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# Analysis, Cloning and Prediction of CIS Acting Regulatory Elements of *Arabidopsis thaliana* Tubulin Gene 4

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**ABSTRACT:** *Tubulin is one of the most abundant member of small family of globular proteins. The most common members of the tubulin family are the proteins that make up microtubules, α-tubulin and β-tubulin. In this study, we report the identification, analysis and characterization of tubulin geneselected from beta family of Arabidopsis thaliana. Out of various genes, the beta-tubulin gene 4 with 2936bp size was selected. The selected gene was analyzed by using various bioinformatics tools. Moreover, various light responsive cis regulatory elements were found in promoter region of this gene. Many of the genes showed its evolution through gene duplication events.Sequence determination of the Arabidopsis gene revealed a pattern of highly conserved regions. The DNA was isolated from young and fresh leaves of Arabidopsis thaliana plants. Moreover, it was searched in NCBI-Genebank repository for identification and analysis.Further, it was cloned in 5.8 kb expression vector (pGR1) for studying the expression of a reporter gene (GUS). The promoters isolated and cloned by these studies can be used for understanding transcriptional regulation and gene expression studies.*

***Keywords:*** *Gene duplication, Genbank, Arabidopsis, pGR1, GUS*

## INTRODUCTION

Use of model plants for exploring new insights in plant genomics has revealed various outstanding facts and discoveries. The flowering plant, "*Arabidopsis thaliana*" is a most common flowering plant used as a model organism in agriculture biotechnology.Many gene duplications are found in evolution of *Arabidopsis.* The size of genome mainly depends on number of genes. However, 11,000 families were found enriched with 25,498 genes encoding proteins. Moreover, Arabidopsis has many new protein families and this was the very first plant genome sequenced.It provides all details about the genetic processes and also about identification of various genes for crop improvement.

For genome wide studies, use of this model plant has remarkable uses and advantages.Its important characteristics are not

only limited to:less generation time, smaller size, increased number of offspring and importantly a small nuclear genome. The comparative studies of *Arabidopsis* and eukaryotic genome showed differential characteristics. The more significant ratio of genes were found to be involved in regulatory pathways. Mainly, the internal metabolism, gene regulation and its pathways are all dependent on functioning of these genes (Borg et al., 2010) .

Microtubules are mainly involved in structural build up and all cellular processes. They are also considered as structural elements of cilia, flagella and cytoskeleton. The microtubule subunits assemble into specialized microtubules according to their diversity (Mayer, 1999). Studies of tubulin genes from different species indicate that the tubulin structure is highly conserved among species. Only little variation is found. This variation is found due to both posttranslational modification of the tubulin heterodimer and primary-structure differencesencoded in multiple tubulin genes (Fulton et al., 1976; Flavin and Murofushi, 1981; L'Hernault and Rosenbaum, 1985) .

The microtubules, which consists of heterodimers of α-tubulin and β-tubulin proteins, are essentially required for morphogenesis of plant cells (Cleveland and Sullivan, 1985). Most of the amino acid sequences of these two subunits resembles about 88% with animals, plants, fungi and protest (Liaud et al., 1992; Parker and Detrich, 1998; Paredez et al., 2006). Arabidopsis thaliana has six tubulin alpha genes that encode four distinct proteins (Kopczak et al., 1992; Rohel, 1998). Whereas at least nine tubulin genes encoding nine different proteins (Snustad et al., 1992). The alpha and beta tubulin proteins show strong sequence conservation with other tubulin genes of animal, plant and protest with >88% amino acid sequence similarity (Dutcher, 2001). The functional importance of microtubules with their dynamic properties is useful to study the complex developmental pathways (Dehmelt and Halpain, 2004; Guzik and Goldstein, 2004).

Such diversity and variation has led to understand the importance of tubulin as a major building block of microtubules. These microtubules are made up of heterodimer of some related alpha and beta subunits (Cleveland and Sullivan, 1985). According to some previous studies, it was suggested that, more than one isotype of each tubulin exists in a cell (Drukman and Kavallaris, 2002). Further, the diversity of microtubules is mainly dependent on 10 residues of the alpha and the last 18 of the beta tubulin C-terminal tails (Duan and Gorovsky, 2002). However, the betatubulin is one of thegenes, responsible for representing the eukaryotic hostcell (Trivinos-Lagos et al., 1993; Liaud et al., 1995). Similarly, some of the fungi, protists and animals have showed existence of single beta-tubulin (MacKay and Gallant, 2002).

The study was focused on in silico identification, analysis through various bioinformatics tools and cloning of tubulin gene 4 (BT-gene 4) of class beta (β) into a modified pJITT166 plant expression vector pGR1 having *GUS* with intron under control of 2X 35S promoter. This vector occupy a size of 5.8 kb followed by CaMV terminator.

## MATERIALS AND METHODS

### Retrieval of Nucleotide Sequence through Bioinformatics Approach

The high expression of tubulin gene in respective dicots,has made it a gene of importance. The use of computational tools under umbrella of synthetic biology approach has made this easy for scientists and researchers to study different expression levels.

The nucleotide sequence of tubulin gene 4 was extracted from NCBI and screened to searchsome regulatory regions.Screening of cis-regulatory elements within tubulin gene and its promoter sequence was performed by Plant CARE software (http://bioinformatics.psb. ugent.be/webtools/ plantcare /html/) which showed presence of various cis regulatory elements, which are involved in several functions. Most of these were acting as a light responsive element.

### Isolation of Tubulin Gene

To isolate the genomic DNA of BT- gene 4, the young leaves of *Arabidopsis thaliana* was selected. The CTAB method was used for isolation of genomic DNA. CTAB facilitates the easy separation of polysaccharides during purification. The isolated BT-gene 4 was amplified by applying conventional PCR.

### Cloning of Beta Tubulin Gene 4 in Plant Expression Vector

After successful PCR reaction, the PCR product was purified, ligated to the general expression vector pGR1 and was further transformed in freshly prepared E.coli (TOP10) cells. The clones were verified by PCR using universal primers. Further, verification was done by sequencing after transformation.

**Table 1: PCRprofile for amplification of tubulin Gene fragment**

| | | |
|---|---|---|
| 1)Initial denaturing temperature | 94$^{o}$C | 4 minutes |
| One cycle | | |
| 2)Denaturing temperature | 94$^{o}$C | 1 minute |
| 3)Annealing temperature | 46$^{o}$C-50 $^{o}$C | 1 minute |
| 4)Extension temperature | 72$^{o}$C | 2 minutes |
| 40 cycles from step 2 to step 4 | | |
| 5)Final extension temperature | 72$^{o}$C | 10 minutes |
| One cycle | | |

# RESULTS

## Sequence Retrieval

The selected tubulin gene of beta family with 2936bp was obtained from NCBI with accession number M21415. The nucleotide sequence was translated to obtain the protein sequence by using bioinformatics software. However,the gene sequence was full and enriched with different types of dispersed motifs. Out of which, the most frequent motif was observed to be light responsive element, with some special features, while others like stress, infection and hormone response motifs were also found scattered throughout the entire promoter region.

**>gi|166639|gb|M21415.1|ATHBT4A**
**A.thaliana beta-tubulin-4 gene, complete cds**

**Fig. 1. Shows the nucleotide sequence for beta tubulin gene 4 from Arabidopsis thalian**

## Translated Sequence

The nucleotide sequence of tubulin gene 4 was translated and explored to obtain the protein model through tair data repository. Tair is a database of genetic and molecular biology data for model plants *Arabidopsis thaliana*. Complete genome and gene structure information can be obtained for all microtubule assembly genes.The assigned identification gene locus for tubulin gene 4 is At5G44340.1.It plays an important function kin GTP binding, GTPase activity and binding of several proteins. The results are shown in fig 2 below:

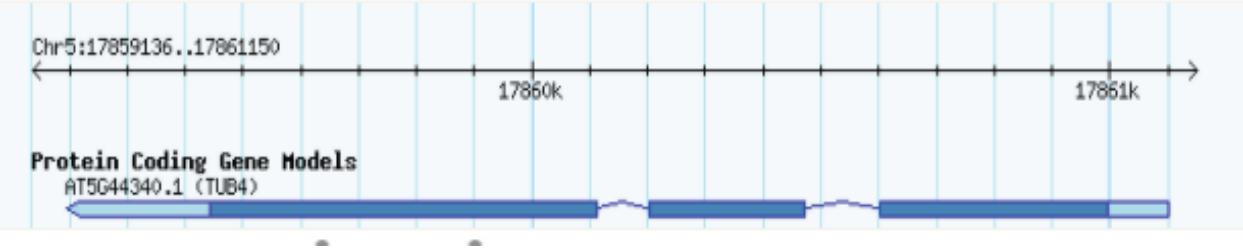


**Fig. 2. Shows the protein coding gene model for selected beta tubulin gene 4.**

## Analysis of Cis regulatory elements

The genome of Arabidopsis thaliana comprise of various conserved regulatory regions. These motif have specific function. The promoter sequence of tubulin gene was searched for prediction of cis acting regulatory regions. The 2936 bp sequence showed different conserved motif with varying functions. The results are represented in figure 3 and table 2 below:

```
>beta-tubulin-4 gene 2936nt your sequence is >1500nt. For cpu reasons it was truncated to 1500nt from the 3'end
+ GTATGGTTTT TGTTAGTTTG GATTTAGGTT TCCAAGTGTG TCATTCATTG GGAGGAGGAA CTGGATCTGG
- CATACCAAAA ACAATCAAAC CTAAATCCAA AGGTTCACAC AGTAAGTAAC CCTCCTCCTT GACCTAGACC

+ AATGGGAACT CTATTGATTT CTAAGATAAG AGAAGAGTAT CCAGATCGTA TGATGATGAC TTTCTCAGTG
- TTACCCTTGA GATAACTAAA GATTCTATTC TCTTCTCATA GGTCTAGCAT ACTACTACTG AAAGAGTCAC

+ TTTCCTTCTC CTAAGGTCTC TGACACTGTT GTTGAGCCAT ACAATGCAAC TCTCTCTGTG CATCAGCTTG
- AAAGGAAGAG GATTCCAGAG ACTGTGACAA CAACTCGGTA TGTTACGTTG AGAGAGACAC GTAGTCGAAC

+ TCGAAAACGC TGACGAGTGT ATGGTTTTGG ACAATGAGGC TCTCTACGAT ATCTGTTTCC GTACCCTCAA
- AGCTTTTGCG ACTGCTCACA TACCAAAACC TGTTACTCCG AGAGATGCTA TAGACAAAGG CATGGGAGTT

+ GCTCGCTAAT CCTACCTGTG AGTACATTTC TCCATTTGCA GCTTGTGTAT TCATGTAGTG TGATTGAGTC
- CGAGCGATTA GGATGGACAC TCATGTAAAG AGGTAAACGT CGAACACATA AGTACATCAC ACTAACTCAG

+ CTGACTTGTT TTTGTGTATT TTGTGTTTTC ATTAGTTGGT GATCTTAACC ATCTCATCTC TGCTACAATG
- GACTGAACAA AAACACATAA AACACAAAAG TAATCAACCA CTAGAATTGG TAGAGTAGAG ACGATGTTAC

+ AGTGGTGTCA CTTGCTGTCT TCGTTTCCCT GGCCAGCTTA ACTCTGACCT TAGGAAACTC GCTGTGAACC
- TCACCACAGT GAACGACAGA AGCAAAGGGA CCGGTCGAAT TGAGACTGGA ATCCTTTGAG CGACACTTGG
```

**Fig. 3. Shows the highlighted motifs in beta tubulin gene 4.**

**Table 2: Shows the cis regulatory elements with functions and location.**

| Name of Motif | Organism | Position | Sequence | Function |
|---|---|---|---|---|
| 3-AF1 binding site | Solanum tuberosum | 1148 | AAGAGATATTT | Light responsive element |
| 5UTR Py-rich stretch | Lycopersicon eesculentum | 1112 | TTTCTTCTCT | Cis-acting regulatory element conferring high transcription |
| AAGAA-motif | Avena sativa | 1116 | GAAGAA | Not found |
| ARE | Zea mays | 4 | TGGTTT | Cis-acting regulatory element conferring high transcription |

## Cloning in TA vector and pGR1 vector

The beta tubulin gene 4 was first cloned in TA vector than in (pGR1) expression vectorand confirmed via PCR.The 2936bp band was obtained after successful PCR reaction.

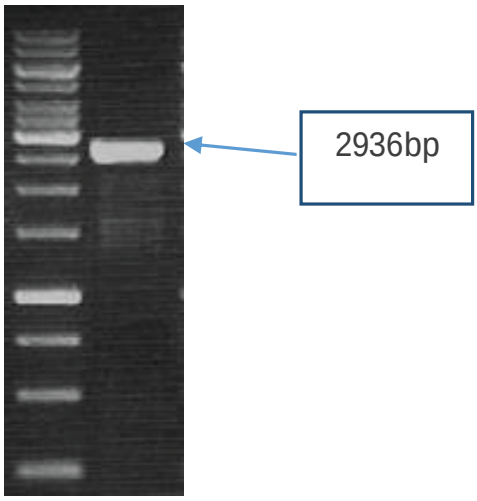


**Fig. 4. Shows clone Confirmation in (pGR1) by PCR. a). M: 1Kb DNA ladder, b) Lane 1: PCR amplification of Beta tubulin gene 4.**

## DISCUSSION

The genome of *Arabidopsis thaliana* is used as a significant model plant for identifying genes. However, out of the 125-megabase genome, the total sequenced region cover 115.4 megabases. The evolution of Arabidopsis involved a number of different whole-genome duplication events.The complete genome contains25,498 genes encoding several proteins from 11,000 families.Multiple new protein families were identified in this model plant, but identification of some novel protein families is still lacking.

Microtubules are necessary for many cellular activities and classified into various subfamilies of tubulins. However, the evolutionary distinct pathways and molecular mechanisms of several tubulins are still not clear. Tubulins are considered as one of the larger component of microtubules that are mainly involved in many cellular processes.

They comprise of α, β, γ, δ, and ε families (Oakley, 2000; McKean and Vaughan, 2001). However, α, β, and γ tubulins are universal and present mostly in all the eukaryotic organisms. Both α and β tubulins gathers in a different orientation and pair up in a head-to-tail heterodimer to form the basic building block of the microtubule. Similarly, the γ-tubulins plays a vital role in the initiation of microtubule assembly (Dutcher, 2003).

At least 15 multiple tubulin α and β genes have found in animals and only one γ tubulin genes. At least 15 α tubulin genes and 21 β tubulin genes but only 3 γ-tubulin genes were reported in human genome (Anthony and Hussey, 1998). Similarly, in maize, the plants do not regenerate if tubulin gene has showed overexpression after transformation, when both of the alpha and beta subunits of tubulin gene were transformed, regeneration occurs (Anthony et al., 1999). In case of fungi, very less tubulin genes were reported. Many of them contains only one gene. However, only few of them have two alpha and two beta genes.

## CONCLUSION

Beta-tubulin, one of the cytoskeletal proteins, has been highly conserved throughout the evolution of eukaryotes. According to some of the previous studies, findings were depicted, with evidence of multiple tubulin genes which code for structurally different tubulins and its families. B-tubulin genes from various species have been identified and analyzed. The selected tubulin gene was found to be rich with various conserved regions. The cloning of this tubulin gene in TA vector was useful for further expression studies. The identified gene can be further cloned in some plant expression vector for getting transgenic *Arabidopsis* plants. This will lead towards success in having enhanced crop production with genetically engineered technologies.

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