Cloning and Sequence Analysis of Actin Gene from *Guzmania*

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Abstract As a house keeping gene, Actin was often used as inner control in quantitative and semiquantitative PCR tests to determine relative expression amount of target genes. Based on EST monoclones of Actin gene obtained from *Guzmania* full length cDNA library, its full length cDNA sequence was obtained by primer walking sequencing. The gene, named for Goactin1 (GenBank accession No. HQ184438), consisted of 1 625 bp cDNA sequence, 1 131 bp ORF (open reading frame) which encoded a protein with 377 amino acids residues, and a putative protein which was 41.7 kD at estimated molecular weight, 5.31 at isoelectric point and had a ‘actin superfamily’ conservative domain. The secondary structure of the protein was composed of random coil, alpha helix, extend strand and beta turn by SPOMA analysis. Furthermore, its tertiary structure was also build based on A chain of 2BTF. Through phylogenetic tree analysis, Goactin1 was gathered to the same group with Actin protein in *Solanum tuberosum*, *Gossypium hirsutum*, *Nicotiana tabacum*, *Brachypodium sylvaticum*, and *Arabidopsis thaliana*.

Keywords *Guzmania* (Guzmania Ruiz&Pav); Actin; Clone

Background

Plant actin was a kind of constitutive expression protein existed broadly, and had high conservatism (Meagher et al., 1999). Since Yan and Shi put forward that actin existed in higher plant for the first time in 1963(Yan and Shi, 1963), at present, actin had been discovered in pollen, root hair, stem cambium layer, stem phloem, vagina cell, leaf epidermal cell, tendril and endocarp tissues of higher plant (Kost et al., 1999), and also was multi-gene family like as that of animal and fungi (Zhang and Liu, 2006; Hussey et al., 2006). Plant actin involved in cell microfilaments formation, whereas, microfilaments were key components of cytoskeleton and participated in important physiological activities such as intracellular cytoplasmic streaming, keep of configuration, movement, division, differentiated, transport of substance, signal transduction and polarity constructing etc. (Ma et al., 2010). As house-keeping gene, Actin can express relative stably to some extent in tissues and cells and had many advantages such as constitutive expression, multiplication easily. Hence, like as other house-keeping genes included GAPDH, 18SrRNA, TBP and β-2-microglobulin, Actin often was used as inner reference in quantitative or semiquantitative PCR reaction to revise expression amount of target genes (Zhu et al., 2006; Yang et al., 2007; Liu, 2003).

*Guzmania*, belonged to Bromeliaceae, was native to tropics and subtropics of American, and was superior quality potted flower plant in flower marketplace. At present, there was a lack of information in public about *Guzmania Actin* gene. Based on full length cDNA library constructed successfully in *Guzmania ostara* and plenty of EST sequences information were obtained by sequencing randomly on a large scale formerly, full length cDNA sequence of Actin gene was obtained, by Primer Walking sequencing to EST monoclonos belonged to Actin gene. Finally, there was a bioinformatics analysis in-depth to the gene. The research laid a foundation to construction of quantitative and semiquantitative PCR technique in ornamental Bromeliaceae.

1 Results and Analysis

1.1 Cloning of Goactin1 gene

Through primer walking sequencing to EST monoclon: [ppfca0_001242.z1.scf], which belonged to Actin gene,
a 1 625 bp full length cDNA sequence was obtained, and named for Goactin1 (GenBank accession number: HQ184438). It had a 1 131 bp ORF (open reading frame) which encoded 377 amino acids (Figure 1).

Figure 1 mRNA sequence and deduced amino acid sequence of Goactin1 gene
1.2 Blast analysis of full length cDNA and putative amino acid sequence

Nucleotide-nucleotide blast analysis showed the full length cDNA sequence of Goactin1 gene had a very high homologue to Actin gene of Populus trichocarpa, Vigna radiata, Betula luminifera, Oryza sativa, Zeamays, Arabidopsis thaliana, Nicotiana tabacum, Gossypium hirsutum, Pisum sativum, Ricinus communis, Pyrus communis, Aegiceras corniculatum, Prunus salicina, Phaseolus vulgaris, Phalaenopsis sp, Picea abies, Larix gmelinii, Mimosa pudica, Diospyros kaki, Plantago major, Linum usitatissimum and Solanum tuberosum. And the homologue of Populus trichocarpa (XM_002311131, GENE: 7467546), Vigna radiate (AF143208.1) and Betula luminifera (FJ410442.1) was the highest, which reached 84 percent. Moreover, by comparing the deduced amino acid sequences (BlastP) with the protein data bank, the result showed that Goactin1 also had very high homologue to actin protein of many other species in the data bank. The maximum number of amino acid homologue was 99 percent from Ricinus communis (XP_002530711.1, EEF31665.1), Solanum tuberosum (CAA39280.1), Gossypium hirsutum (AAC31886.1), Populus trichocarpa (XP_002308365.1, XP_002322664.1, ABK92789.1, XP_002311167.1, EEE88534.1, XP_002316289.1, ABK92513.1, EEF02460.1), Caragana Korshinskii (ACK87035.1) and Nicotiana tabacum (ACH69153.1, CAA45149.1).

1.3 Characteristic analysis of Goactin1 protein

ProtParam analysis showed Goactin1 theoretical molecular weight and isoelectric point were 41.7 kD and 5.31 respectively, and there was 50 negative charge amino acids (Asp +Glu) and 38 positive charge amino acids (Arg +Lys). The protein was composed of Alpha helix (39.26%), Random coil (33.69%), Extend strand (20.42%)and Beta turn (6.63%) in secondary structure analyses by SPOMA program (Combet et al., 2000) (Figure 2). Through conservative domain analysis by cdart program (NCBI), A conservative domain: Actin superfamily, was discovered (Figure 3), which included 6 ATP binding sites, 11 profilin binding sites, and 9 gelsolin binding sites (Marchler-Bauer et al., 2009; Marchler-Bauer and Bryant, 2004).

1.4 Tertiary structure of Goactin1 protein

Based on 3D structure of 2BTF A chain, a tertiary structure model of Goactin1 was established by ESyPred 3D program (Figure 4), and there was 88.3% homologue between them (Lambert et al., 2002).

1.5 Molecular phylogenetic tree analyses of Goactin1

Based on multisequencing comparison between Goactin 1 and actin amino acid sequence of other species by ClustalX (1.81) program, circular molecular phylogenetic tree was established use MEGA 4.0 program (Neighbor Joining method). Analytical result was showed at figure 5. With Solanum tuberosum (gi|231503|, gi|231496|), Gossypium hirsutum (gi|54035683|), Nicotiana tabacum (gi|197322805|, gi|461465|), Brachypodium sylvaticum (gi|226858185|), Arabidopsis thaliana (gi|15231447|, gi|15238387|, gi|28393806|), Goactin1 were gathered...
to the same group.

Figure 5 Molecular evolution analyses in putative amino acid sequence of Goactin1

2 Discussion

Actin with high conservatism was one of the most antiquity and ubiquitous proteins (Meagher et al., 1999), which was vital for plant natural morphochoresis that action of Actin in deciding cell division, keeping cytoskeleton structure, guiding cell elongation and cell wall deposition as major component of cell microfilaments (Zhou et al., 2001). At present, Actin gene had been cloned in Arabidopsis thaliana, Oryza sativa, Zea mays, Populus trichocarpa, Selaginella moellendorffii, Ricinus communis, Olea europaea, physcomitrella patens, Microur a sikkimensis, Agropyron mongolicum, Cucumis sativus, Malus robusta, Dunaliella salina, Helianthus annuus etc, based on result of GenBank data base and document search. In Blast analyses, 84 percent homologue to Populus trichocarpa (XM_002311131, GENE:7467546), Vigna radiate (AF143208.1) and Betula luminifera (FJ410442.1). It was obvious that cDNA homologue was far less than putative amino acid sequence. All these showed that though the nucleotide sequence had big variations in different plants, its amino acid sequence still kept high stability and uniformity, which ensured plant growth and development process on the rails.

3 Materials and Methods

3.1 Materials

Plant materials came from the early floral organ of Ostara (Guzmania cultivar).

3.2 Methods

Since 2008, we had constructed successfully full length cDNA plasmid library of Ostara floral organ, and obtained 1758 high quality sequences by 5’EST sequencing to 2004 positive clones picked at random (Liu et al., 2009). After all sequences were analyzed by Blast, 10 EST involved in Actin gene were obtained, which included three single EST such as: [ppfca0_000981.z1.scf], [ppfca0_000514.z1.scf], [ppfca0_000958.z1.scf] and two contigs that the first contig included [ppfca0_0000514.z1.scf], [ppfca0_000958.z1.scf] and two contigs that the first contig included [ppfca0_0001_C12.ab1], [ppfca0_001242.z1.scf], [ppfca0_000453.z1.scf] monoclonal, and the second contig included [ppfca0_001161.z1.scf], [ppfca0_001242.4.z1.scf], [ppfca0_000774.z1.scf], [ppfca0_001078.z1.scf] monoclonal.

And then, through Primer Walking sequencing to every single EST and contig in Hangzhou Genomics Institute, four full length cDNA sequences of Actin gene were obtained, one of which was analyzed and published in the paper, the rest would be published in other papers.

Some characters involved in cDNA sequence, amino
acid sequence, conservative domain and genetic relationship, were analyzed by diversified bioinformatics methods, for instance, prediction of amino acid sequence by ExPASy combined with BlastP, multisequencing comparison of nucleotide and amino acid by Blast, estimation of protein molecular weight and isoelectric point by ProtParam, prediction of protein tertiary structure by ESyPred 3D and phylogenetic tree analyse by MEGA 4.0 combined with ClustalX (1.81) software.

Author Contributions
Jianxin Liu was deviser, superintendent and principal transactor; Yaying Ge, Danqing Tian and Zhi Zhang participated in experimental design, test result analysis and writing of the preliminary draft; Huaqiao Ding, Fuquan Shen and Weiyong Wang provided test materials and analyzed data. All authors had read and approved the final version.

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