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A cDNA Sequence Encoding Actin Gene in Moso Bamboo Shoot and its Phylogenetic Analysis

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Abstract: Actin is one of the most ubiquitous and conserved eukaryotic protein. All eukaryotic cells, from yeast to plants to animals, have an internal framework called the cytoskeleton. The plant actin cytoskeleton is central to many different sub-cellular processes and actin genes have been the focus of numerous scientific studies because of their involvement in basic cellular processes. In order to explain the structure and evolution of monocot actin gene family we have cloned and sequenced a 515 bp cDNA sequence from moso bamboo (*Phyllostachys edulis*) shoot. Phylogenetic analysis of the bamboo actin along with asparagus actin and those previously published in the database, indicates that monocots actin genes are highly conserved and actin multigene family underwent many gene duplication events early in the evolution of angiosperms.

Key words: Actin, bamboo shoot, cDNA sequence, phylogeny

INTRODUCTION

Higher plants contain families of actin encoding genes which are divergent and differentially expressed. They are involved in numerous cytoskeletal processes affecting development, including cell division plane determination, cell elongation and cell wall deposition. Progress in understanding the functions and evolution of plant actins has been hindered by the large size of the actin gene families[1]. Among plant cytoskeletal gene families in Arabidopsis, there are at least 10 actins, nine α-tubulins, six β-tubulins, six profilins and dozens of myosins. However, the expression of many of these gene family members overlaps considerably. In angiosperms, actin is encoded by a relatively large, diverse and dispersed multigene family comprising 8-40 genes[2]. As in most other eukaryotes, the multiple copies of actin genes in angiosperms are thought to allow a more diverse pattern of gene regulation, rather than direct the production of a large amount of actin[3]. Based on the shared intron positions and the many short amino acid sequences unique to angiosperm sequences, it is believed that all angiosperm actin sequences are derived from a single ancestral sequence. Relative to other nuclear genes, actin genes are highly conserved in both size and sequence[4]. However, relative to mammalian actins, angiosperm actin genes show a greater sequence divergence. It has been suggested that angiosperm actin are evolving at the same rate as those in fungi and mammals and that their relatively high sequence divergence is due to a more ancient origin.

Our previous report[5] focused on the sequencing and sequence data analysis of an asparagus actin gene in order to improve our understanding about structure of actin gene in asparagus. Here we report a cDNA sequence encoding actin gene in moso bamboo shoot. Phylogenetic analysis also has been used to gain some understanding of the pattern of actin gene evolution.

MATERIALS AND METHODS

RNA Isolation and PCR Amplification: Total RNA was extracted from moso bamboo shoots using Hot Borate method adapted from Wan and Wilkins[6]. The first strand cDNA was synthesized from 2 g of the total RNA by reverse transcriptase with Oligo-(dT) primer according to the instruction of SUPER SCRIPT™ Preamplification System for First Strand cDNA Synthesis (GIBCOBRL, Tokyo, Japan). PCR was performed in a total volume of 25 μl containing the first strand cDNA reaction products, 10xPCR Buffer, $MgCl_2$, dNTP, First Start Taq DNA Polymerase (Roche) and primers. The primers (5'- GARAARATGACNCARATHATG-3' as the upstream primer and 5'-TCNACRTCRCAYTTCATDAT-3' as the down stream primer) were designed and synthesized on the basis of amino acid domains (EKMTQIM and IMKCDVD respectively) conserved in various actin genes. The *Sal 1* and *Not1* restriction site sequences were

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also included at 5'-end of the sense and antisense primer, to facilitate cloning of PCR product. The PCR procedure started with 10 min at 95°C and was carried out 35 cycles of 30 s denaturation at 95°C, 30 s annealing at 50°C and 30 s extension at 72°C and 10 min at 72°C with ASTEC Program Temperature Control System PC-700. The PCR products were confirmed by agarose gel electrophoresis.

Cloning and sequencing of cDNA: Cloning and sequencing of cDNA was performed as in Bhowmik *et al.* (2003). The amplified cDNA was ligated to the plasmid pSPORT1 and cloned into *Escherichia coli* (DH-5α) *Not* 1-*Sal* 1–cut (BRL, Tokyo, Japan). Sequencing was performed by the cycle sequencing method using GATCR-Bio Cycle sequencing Kit and a DNA sequencer GATC 1500 Long-Run system (GATC Gmbh, Konstanz, Germany).

Sequence data analysis: Sequence analysis was performed using computer software GENETYX-MAC Ver.7. Homology searches with the Genbank and the EMBL databases were performed using the homology program in the software. The phylogenetic tree was also constructed with the UPGMA method in the software.

GenBank accession number: The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB 114677.

RESULTS

Isolation of actin gene: Clones of moso bamboo actin genes were obtained by PCR using a pair of primers designed around two evolutionary conserved actin gene regions that are rich in amino acids encoded by only one or two codons. The cDNA pBA-AC is a partial clone encoding actin in moso bamboo shoot. The sense primer hybridizes to the region from the first nucleotide of EKMTQIM and the antisense primer hybridizes to the IMKCDVD.

Actin gene sequence: The encoded mRNA of pBA-AC is 515 bp long which is shown in Fig. 1. It is highly homologus to actin genes of other plants in the database. The pBA-AC sequence is 75.5% identical to actin from rice (X15864), 81.0% identical to actin from banana (AB022041) and 75.7% to maize actin (U60507). Allowing for conservative amino acid substitutions, the similarities are 91.3, 94.7 and 94.7% for rice, banana and maize sequence, respectively.

Phylogeny of actin gene: The moso bamboo actin gene sequenced in this study was aligned with 13 existing actin sequences from maize, soybean, potato, tobacco, rice, pea, sorghum, tomato and banana. This alignment was used to generate phylogenetic tree. Nucleotide

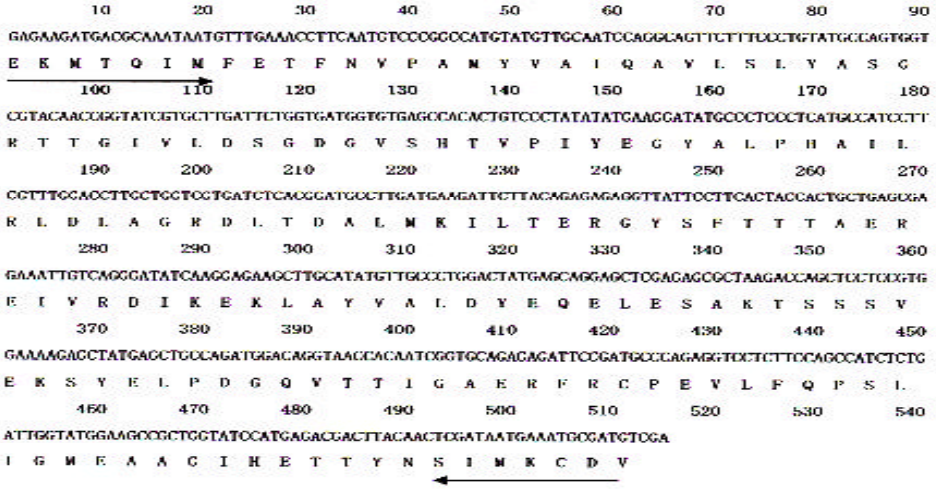

Fig. 1: Nucleotide sequence and deduced amino acid sequence of the cDNA clone corresponding to pBA-AC. The predicted amino acid sequence is given in single-letter code for each amino acid. The arrows indicate the positions of degenerated primers (sense, antisense) used for RT-PCR. Numbering refers to total nucleotide residues on each line

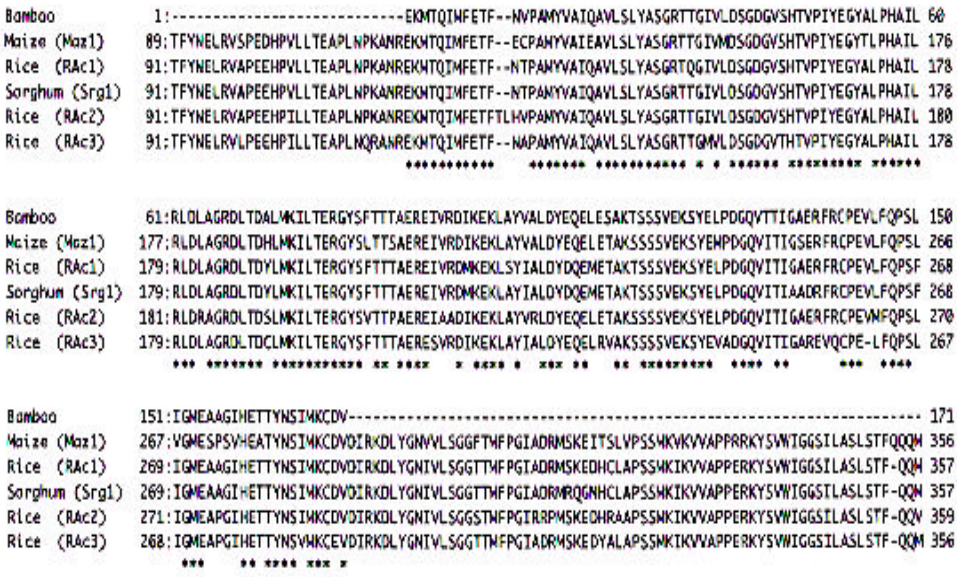

Fig. 2: Comparison of the deduced amino acid sequences from moso bamboo shoot (AB114677), maize (Maz1) (J01238), rice (RAc1)(X15865), sorghum (Srg1) (X79378), rice (RAc2) (X15864) and rice (RAc3) (X15862) by multi alignment. The amino acid residues are numbered at the beginning and end of the sequences on each line. Asterisks (*) denote the amino acid residues those are identical. Dashes in the amino acid sequences represent gaps introduced to maximize alignment of the polypeptides

Table 1: Percentage of nucleotide and deduced amino acid homology between actin from moso bamboo shoot and other plants in the databases

Plants	Nucleotide	Amino acid
Rice (RAc1) (X15865)	75.2	93.6
Rice (RAc2) (X15864)	75.5	91.3
Rice (RAc3) (X15862)	74.9	88.3
Maize(Maz1) (J01238)	73.4	88.9
Maize(Maz89)(U60508)	75.5	94.7
Maize(Maz95)(U60507)	75.7	94.7
Sorghum(Srg1)(X79378)	75.0	93.6
Banana (AB022041)	81.0	94.7
Tomato(Tom41)(U60480)	73.1	92.4
Tobacco (X63603)	76.2	93.6
Pea (X68649)	82.5	93.6
Potato(Pot46)(M29232)	72.8	89.6
Soybean (J01298)	71.0	84.2

Moso bamboo actin (AB114677) is calculated as 100%

sequences, rather than amino acid sequences, were used to construct tree because they had twice the number of variable. The order of sequence input had no effect on the topology of the tree. The topology in the tree suggests that there might be two main lineages of angiosperm actin genes, one group composed exclusively of dicot actin genes and a second group composed of both monocot and dicot actin genes. Bamboo actin falls into the second main lineage like asparagus actin (Table 1, Fig. 2).

DISCUSSION

Actin genes have been the focus of numerous scientific studies because of their involvement in basic cellular processes. The amino and carboxyl termini of plant actins are conserved. Based on this fact, we designed degenerate primers using the plant actin sequences already available in the database. Our moso bamboo actin was a partial clone and encoded 515 bp of mRNA. Although it was of similar length like asparagus actin but when comparison of the sequence was done it varied in homology with other monocot actin. Actin nucleotide sequences vary by as much as 21-25% both within and between species[7]. This suggests that the actin multigene family is equally old in all lineages. The fact that this level of variation is also observed between the *Volvox* actin gene and all the angiosperm actin genes suggests that variation in nucleotide sequence between some angiosperm actin genes is saturated. Actin sequences in monocots vary from 0-14% at the amino acid level. Within a species, the variation in amino acid sequences is 2-12% in maize.

The GC content of monocot actin genes used in this study varies with in a relatively narrow range. The average GC content of dicot actin genes is lower than that of monocot actin genes. Drouin and Moniz[7] suggested that monocot and dicot actin genes have different codon usages patterns and the difference in GC content between monocot and dicot actin genes is solely due to a GC bias at the third positions of codons.

The phylogenetic analysis of moso bamboo actin gene presented here showed that, despite the fact that the

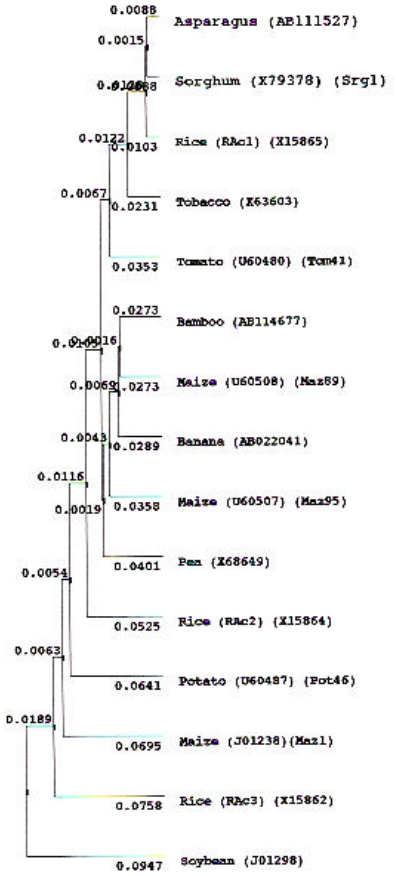

Fig. 3: Phylogenetic tree of the alignment of pBA-AC deduced amino acid sequence with other actin gene in the database. Protein sequences were aligned using UPGMA and a phylogenetic tree was constructed using GENETYX-MAC software. The GenBank accession numbers are shown in the parentheses

actin multigene family underwent several gene duplication events that preceded the monocot-dicot divergence, most of the monocot actin genes clustered together on the tree. This clustering of the monocot actin genes suggests that these sequences may have undergone concerted evolution early in the evolution of monocots. These observations of similarities between cytoskeletal actin genes from different monocots support the notion that monocots actins have evolved independently and they are functionally conserved (Fig. 3).

The work presented here has laid the groundwork for further characterization of the evolution and function of actin genes in moso bamboo shoot.

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