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Phylogeny of actin and tubulin gene homologs in diverse eukaryotic species

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Abstract

Actin and tubulin are cytoskeleton proteins, which are important components of the celland are conserved across species. Despite their crucial significance in cell motility and cell division the distribution and phylogeny of actin and tubulin genes across taxa is poorly understood. Here we used publicly available genomic data of 49 model species of plants, animals, fungi and Protista for further understanding the distribution of these genes among diverse eukaryotic species using rice as reference. The highest numbers of rice actin and tubulin gene homologs were present in plants followed by animals, fungi and Protista species, whereas ten actin and nine tubulin genes were conserved in all 49 species. Phylogenetic analysis of 19 actin and 18 tubulin genes clustered them into four major groups each. One each of the actin and tubulin gene clusters was conserved across eukaryotic species. Species trees based on the conserved actin and tubulin genes showed evolutionary relationship of 49 different taxa clustered into plants, animals, fungi and Protista. This study provides a phylogenetic insight into the evolution of actin and tubulin genes in diverse eukaryotic species.

Keywords: Actin, cytoskeleton protein, eukaryotes, phylogenetic tree, rice, tubulin

Introduction

The complexity and diversity of life has gradually increased over 4.1 billion years of evolution on earth. Microscopic fossils and molecular evidence indicate that the earth has been considerably inhabitable for 3.9 billion years (Bell et al. 2015; Abramov and Mojzsis 2009). Different studies have indicated availability of oxygen in the atmosphere around 2.4 billion years ago which is a fundamental constituent of all living organisms (Farquhar et al. 2000). Genomic resources have highlighted the role of double-membrane prokaryotes, including cyanobacteria in building the Earth's oxygen atmosphere, originating after the establishment of most major prokaryotic groups (De 2000). The advancement in genome sequencing technology starting from Sanger to Illumina to PacBio SMRT has resulted in huge genomic resources in the form of raw or curated data available in the public domain, e.g., National Centre for Biological Information (NCBI, https://www.ncbi.nlm.nih.gov/), DNA Data Bank of Japan (DDBJ, https://www.ddbj.nig.ac.jp/index-e.html) and European Molecular Biology Laboratory (EMBL, https://www.embl.org/) providing tremendous opportunity for functional characterization and evolutionary analysis of Archaebacteria, Eubacteria and Eukaryotes (Rothberg and Leamon 2008; Feng et al. 2015; Reuter et al. 2015; Rhoads and Au 2015). Broadly, the evolution of genes and genomes is driven by natural selection on the genetic variation caused by duplication, deletion, substitution, insertion, inversion or translocation of DNA segments and ultimately new gene functions by neo-functionalisation, sub-functionalisation and pseudogenisation (Gilbert et al. 1997; Kaessmann 2010; Assis and Bachtrog 2013; Zwart et al. 2014; Hughes et al. 2014; Mahmudi et al. 2015; and Sandve et al. 2018).

Analysis of multigene families containing set of genes evolved by gene duplication and divergence from common ancestors are useful for evolutionary studies. The skeletal structure of the cell is made up of cytoskeleton proteins belonging to three different categories: (i) microfilaments - made up of actin; (ii) intermediate filament - made up of lamin, desmin and

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crescentin (Ausmees et al. 2003); and (iii) microtubules - made up of tubulin. Actin is mainly involved in the cell movement, cytoskeletal structure, intra-cellular transportation and cell division (Bramhill 1997) conserved among all taxa of life including archaea and bacteria (Ettema et al. 2011; Dawson and Paredez 2013). A number of actin genes are expressed throughout the plant tissues in different isovariant forms which indicate the variability of the gene family. Earlier two actin isotypes were reported in red algae (*Palmaria palmata* and *Porphyra purpurea*) as well as in green algae *Chlamydomonas* (Le Gall et al. 2005; Slajcherova et al. 2012). Interestingly, 21 actin isovariants have been reported in complex monocot species e.g., maize (Slajcherova et al. 2012). Like actins, tubulins are highly dynamic composed of repetitive hetero dimers of α1/α2 and α1/β2 tubulins and are involved in different cellular processes like cell division, cell motility and cell transportation (Nick 1998). The α-and β-tubulin subunits heterodimerize in a head-to-tail fashion, but γ-tubulin which is related to α-and β-tubulin but does not heterodimerize with any other α-, β-tubulin subunit which play a key role in the nucleation of microtubule polymerization (Oakley 1992; Nogales et al. 1998; O'Toole et al. 2012). Earlier, different isotopes of the tubulin have been characterized and reported in different plant species e.g., in maize six copies of α and eight copies of β have been reported (Villemur 1992). The homology based structural and functional characterization of tubulin genes may be helpful in identifying the direction of evolution of these conserved genes. In the present study, we performed the comparative genomic analysis to identify the copy number of actin and tubulin genes in rice and their homologs across eukaryotic taxa. Further, we developed the conserved actin, tubulin gene sequence based phylogenetic tree to study the evolutionary interrelationship among them.

Table1. Number of rice actin and tubulin gene homologs present in 49 diverse eukaryotic species

S.No.	Species	Actin	Tubulin
1	Rice (*Oryza sativa*)	19	18
2	Maize (*Zea mays*)	19	18
3	Broomcorn (*Sorghum bicolor*)	16	17
4	Wheat (*Triticum aestivum*)	19	17
5	Barley (*Hordeum vulgare*)	19	17
6	Purple false brome (*Brachypodium distachyon*)	16	15
7	Banana (*Musa acuminate*)	19	18
8	Soyabean (*Glycine max*)	19	18
9	Pigeonpea (*Cajanus cajan*)	19	16
10	Barrel clover (*Medicago truncate*)	17	16
11	Grape (*Vitis venifera*)	18	17
12	Thale Cress (*Arabdiopsis thaliana)*	19	16
13	*Tomato (Solanum lycopersicum)*	19	16
14	Black cottonwood (*Populus trichocarpara*)	17	16
15	Southern yellow pine (*Pinus taeda*)	16	16
16	White spruce (*Picea glauca)*	18	16
17	Bryophyta (*Physcomitrella patens*)	16	16
18	Green algae (*Chlamydomonas reinhardtii*)	10	16
19	Human (*Homo sapiens*)	12	13
20	Chimpanzee (*Pan troglodytes*)	12	13
21	Gorilla (*Gorilla gorilla*)	12	13
22	Orangutan (*Pongo abelii*)	12	13
23	House Mouse (*Mus musculus*)	12	13
24	Cow (*Bos torus*)	12	13
25	Zebrafish (*Danio rerio*)	12	13
26	African clawtoed frog (*Xenopus laevis*)	12	13
27	Sea squirt (*Ciona intestinalis*)	11	13
28	Chicken (*Gallus gallus*)	12	13
29	Chinese softshell turtle (*Pelodiscus sinensis*)	12	13
30	Green anole(*Anolis caroliensis*)	12	12
31	Silk moth (*Bombyx mori*)	10	13
32	African malaria mosquito (*Anopheles gambiae*)	12	13
33	Honey bee (*Apis mellifera*)	12	13
34	Fruit fly (*Drosophilla melanogaster*)	12	13
35	Nematode (*Caenorhabditis elegans*)	11	13

Materials and methods

Identification of conserved actin and tubulin genes in eukaryotic species

Actin and tubulin genes of 49 model eukaryotic species were analyzed including 18 plants, 20 animals, seven fungi and four Protista species (Table 1). Unigene sequences were downloaded from various public database, *viz*., NCBI (NCBI, http://www.ncbi.nlm.nih.gov/unigene), Broad Institute of Microbial genome (https://www.broadinstitute.org/scientific-community/data) and Ensembl (http://asia.ensembl.org/index.html). For identification of actin and tubulin

36	Starlet sea anemone (*Nematostella vectensis*)	12	13
37	*Hydra magnipapillata*	13	11
38	Purple Sea urchin (*Strongylocentrotus purpuratus*)	12	13
39	Yellow koji mold (*Aspergillus oryzae*)	11	13
40	*Fusarium oxysporum*	11	13
41	Nerve spore (*Neurospora crassa*)	10	13
42	*Puccinia graminis*	10	13
43	*Rhizopus oryzae*	12	13
44	Rice blast fungus (*Magnaporthe grisea*)	10	13
45	*Cryptococcus gattii*	11	13
46	*Salpingoeca rosetta*	11	13
47	Slime mold (*Dictyostelium discoideum*)	12	11
48	*Toxoplasma gondii*	10	13
49	Water mold (*Phytophthora infestans*)	11	13

genesin the rice genome 36,002 EST sequence (NCBI, ftp://ftp.ncbi.nlm.nih.gov/repository/UniGene/Oryza_sativa/)supportedwith CDS sequences (Rice Genome Annotation Project Database, http://rice.plantbiology.msu.edu/) of rice were used. and ultimately CDS locus id annotation was preferred for the identification of actin and tubulin genes. The rice actin and tubulin unigenes sequences were used as query in a locally configured BLASTN (Altschul et al. 1990) programme with pre-optimized parameters (Singh et al. 2004) against unigene sequences of other 48 species as subject. The search parameters for finding rice actin and tubulin gene homologs in 48 other species were fixed 100 bit score and 60% sequence identity.

Construction of phylogenetic trees of actin and tubulin genes and species

For construction of phylogenetic trees of rice actin and tubulin genes nucleotide sequences of all actin and tubulin genes of rice were aligned separately using high speed multiple sequence alignment program inbuilt in mafft v.7.409 (Multiple Alignment using Fast Fourier Transform, Katoh 2002) programme with default parameters. For construction of species phylogenetic trees actin and tubulin genes conserved in all 49 species were aligned. Phylogenetic trees were constructed using maximum likelihood (ML) based method implemented in RAxML (Randomized Axelerated Maximum Likelihood, Stamatakis 2014) software. Statistical reliability for individual node support was determined from 500 replicates of non-parametric bootstrap with GTRGAMMA (General Time-Reversible + G discrete Gamma distribution) model. Output of each tree was visualized using Figtree v 1.4.0 software.

Results and discussion

Distribution of actin and tubulin genes in 49 diverse eukaryotic species

A comparative genome analysis of 49 diverse plants, animals, fungi and Protista species revealed that actin and tubulin genes are widely distributed and some of these are conserved across species. There were 19 actin genes in *Oryza sativa*, most of which were conserved in the plant species (Fig. 1). Eight angiosperm species namely, wheat, barley, maize, banana, soybean, pigeonpea, tomato and *Arabispsis* have all the 19 genes, grape and pine have 18 genes each, *Medicago* and *Populus* have 17 genes each while four species, broomcorn, purple false brome, southern yellow pine and bryophyte have 16 genes each. In contrast, unicellular green alga *Chlamydomonas reinhardtii* has only ten rice actin gene homologs (Fig. 1). Earlier, Bhattacharya et al. (2000) reported only 1-2 copy of actin genes in green algae and 2-3 copies in bryophyta species. However, consistent with our analysis recent genome wide studies have reported 21 actin genes in maize, 15 actin genes in flax (Slajcherová et al. 2012, Pydiura et al. 2018). Further, our pairwise analysis showed that 10-13 rice actin gene homologs were present in twenty animal species, 10-12 homologs in seven fungal and four Protista species. Overall 10 rice actin genes were conserved in all the 49 eukaryotic species analyzed in this study, namely LOC_Os01g73310.1, LOC_Os01g64630.1, LOC_Os03g61970.1, LOC_Os03g50885.1, LOC_Os05g01600.1, LOC_Os05g36290.1, LOC_Os10g36650.1, LOC_Os11g06390.1, LOC_Os12g44350.1 and LOC_Os12g06660.1 (Table 2). The Gene ontology based functional analysis revealed that the identified conserved genes were involved in different functional activities like ATP binding, motor activity, protein kinase and actin filament structure (Table 2).

Similarly, we identified 18 tubulin genes in rice distributed on eight chromosomes of rice: (LOC_Os01g18050.1, LOC_Os01g59150.1, LOC_Os02g07060.1, LOC_Os03g01530.1, LOC_ Os03g

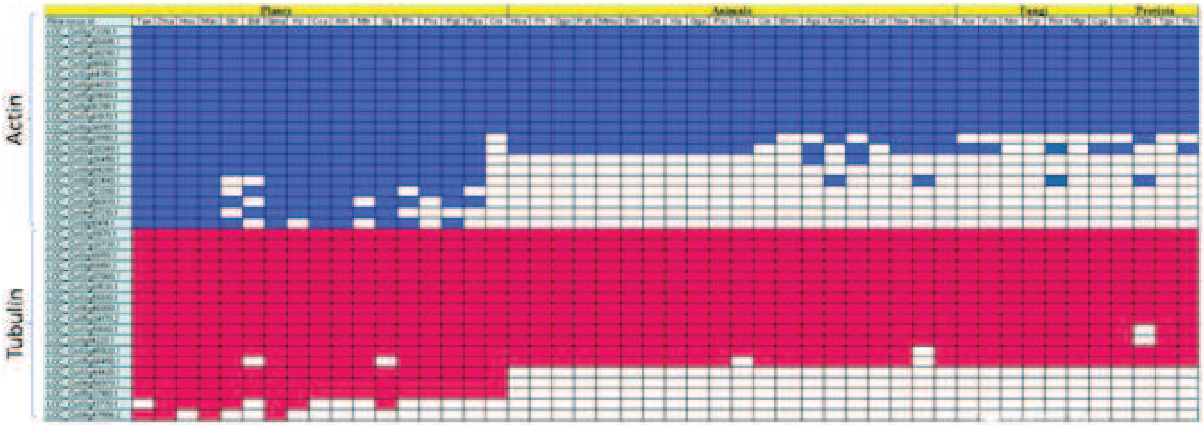

Fig. 1. **Distribution of rice actin and tubulin gene homologs in 49 diverse eukaryotic species. Colored and blank cells represent presence and absence of gene, respectively**

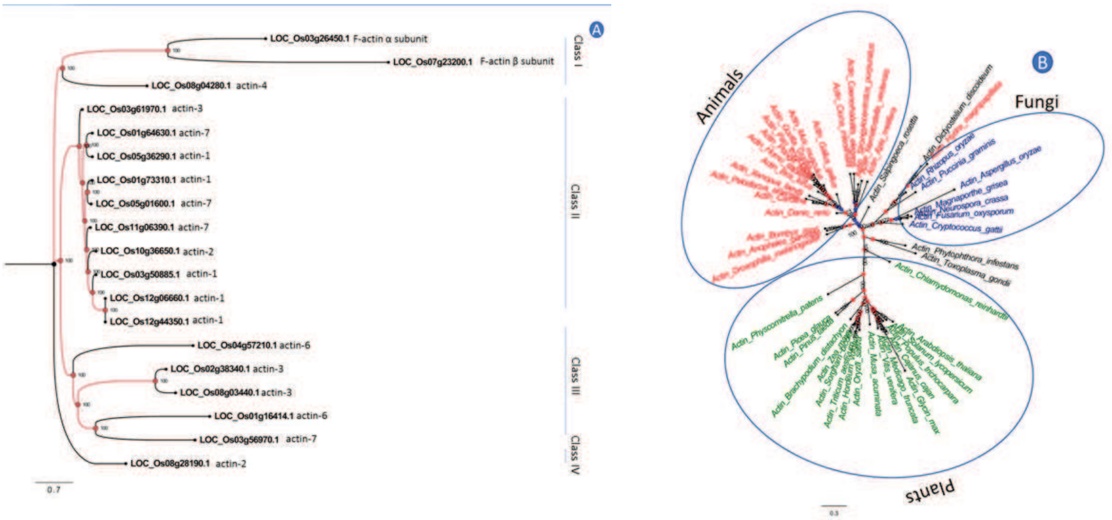

Fig. 2. **Maximum likelihood based phylogenetic tree of actin genes. (A) Minimum-evolution tree of 19 actin genes of rice obtained using RAxML. Four major clusters are labeled I-IV, all nodes are supported with bootstrap value of 100. Clustre II genes were conserved in 49 eukaryotic species, (B) Species tree of developed using ten conserved concatenated rice actin gene homologs in 49 eukaryote species. Species are color coded with plants, animals, fungi and Protista shown in green, red, blue and black, respectively**

11970.1, LOC_Os03g13770.1, LOC_Os03g 44420.1, LOC_Os03g45920.1, LOC_O s03g51600.1, LOC_Os 03g56810.1, LOC_Os04g47906.2, LOC_Os04g 56970.1, LOC_Os05g06450.1, LOC_Os05g 34170.2, LOC_Os05g37160.1, LOC_Os06g 46000.1, LOC_Os 07g38730.1 and LOC_Os 11g14220.1). Among the 18 rice tubulin genes four, eight and two genes were belonging to α, β and γ category, respectively, while three genes were homologs of prokaryotic tubulin/FtsZ (filamentous temperature-sensitive protein Z), which structurally resemble tubulin. Apart from this one uncharacterized tubulin gene was also present in rice. Comparatively, β-tubulin genes were predominant over other categories of tubulin genes in rice. We did not observe any other category of tubulin genes, e.g., δ, ε, ζ and η in rice. Earlier these isovariant are reported in Protista and Chytridiomycetes class of fungi (Ivens et al. 1998; Dupuis-Williams et al. 2002; Zhao et al. 2014). Genome wide comparative analysis has shown the presence of rice α, β and γ tubulins present in all eukaryote species. Among 18 plant species, three namely, maize, banana and soybean possessed all the 18 tubulin genes, wheat, barley, broomcorn and grape have 17 genes each, while nine species namely, pigeon pea, *Arabidopsis*, barrelclover, tomato, black cottonwood, southern yellow pine, white spruce, bryophyte and green algae have 16 genes each (Fig. 1). Further, pairwise analysis showed 11-13 tubulin genes were present in 20 animal species and four protista species while 13 genes each were present in

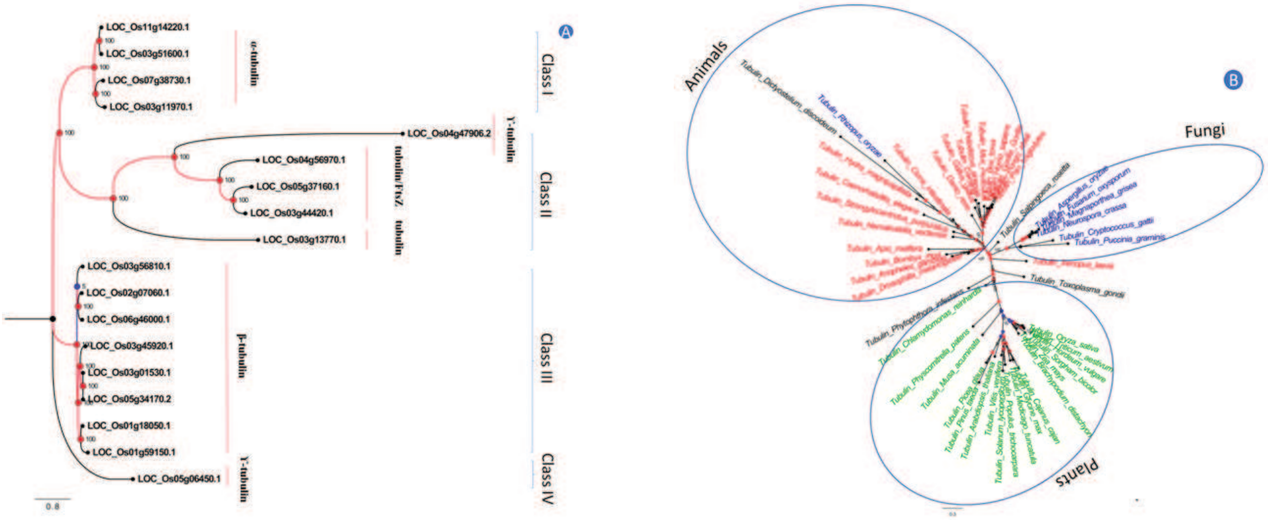

Fig. 3. Maximum likelihood based phylogenetic tree of tubulin genes. (A) Tubulin gene tree. The developed tree was based on 18 rice homologs of tubulin gene sequence, clustered into four groups i.e. Class I - IV. The red dot at each node showed the 100 bootstrap value. The scale bar represents 0.8 nucleotide changes per position, (B) Species tree of tubulin gene was based on nine conserved concatenated rice tubulin gene homologs in 49 different species. The phylogenetic tree showed the 49 species were clustered into plant, animal and fungi group while Protista species was distributed across the three major taxa like plants, animals and fungi. The bootstrap value of each node was in between 97-100. The scale bar of species tree represents 0.3 nucleotide changes per position

Table 2. Rice actin genes conserved in 49 diverse eukaryotic species. Gene ontology based gene annotation showed actin genes involved in the ATP binding, structural constituent of cytoskeleton

S.No.	*Oryza sativa* locus Id.	Unigene-length (bp)	Annotation
1.	LOC_Os05g01600.1	1134	actin-7(ATP-binding)
2.	LOC_Os05g36290.1	1131	actin-1
3.	LOC_Os01g73310.1	1134	actin-1 (ATP-binding)
4.	LOC_Os03g61970.1	1134	actin-3 (ATP-binding)
5.	LOC_Os03g50885.1	1134	actin-1 (ATP binding)
6.	LOC_Os11g06390.1	1068	actin-7 (ATP binding)
7.	LOC_Os12g44350.1	1136	actin-1 (ATP binding)
8.	LOC_Os12g06660.1	1078	actin-1
9.	LOC_Os10g36650.1	1428	actin-2 (ATP binding)
10.	LOC_Os01g64630.1	843	actin-7 (ATP binding)

seven fungal species. Most of the rice β and β tubulins isovariants were conseved among all the plants, animals, fungi and Protista species except two α (LOC_Os03g 51600.1, LOC_Os11g14220.1) and one β tubulins (LOC_Os03g45920.1) which were not conserved in *D. discoideum* and *H. magnipapillata* species, respectively (Fig. 1). Similarly, out of two γ-tubulin, one of them with rice locus id LOC_Os05g 06450.1 was conserved across the eukaryotes except from *B. distachyon, S. lycopersicum, A. caroliensis* and *H. magnipapillata*. Another rice γ-tubulin with locus id LOC_Os04g47906.2 was conserved in only four different species namely, *T. aestivum, Z. mays, M. acuminate* and *G. max.* The three rice homologs of tubulin/FtsZ gene were present in selected eighteen plant species only. Earlier genome-wide study have shown the distribution of α, β and γ-tubulins in different plant, animal and fungi species for example, flax (*Linum usitatissimum*) genome contains six α, thirteen β and two γ-tubulin genes (Pydiura et al. 2018). Similarly, *Arabidopsis thaliana* contains six α, nine β and two γ-tubulin genes (Kopczak et al. 1992; Snustad et al. 1992; Liu et al. 1994; Pastuglia et al. 2006) while *Populus* has eight α-tubulin and twenty β-tubulin genes (Oakley et al. 2007). There were nine rice tubulin genes conserved in all 49 species, among which two were for α tubulins and seven for β-tubulins (Table 3). The sequence of tubulin genes are highly conserved as reported earlier in different monocot and dicot plant species, e.g. rice (Jeon et al. 2000; Yoshikawa et al. 2003), *Populus* (Oakley et al. 2007) and *Arabidopsis* (Ludwig et al. 1987; Snustad et al. 1992).

Table 3. Rice tubulin genes conserved in 49 diverse eukaryotic species. Gene ontology based gene annotation showed tubulin genes involved in GTPase activity, GTP binding and structural constituent of cytoskeleton

S.No.	*Oryza sativa*locus Id.	Unigene-length (bp)	Annotation
1.	LOC_Os01g59150.1	1344	tubulin β-chain
2.	LOC_Os01g18050.1	1345	tubulin β-5 chain
3.	LOC_Os02g07060.1	1344	tubulin β-5 chain
4.	LOC_Os07g38730.1	1353	tubulin α-6 chain
5.	LOC_Os03g56810.1	1335	tubulin β-7 chain
6.	LOC_Os03g11970.1	1350	tubulin α-2 chain
7.	LOC_Os05g34170.2	1335	tubulin β-5 chain
8.	LOC_Os03g01530.1	1319	tubulin β-5 chain
9.	LOC_Os06g46000.1	1341	tubulin β-chain

Phylogeny of actin and tubulin genes in rice and across eukaryotic species

To understand the evolutionary relationship of actin and tubulin genes in rice and across the eukaryotic taxa of life, we developed unrooted maximum likelihood (ML) phylogenetic trees. The ML tree of 19 rice actin genes was a robust consensus tree with bootstrap value of 100 at each node (Fig. 2A). The nineteen rice actin genes were grouped into four major classes named I-IV. Class I included a pair of filamentous actin (F-actin) genes grouped together along with another actin gene LOC_Os08g04280.1 coding for isoform actin-4. Class II was the largest group containing ten actin genes located on six different rice chromosomes coding for actin isoforms 1, 3, 5 and 7. Class II is the most conserved group of actin genes present in all 49 eukaryotic species (Fig. 1). Class III consisted of five actin genes located on five different chromosomes and coding for isoforms 3, 6 and 7. Class IV consisted of a single actin gene LOC_Os08g28190.1 that was distantly related to the other actin genes.

A phylogenetic tree of 49 eukaryote species based on the 10 conserved actin genes was constructed (Fig. 2B). The tree grouped 49 species into three major clades, representing plants, animals and fungi while four Protista species were interspersed among the three groups. Plant lineages of actin genes were further subdivided into angiosperm, gymnosperm, bryophyta and green alga. *C. reinhardtii* was the sister clade of the plant species and very close to the midpoint of the tree showing its ancient origin (Merchant et al. 2007). Among the 20 animal species 19 were clustered together while *Hydra* was grouped with amoeba and both were nested in the fungal clade close to *Puccinia* and *Rhizopus*. Further, the major 19 animal clade containing 19 species was subdivided in to three different sub-cluster, representing insects, vertebrates and other invertebrate's animals belong to Anthozoa (*N. vectensis*), Echinoidea (*S. purpuratus*), Chromadorea (*C. elegans*) and Ascidiacea (*C. intestinalis*) class. The two Protista species were located near the mid point of the tree indicating their primitive evolutionary position as compared to the higher eukaryotes (http://bioweb.uwlax.edu/bio203/s2008/parks_chri/Classification.htm).

The ML phylogenetic of 18 rice tubulin genes grouped into four major classes named I-IV (Fig. 3A). Class I consisted of four genes coding for γ-tubulin, class II consisted of five genes coding for one α-tubulin, three tubulin/FtsZ and one uncharacterized tubulins; Class III consisted of eight genes coding for β-tubulin. This was also the largest class conserved in all 49 eukaryotic species. Class IV consisted of single tubulin gene (LOC_Os03g13770.1) coding for γ-tubulin distantly related to the other tubulin genes. The developed tree resolved all the nodes and sub nodes with strong bootstrap values (Fig. 3A).

To further analyse the evolutionary relationship of tubulin gene across the species we developed a species phylogenetic tree by using concatenated nine tubulin gene sequences which were conserved across the taxa. The maximum likelihood-based tree showed the 49 species were grouped into three major clades like plant, animal, fungi, however, among four protista species (*Salpingoeca rosetta, Dictyostelium discoideum, Toxoplasma gondii, Phytophthora infestans*) two of them namely, *Dictyostelium discoideum and Salpingoeca rosetta* were grouped with animal and in between the animal and fungi clade respectively (Fig. 3b). Like actin, tubulin genes containing 18 plant species grouped into monocot and dicot clades separately but some ambiguous result was also observed, for example grouping of dicot species *Arabidopsis* with gymnosperm, banana was grouped outside from the angiosperm and gymnosperm plant species. All 20 animal species grouped together in three different subclades except *Xenopus laevis* (Fig. 3b). Out of seven fungi species, six were clustered together while one of them like *Rhizopus oryzae* was exceptionally cluster with amoeba in animal clade (Fig. 3b). The phylogenetic tree results indicate the cluster based functional diversification and evolution of tubulin genes in rice and other distantly related species.

Authors' contribution

Conceptualization of research (NKS, PKJ); Designing of the experiments (NKS, AS, PKJ); Contribution of experimental materials (PKJ, AS); Execution of field/ lab experiments and data collection (PKJ); Analysis of data and interpretation (PKJ, NKS); Preparation of manuscript (PKJ, NKS).

Declaration

The authors declare no conflict of interest.

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Reference

Abramov O. and Mojzsis S. J. 2009. Microbial habitability of the Hadean Earth during the late heavy bombardment. Nature, **459**: 419-422.

Altschul S. F., Gish W., Miller W., Myers E. W., and Lipman D. J. 1990. Basic local alignment search tool. J. Mol. Biol., **215**: 403-410.

Assis R., and Bachtrog D.2013. Neofunctionalization of young duplicate genes in Drosophila. Proc. Natl. Acad. Sci. U S A., **110**: 17409-17414.

Ausmees N., Kuhn J. R. and Jacobs-Wagner C. 2003. The bacterial cytoskeleton: an intermediate filament-like function in cell shape. Cell, **115**: 705-713.

Bell E. A., Boehnke P., Harrison T. M. and Mao W. L. 2015. Potentially biogenic carbon preserved in a 4.1 billion-year-old zircon. Proc. Natl. Acad. Sci. USA., **112**: 14518-14521.

Bhattacharya D., Aubry J., Twait E. C. and Jurk S. 2000. Actin gene duplication and the evolution of morphological complexity in land plants. J. Phycol. **36**: 813-820.

Bramhill D. 1997. Bacterial cell division. Annu. Rev. Cell Dev. Biol., **13**: 395-424.

Dawson S.C. and Paredez A.R. 2013. Alternative cytoskeletal landscapes: cytoskeletal novelty and evolution in basal excavate protists. Curr. Opin. Cell Biol., **25**: 134-141.

De Marais D. J. 2000. Evolution. When did photosynthesis emerge on Earth?. Science, **289**: 1703-1705.

Dupuis-Williams P., Fleury-Aubusson A., de Loubresse N. G., Geoffroy H., Vayssie L., Galvani A., Espigat A. and Rossier J. 2002.Functional role of epsilon-tubulin in the assembly of the centriolar microtubule scaffold. J. Cell Biol., **158**: 1183-1193.

Ettema T.J., Lindas A.C. and Bernander R. 2011. An actin-based cytoskeleton in archaea. Mol. Microbiol., **80**: 1052-1061.

Farquhar J., Bao H. and Thiemens M. 2000. Atmospheric influence of Earth's earliest sulfur cycle. Science, **289**: 756-759.

Feng Y., Zhang Y., Ying C., Wang D. and Du C. 2015. Nanopore-based fourth-generation DNA sequencing technology. Genomics Proteomics Bioinformatics, **13**:4-16.

Gilbert W., de Souza S. J.and Long M. 1997. Origin of genes. Proc. Natl. Acad. Sci. USA., **94**: 7698-7703.

Hughes T. E., Langdale J. A. and Kelly S. 2014.The impact of widespread regulatory neofunctionalization on homeolog gene evolution following whole-genome duplication in maize. Genome Res., **24**: 1348-1355.

Ivens A. C., Lewis S. M., Bagherzadeh A., Zhang L., Chan H. M. and Smith D. F. 1998. A physical map of the Leishmania major Friedlin genome. Genome Res.,**8**: 135-145.

Jeon J. S., Lee S., Jung K. H., Jun S. H., Kim C. and An G. 2000. Tissue-preferential expression of a rice alpha-tubulin gene, OsTubA1, mediated by the first intron, Plant Physiol., **123**: 1005-1014.

Kaessmann H. 2010. Origins, evolution, and phenotypic impact of new genes. Genome Res., **20**: 1313-1326.

Katoh K., Misawa K., Kuma K. and Miyata T. 2002. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res., **30**: 3059-3066.

Kopczak S.D., Haas N.A., Hussey P.J., Silflow C.D. and Snustad D.P. 1992. The small genome of Arabidopsis contains at least six expressed alpha-tubulin genes. Plant Cell, **4**: 539-547.

Le Gall L., Lelong C., Rusig A. M., Favrel P. 2005. Characterization of an actin gene family in *Palmaria palmata* and *Porphyra purpurea* (Rhodophyta). Cah. Biol. Mar., **46**: 311-322.

Liu B., Joshi H.C., Wilson T.J., Silflow C.D., Palevitz B.A. and Snustad D.P. 1994. Gamma-Tubulin in Arabidopsis: gene sequence, immunoblot, and immune fluorescence studies. Plant Cell, **62**: 303-314.

Ludwig S. R., Oppenheimer D. G., Silflow C. D. and Snustad D. P. 1987. Characterization of the alpha-tubulin gene family of Arabidopsis thaliana. Proc. Natl. Acad. Sci. USA., **84**: 5833-5837.

Mahmudi O., Sennblad B., Arvestad L., Nowick K.and Lagergren J.2015. Gene-pseudogene evolution: a probabilistic approach. BMC Genomics, **16** (Suppl. 10): S12.

Merchant S.S., Prochnik S.E., Vallon O., Harris E.H., Karpowicz S.J., Witman G.B., Terry A., Salamov A., Fritz-Laylin L.K., Maréchal-Drouard L., et al. 2007. The Chlamydomonas genome reveals the evolution of key animal and plant functions. Science, **318**: 245-250.

NickP. 1998. Signaling to the microtubular cytoskeleton in plants. Int. Rev. Cytol.,**184**: 33-80.

Nogales E., Wolf S. G. and Downing K. H. 1998. Structure of the alpha beta tubulin dimer by electron crystallography. Nature, **391**: 199-203.

Oakley B. R. 1992. Gamma-tubulin: the microtubule organizer? Trends Cell Biol., **2**: 1-5.

Oakley R. V., Wang Y. S., Ramakrishna W., Harding S. A. and Tsai C. J. 2007. Differential expansion and expression of alpha- and beta-tubulin gene families in *Populus*. Plant Physiol.,**145**: 961-973.

O'Toole E., Greenan G., Lange K. I., Srayko M. and Müller-Reichert T. 2012. The role of β-tubulin in centrosomal microtubule organization. PLoS One, **7**: e29795.

Pastuglia M., Azimzadeh J., Goussot M., Camilleri C., Belcram K., Evrard J. L., Schmit A. C., Guerche P. and Bouchez D. 2006. Gamma-tubulin is essential for microtubule organization and development in *Arabidopsis*. Plant Cell, **18**: 1412-25.

Pydiura N., Pirko Y., Galinousky D., Postovoitova A., Yemets A., Kilchevsky A. and Blume Y. 2018. Genome-wide identification, phylogenetic classification, and exon-intron structure characterization of the tubulin and actin genes in flax (*Linum usitatissimum*), Cell Biol. Int., **9999**: 1-10.

Reuter J. A., Spacek D. V. and Snyder M. P. 2015. High-throughput sequencing technologies. Mol. Cell., **58**: 586-597.

Rhoads A. and Au K. F. 2015. PacBio Sequencing and Its Applications. Genomics Proteomics Bioinformatics, **13**: 278-289.

Rothberg J. M. and Leamon J. H. 2008. The development and impact of 454 sequencing. Nat. Biotechnol., **26**: 1117-1124.

Sandve S. R., Rohlfs R. V. and Hvidsten T. R. 2018. Subfunctionalization versus neofunctionalization after whole-genome duplication. Nat. Genet., **50**: 908-909.

Shah D. M., Hightower R. C. and Meagher R. B. 1983. Genes encoding actin in higher plants: intron positions are highly conserved but the coding sequences are not. J. Mol. Appl. Genet., **2**: 111-126.

Singh N. K., Raghuvanshi S., Srivastava, S. K., Gaur A., Pal A. K., Dalal V., Singh A., Ghazi I. A., Bhargav A., Yadav M., Dixit A., Batra K., Gaikwad K., Sharma T. R., Mohanty A., Bharti A. K., Kapur A., Gupta V., Kumar D., Vij S., Vydianathan R., Khurana P., Sharma S., McCombie W. R., Messing J., Wing R., Sasaki T., Khurana P., Mohapatra T., Khurana J. P. and Tyagi A. K. 2004. Sequence analysis of the long arm of rice chromosome 11 for rice-wheat synteny. Funct. Integr. Genomics, **4**: 102-117.

Slajcherova K., Fiserova J., Fischer L. and Schwarzerova K. 2012. Multiple actin isotypes in plants: diverse genes for diverse roles? Front. Plant Sci., **3**: 1-8.

Snustad D. P., Haas N. A., Kopczak S. D. and Silflow C. D.1992. The small genome of Arabidopsis contains at least nine expressed beta-tubulin genes. Plant Cell, **4**: 549-556.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, **30**: 1312-1313.

Villemur R., Joyce C. M., Haas N. A., Goddard R. H., Kopczak S. D., Hussey P. J., Snustad D. P. and Silflow C. D. 1992. Alpha-tubulin gene family of maize (*Zea mays* L.). Evidence for two ancient alpha-tubulin genes in plants. J. Mol. Biol., **227**: 81-96.

Yoshikawa M., Yang G., Kawaguchi K. and Komatsu S. 2003. Expression analyses of beta-tubulin isotype genes in rice. Plant Cell Physiol., **44**: 1202-1207.

Zhao Z., Liu H., Luo Y., Zhou S., An L., Wang C., Jin Q., Zhou M. and Xu J. R. 2014. Molecular evolution and functional divergence of tubulin superfamily in the fungal tree of life. Sci. Rep., **4**: 6746.

Zwart M. P., Willemsen A., Daros J. A. and Elena S. F. 2014. Experimental evolution of pseudogenization and gene loss in a plant RNA virus. Mol. Biol. Evol., **31**: 121-134.