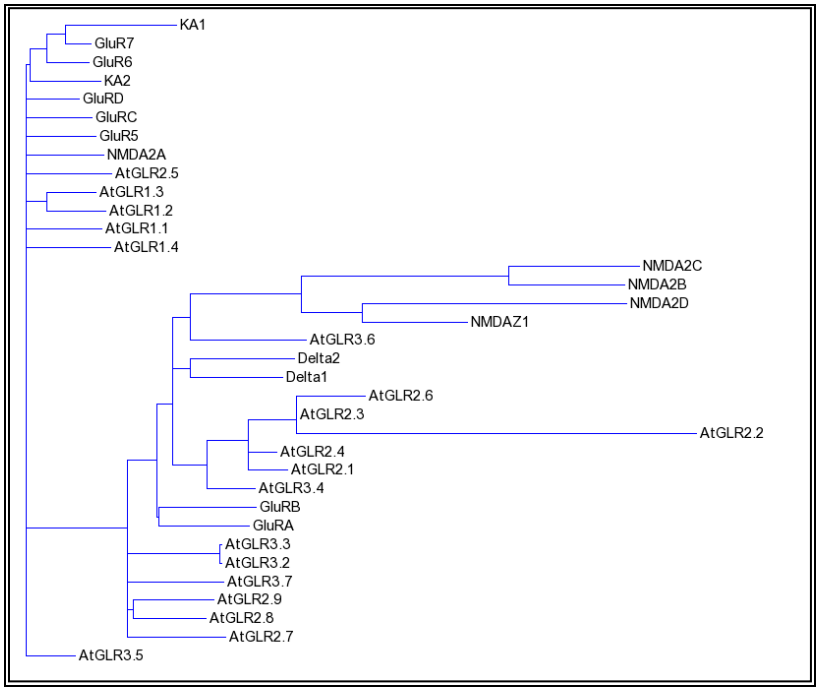

Figure2: Phylogenetic tree constructed for total Data Sets taken

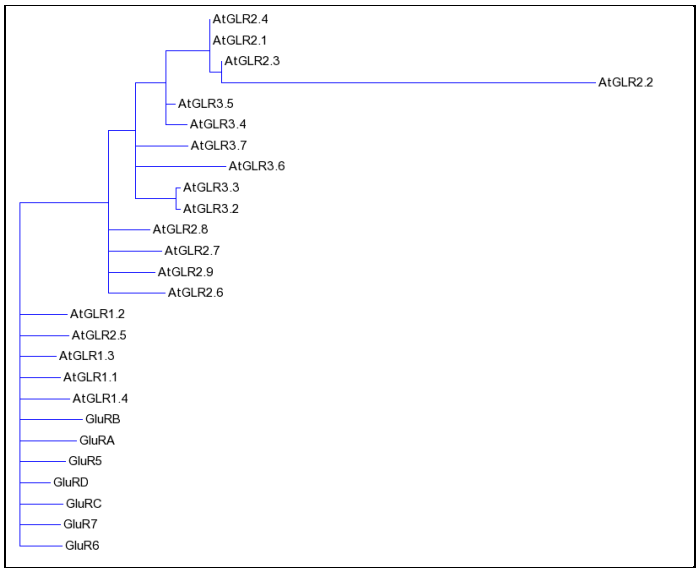

Figure 3: Phylogenetic tree constructed of all the 19 AtGLR genes of Arabidopsis thaliana and 8 iGluR genes (GluRA, GluRB, GluRC, GluRD, GluR5, GluR6, GluR7, GluR8) of Rat.

Conclusions

The analysis throughout the paper confirms in silico the recent biological claim that the iGluR family of the rat, a mammal and AtGLR family of Arabidopsis, a plant have some common functionalities in neurotransmission and have been conserved during the long process of evolution from a common ancestor. The graph theoretic approach which is applied to investigate evolutionary relationship among all the primary protein sequences taken is a completely alignment free method and hence the time complexity is directly proportional to the sequence length N, that is O(N).

Supporting Information

Table 11: Common patterns found in all the primary protein sequences with regions respectively.

Table 18: Dissimilarity matrix of all primary protein sequences taken to investigate evolutionary distance between them.

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