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COMPREHENSIVE PHYLO-GENETIC ANALYSIS OF –PEX11 GENE FAMILY IN *Oryza sativa*

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Peroxin gene 11 has been revealed to have important functions involving photorespiration, beta degradation and peroxisomes biogenesis. In this study we show 5 assumed genes present in *Oryza sativa* gnome PEX11(1-5) and each contains one conserved motif. The PEX11 sequence from *Oryza sativa* and other species are divided into three major categories. Despite of its important functions a very limited knowledge is known about PEX11 gene family in rice. This study confirms the five different PEX11 genes in rice and each gene contains one particular conserved domain. Among other members PEX11-2 and PEX11-3 are more closely related and shows recent duplications. Different analysis was done in this study to better understand the structure and phylo-genetic relations of PEX11 genes and its evolutionary analysis. Our results showed that PEX11 genes have conserved domains but have diversification not only in sequences but also in functions.

Keywords: PEX11 gene; phylogenetic analysis; *Oryza sativa*; evolutionary analysis; peroxisome biogenesis.

1. INTRODUCTION

Peroxins is a gene family which represents different protein families found in an organelle known as peroxisome [1]. Present in almost all eukaryotic cells, a peroxisoome is a membrane bounded organelle having various functions and called oxidative organelles. Peroxin gene families are involved in different kind of functions such as cytoplasm protein recognition which contains PTS (peroximal targeting signals). Peroxins tag these proteins and they are transported from cytoplasm to perixomes by peroxins.Being diverse in structure peroxins are classified into various protein families such as PEX1, PEX2, PEX3 etc. their major role is in the breakdown of fatty acids which are of long chains into smaller molecules through a process of beta oxidation. The long fatty acids are converted into medium chain fatty acid with the help of peroxins in animal cells. These medium sized fatty acids are then transported to mitochondria where they are broken down into CO2 and water. Deficiencies of peroxins are associated with several severe disorders [2].

PEX genes encode the protein machinery which is required by the proper assembly of peroxisomes. Proliferation of the peroxisome is regulated by PEX11 whereas PEX 3, 16 and 19 are involved in assembly

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and maintenance of organelle. PEX 1, PEX 2, PEX 3, PEX 5, PEX 6, PEX 7, PEX 9, PEX 10, PEX 11A, PEX 11B, PEX 12, PEX 13, PEX 14, PEX 16, PEX 19, PEX 26 are the genes that encode peroxin proteins. The content of protein of the peroxisome varies in different organisms. The endo-symbiotic origin of peroxins was suggested because of the presence of these proteins in many species. It was assumed that peroxisomes were originated from bacteria as a result of invading larger cells as parasites. However with the passage of time and with the recent discoveries this view of endo-symbiotic origin is challenged. Recent studies suggest that peroxisomes have action-bacterial origin. However this suggestion is still controversial [3-14].

Peroxisomes are mostly spherical in plants. Plasticity in function is one of the remarkable features of peroxisomes in higher plants [15]. A large number of peroxisomes are involved in photorespiration, nitrogen metabolism, plant hormone metabolism and interaction of pathogens and plants [16-19]. In higher plantsperoxins are highly conserved, among them PEX11 was the first to be isolated and is best understood. PEX11 proteins are involved in proliferation of peroxisome in plants [20-22]. The Absence of PEX11 in plants leads to the formation of two or more giant peroxisomes which than caused cessation of cell growth [20,23]. Similarly over expression of PEX11 leads to the increased number of peroxisomes per cell [20,23]. Despite of the discovery of PEX11 10 years ago, its mode of mechanism is still not completely understood [24]. Rice is considered as one of the most important and model plant of monocot species for various genomic studies. However limited study and knowledge of PEX11 gene is found in rice plant. In this paper PEX11 sequences was identified and their phylo-genetic analysis was done [2].

2. MATERIALS AND METHODS

2.1 Recognition and Sequence Retrieval of PEX11 Gene in Rice

The sequences of PEX11 in rice were identified from National center for biotechnological information (https://www.ncbi.nlm.nih.gov/). The sequence was used as a query in comparison to non redundant sequence of protein in BLASTP program to get supposed peroxin 11 in rice and other species. All the sequences were than inspect for the conserved PEX11 domain by using the online tool called as a Simple Modular Architecture Research tool (http://smart.embl-heidelberg.de/) and PFAM domain (http://pfam.xfam.org), conserved domain was also identified by using NCBI (https://www.ncbi.nlm.nih.gov/Structure/bwrpsb/bwrpsb.cgi). SMART and PFAM tools are used for the analysis of architecture and functions of domains and motifs. Multiple sequence alignment MSA was performed to confirm the conserved domains of PEX11. The full length PEX11 proteins from rice species were aligned using MEGA 7.0.21 and the evolutionary tree was build. A maximum likelihood tree was build and exported in newick format. Both kind of tree was exported i-e circular and radiant. The tree was build in order to study the evolutionary relationship of PEX11 gene in other species. The full chromosome detail of PEX11 was identified by using NCBI tool. All these steps were performed in order to remove all those sequences which are not conserved in terms of domains. All the genomic information of PEX11 gene for example gene location on chromosome, protein length and their protein cdna sequences was obtained from NCBI. In this study we show 5 assumed genes present in ARYZA rae gnome and each contains one conserved motifs. The PEX11 sequence from rice and other species are divided into three main categories.

2.2 Alignments, Phylo-genetic Analysis and Motif Identification of Peroxin11 Gene Family

Phylo-genetic analysis and tree building by using both maximum likelihood and neighbor joining approaches was used to build evolutionary tree by using MEGA7.0 software. All the protein sequences obtained from BLASTp from NCBI was subjected to CLUSTAL omega program using MEGA 7 tool. By using MEGA 7 phylo-genetic tree was constructed. Different forms of rooted and un-rooted tree and branch length of tree as well as divergence time was obtained.

2.3 Analysis of Structure of Gene and Conserved Domains & Motifs

In order to confirm the conserved motifs of PEX11 in different species a meme tool was used (https://meme-suite.org/meme/tools/meme) .To illustrate the exon/intron organization the gene structure display server (GSDS) program which is an online tool was (http://gsds.cbi.pku.edu.cn/).

3. RESULTS

3.1 Sequence Retrieval and Evolutionary Analysis of PEX11

All the sequence of PEX11 gene family was retrieved from NCBI in Fasta format. The retrieved sequences were subjected to NCBI-BLAST and all the available sequences in different organisms were downloaded.

PFAM data base was used to inspect the conserved domains for the PEX-11 gene homologos in rice, Arabidopsis and other species. The results show that in rice PEX-11 has five members. These members are distributed on chromosome 1, 3, 4 and 6. About 100 PEX11 homologous sequences were used for building phylo-genetic tree. Results showed that PEX11 homologous sequences were were divided into three main categories having further different subgroups. These three major groups includes fungi mammals and yeast in group one and group two contains only plant species while group three contains Arabidopsis and rice. All these three groups are further classified into subgroups containing different number of species. From phylo-genetic tree it is observed that OsPEX11-2 and OsPEX11-3 are more closely related than others. PEX11-2 and PEX11-3 have 85.25 cDNA similarities.

3.2 Phylo-genetic Analysis of PEX11 Gene Family

Phylo-genetic analysis of PEX11 gene family was done by using MEGA 7 software to generate evolutionary tree. Phylogenetic analysis was generated by using 100 sequences from different species. Results showed that PEX11 gene family is broadly classified into many groups including yeast, fungi, animals and plants. Results showed that PEX11 homologous sequences were classified into three major groups having further different subgroups. These three major groups include fungi mammals and yeast in group one and group two contains only plant species while group three contains Arabidopsis and rice. All these three groups are further classified into subgroups containing a different number of species. From phylo-genetic tree it is observed that OsPEX11-2 and OsPEX11-3 are more closely related than others. PEX11-2 and PEX11-3 have 85.25 cDNA similarities.

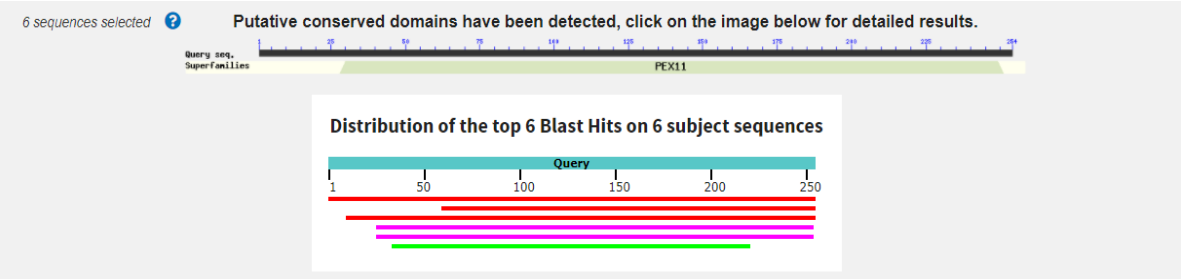

Fig. 1. Conserved hits by using BLAST

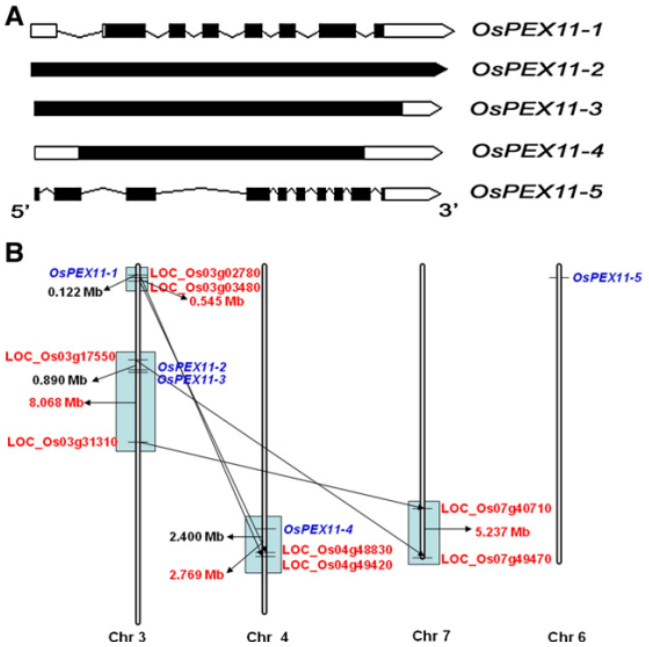

Fig. 2. Distribution of PEX11 on different chromosome

Table 1. Proposed nomenclature and important features of PEX11 genes

Proposed names	Gene Locus	Protein accession #	RNA accession#	Exons	Chr #	ORF length	Amino acid length	Start of Genomic Location	Conserved domains in protein sequence
PEX11OS2	LOC9267541	XP_015630854.1	XM_015775368.2	1	3	762	254	10645772	pfam05648
PEX11OS1	LOC4331408	XP_015630823.1	XM_015775337.2	8	3	711	237	958077	pfam05648
PEX11OS3	LOC4332579	XP_015630855.1	XM_015775369.2	1	3	726	242	10647657	pfam05648
PEX11OS4	LOC4336511	XP_015634264.1	XM_015778778.2	1	4	666	222	26720014	pfam05648

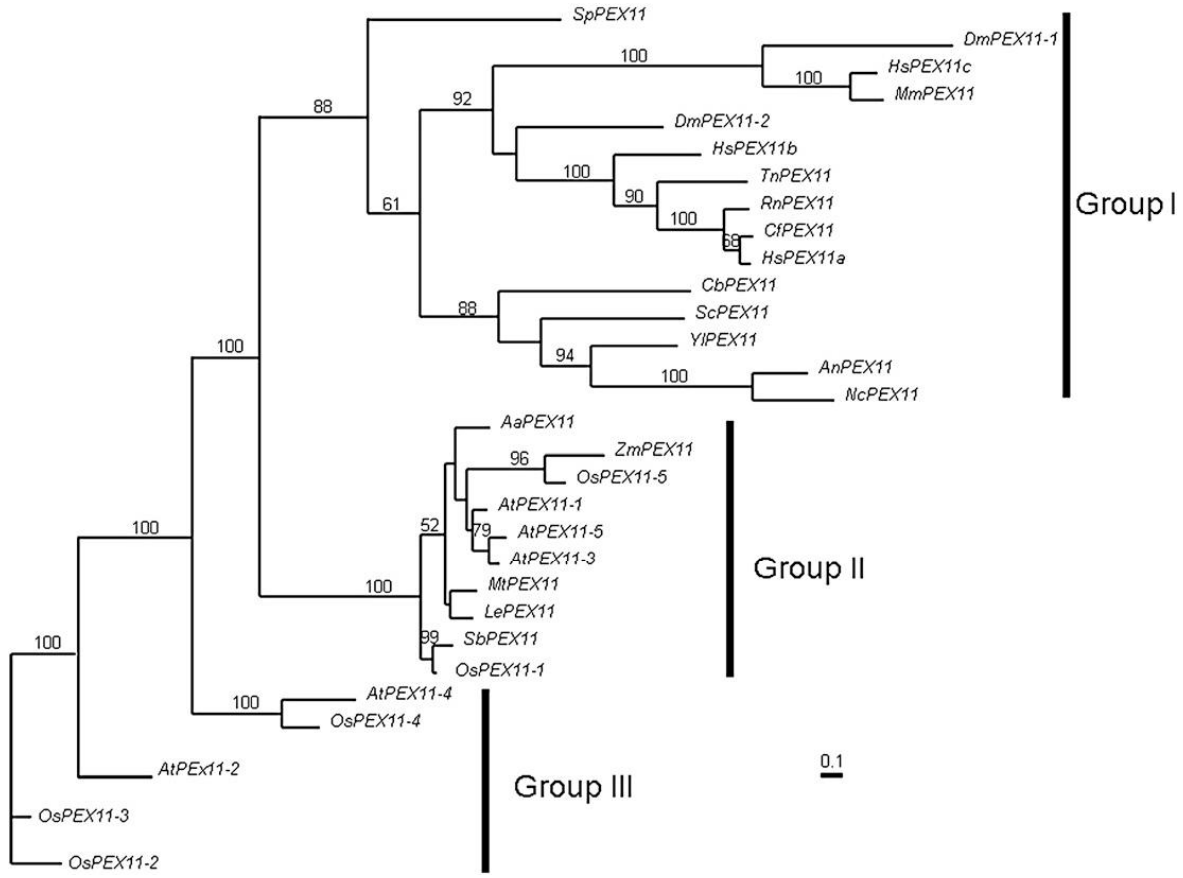

Fig. 3. Phylogenetic tree

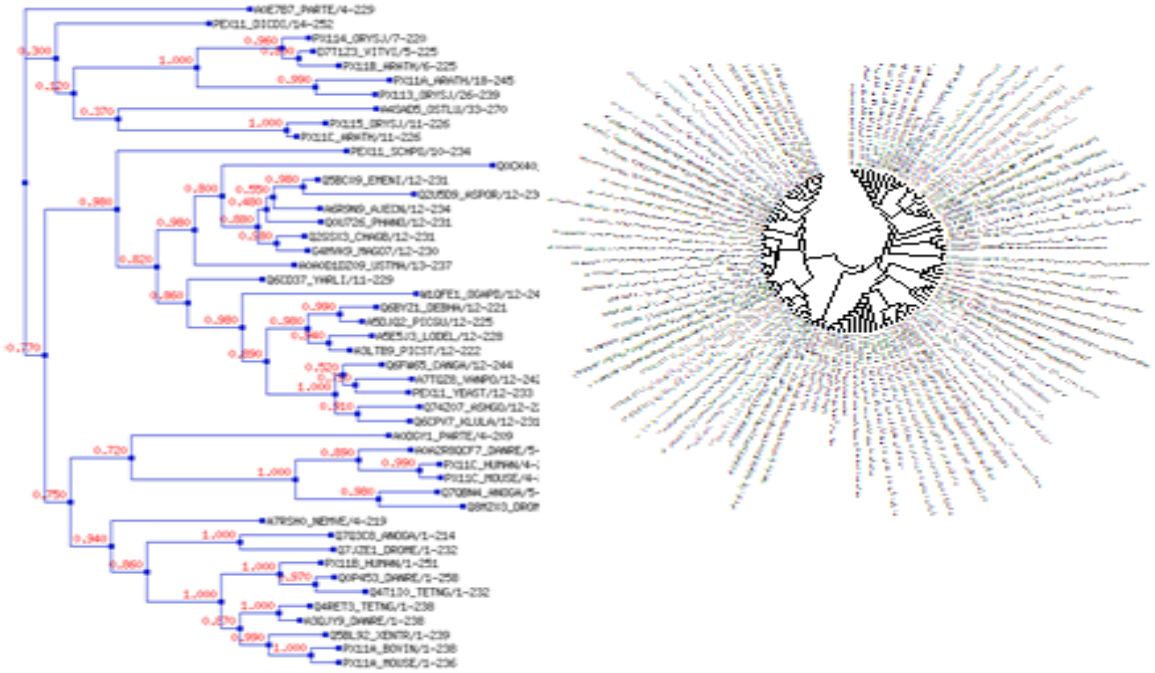

Fig. 4. Phylogenetic tree using MEGA7

3.3 Analysis of Gene Structure of PEX11 Genes

The intron/exons positions remains conserved in orthologous genes with respect to evolution while in case of paralogous genes the structure of intron/exons are relatively less conserved. To analyze gene structure and position of introns and exons in PEX11 genes we compare their cDNA sequences and genomic DNA sequences by using online tool gene structure display server (GSDS) program (http://gsds.cbi.pku.edu.cn/). The gene structure shows that introns are less overall while there is much exonic region. The overall gene structure model of PEX11 gene was also obtained from trRosetta software. We get results and analyzed that *Oryza sativa* both have five members of PEX11 gene family. the distribution of five members of PEX11 in rice are on chromosome 3(1-3),4 AND 6. Among these PEX11-2 and PEX11-3 are more closely related and undergoes recent duplications. We analyzed the genome structure of PEX11 gene family by using GSDS and compares their genomic vs cDNA sequences and found that coding sequences of PEX11-2,3,4 are not interrupted by non coding sequences.

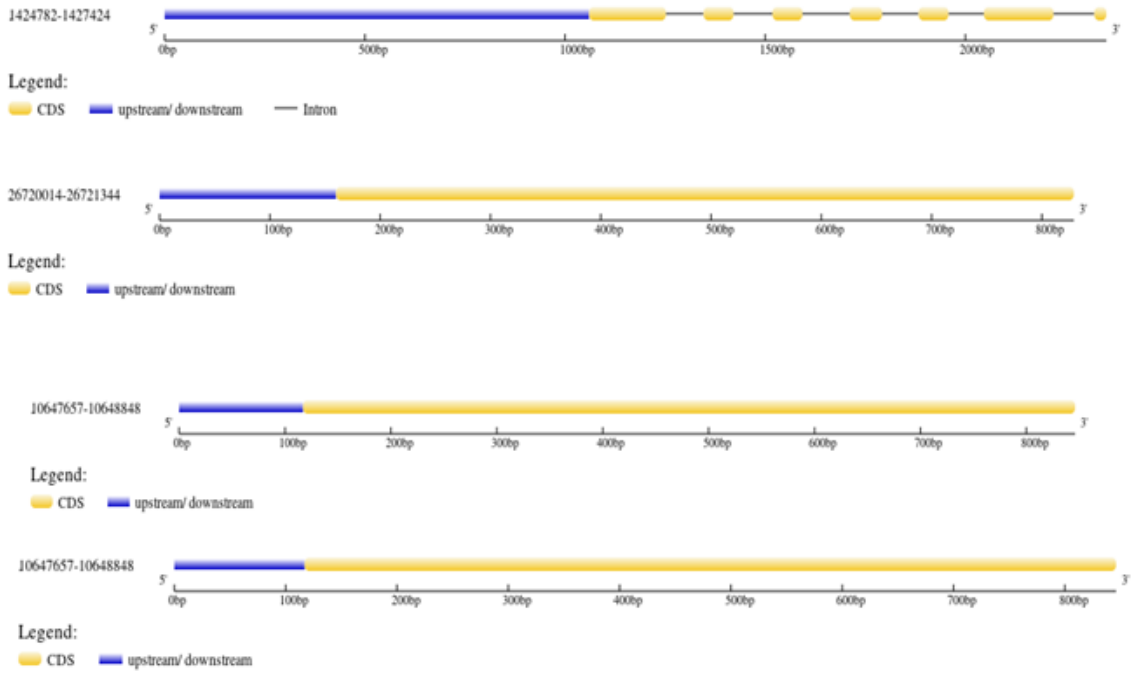

Fig. 5. Gene structure and intron/exon positions analyzed by GSDS

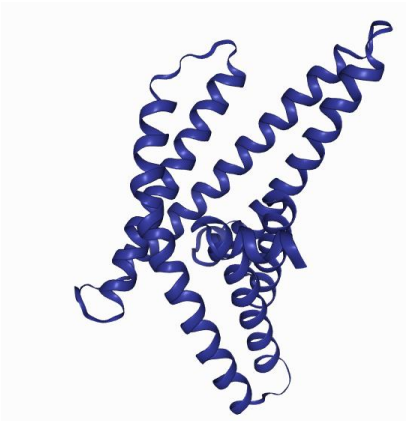

Fig. 6. Structural model of PEX11 gene

3.4 Analysis of Conserved Domains

Analysis of conserved domains of proteins could help to understand protein functions exactly. The conserved domains were analyzed by using different software such as pFAM, MEME. As a result only one conserved motif was identified. Along with conserved domains, the full chromosomal map of PEX11 genes was obtained from NCBI. Results from PFAM analysis suggest that all the members of PEX11 gene family in rice contain one particular conserved domain. Expasy analysis gives us results which shows that there is a great variation in chemical properties of all these five members. Analysis was also done by using MEME program which shows three different motifs. Theses motif locations were well matched with the conserved sequences obtained from MSA.

Fig. 7. Conserved motifs and their location identified By MEME software

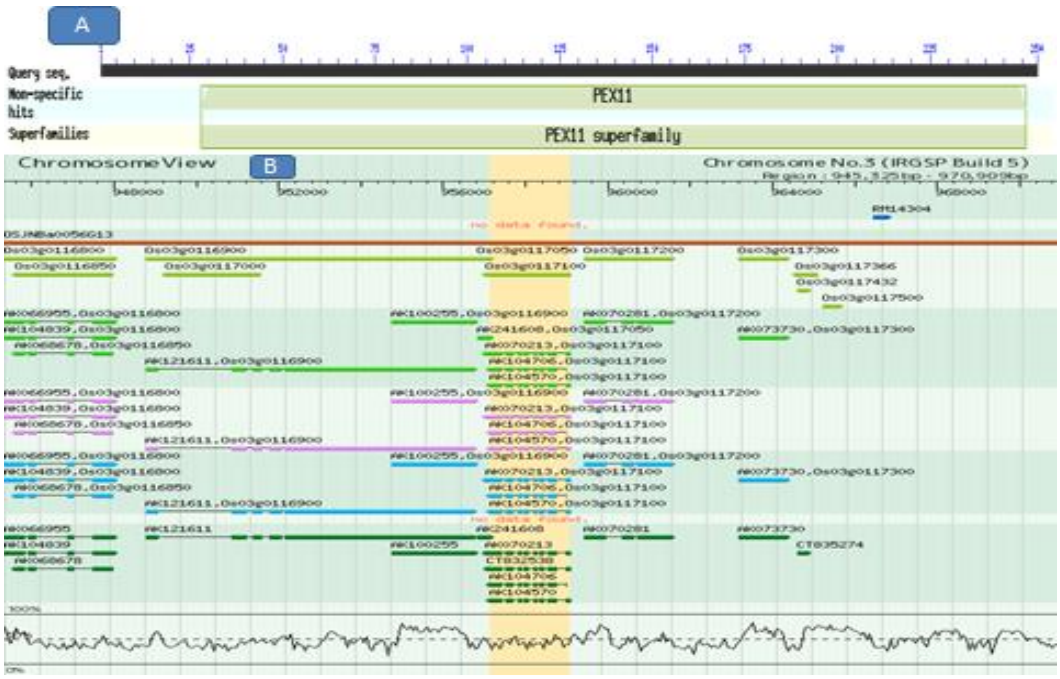

Fig. 8. Conserved Domain and chromosome view obtained from NCBI

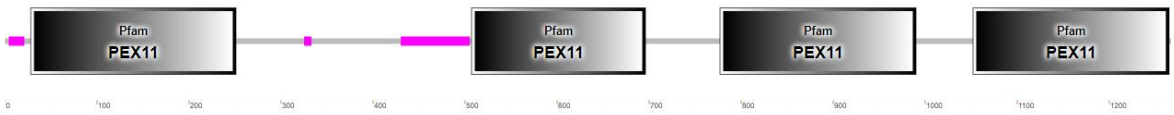

Fig. 9. PFAM conserved domains

Fig. 10. Conserved domain logo obtained from WebLogo

4. DISCUSSION

Peroxisomes were involved in detoxification of cells by decreasing expression level of oxygen. But this was poisonous to various other life forms. Later on peroxisomes were evolved to take various other functions such as photorespiration and beta oxidation. At first peroxisomes were identified in mice but later on its presence in other species was confirmed. In this research PEX11 sequences from 101 species are obtained which contains conserved domains from three large group's i-e fungus, mammals and plant species. It is concluded from phylogenetic analysis that the PEX11 genes had a single origin and later it was evolved independently. Among these three groups the PEX11 gene family seems to be largest group and it is the largest protein family in *Oryza sativa* species. However, recently several gene duplications occur in PEX11 gene family. These duplications show that OSPEX11-2 and OSPEX11-3 becomes part of chromosomes 3 recently. Several rounds of genomic duplications have been found in all species of rice. We get results and analyzed that *Oryza sativa* both have five members of PEX11 gene family. the distribution of five members of PEX11 in rice are on chromosome 3(1-3),4 AND 6. Among these PEX11-2 and PEX11-3 are more closely related and undergoes recent duplications. We analyzed the genome structure of PEX11 gene family by using GSDS and compares their genomic vs cDNA sequences and found that coding sequences of PEX11-2,3,4 are not interrupted by non coding sequences. Results from PFAM analysis suggest that all the members of PEX11 gene family in rice contain one particular conserved domain. Expasy analysis gives us results which shows that there is a great variation in chemical properties of all these five members. Analysis was also done by using MEME program which shows three different motifs. Theses motif locations were well matched with the conserved sequences obtained from MSA. This study helps to the better understanding of PEX-11 gene family in rice. Still there requires a lot of room of experiments for further analysis and understanding of PEX11 gene family in plants.

5. CONCLUSION

This study represents several members of PEX11 genes in different species particularly in rice spread on different chromosomes. This gene is highly conserved in plants mammals and fungi. The highly conserved manner of PEX11 gene family reveals that it has important functions in diverse taxonomic groups. Different analysis gives us the complete map of PEX11 gene, its phylo-genetic relationship, intron/exon locations, structure of gene as well as conserved domains which is helpful for further study of PEX11 gene family functions. Since till now there is a little knowledge about PEX11 gene family due to limited study and experiments, this study may help to better understand the functions of PEX11 gene.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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