

ACTIN GENE IDENTIFICATION FROM SELECTED MEDICINAL PLANTS FOR THEIR USE AS INTERNAL CONTROLS FOR GENE EXPRESSION STUDIES

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Abstract

Internal control genes are the constitutive genes which maintain the basic cellular functions and regularly express in both normal and stressed conditions in living organisms. They are used in normalization of gene expression studies in comparative analysis of target genes, as their expression remains comparatively unchanged in all varied conditions. Among internal control genes, *actin* is considered as a candidate gene for expression studies due to its vital role in shaping cytoskeleton and plant physiology. Unfortunately most of such knowledge is limited to only model plants or crops, not much is known about important medicinal plants. Therefore, we selected seven important medicinal wild plants for molecular identification of *actin* gene. We used gene specific primers designed from the conserved regions of several known orthologues or homologues of *actin* genes from other plants. The amplified products of ~370-380 bp were sequenced and submitted to GeneBank after their confirmation using different bioinformatics tools. All the novel partial sequences of putative *actin* genes were submitted to GeneBank [*Parthenium hysterophorus* (KJ774023), *Fagonia indica* (KJ774024), *Rhazya stricta* (KJ774025), *Whithania coagulans* (KJ774026), *Capparis decidua* (KJ774027), *Verbena officinalis* (KJ774028) and *Aerva javanica* (KJ774029)]. The comparisons of these partial sequences by Basic Local Alignment Search Tool (BLAST) and phylogenetic trees demonstrated high similarity with known *actin* genes of other plants. Our findings illustrated highly conserved nature of *actin* gene among these selected plants. These novel partial fragments of *actin* genes from these wild medicinal plants can be used as internal controls for future gene expression studies of these important plants after precise validations of their stable expression in such plants.

Key words: *Actin* gene, Medicinal plants, Gene expression control.

Introduction

Sequencing the whole genome of an organism not only describes the sequence of a DNA but also enable us to understand the role of each gene at genomic, transcriptomic and proteomics level. There are various ways of gene expression studies in stress responsive plants like microarrays, ribonucleic acid (RNA)-seq and realtime PCR etc. (Shinwari *et al.*, 1998; Kim *et al.*, 2014). In such analysis typically a normal gene from control and a diseased/stressed gene can be compared quantitatively (Nakashima *et al.*, 2000). These kinds of relative studies need appropriate and validated controls from the selected species to compare finally with some genes for normalizations. Hence, analyzing the regulated expression of a gene is very important for biological and medical research grasping the foundation of molecular biology (Feng *et al.*, 2012; Wan *et al.*, 2010; Narusaka *et al.*, 2003). Housekeeping genes (HKG) is a term applied to those genes that contribute to the most basic form of cellular activities and demonstrate in general the invariant expression (Butte, 2001; Banaras *et al.*, 2012). Their expression in all tissues and cell types at the same time remains same even in different environmental conditions (Bar *et al.*, 2009). Fundamentally, they are accountable for maintenance of cellular processes such as components of the cytoskeleton, synthesis of ribosome subunits, protein degradation and in protein folding etc. Thus they can be treated as endogenous references or reference genes or internal control genes (Rubie *et al.*, 2005; Aman *et al.*,

2013). They are used in normalization studies of a target gene at qRT-PCR which lessen the variations making it more trustworthy technique (Huggett *et al.*, 2005). Precise selection of housekeeping genes is needed for effective qPCR (Jain *et al.*, 2006). It can normalize the mRNA or level of protein expression stably expressed constitutively at high levels in most tissues and cells of different species in all target samples (Mehta *et al.*, 2010; Kidokoro *et al.*, 2009).

Actin proteins mainly help in transcription regulation, reshaping chromatin and in trafficking of nucleoplasm (Bettinger *et al.*, 2004). It maintains the cytoskeleton and physiology of eukaryotic plants thus stabilizing the shape of cell (Paniagua *et al.*, 2007). It is also a part of microfilament formation, taking part in movement of organelles, differentiation of cell, transport of substances, keeping configuration, signal transduction, cellular morphogenesis i.e. cell division or elongation of cell, polarity construction and intracellular cytoplasmic streaming etc (Higaki *et al.*, 2007). There are certain ways of integrating regulation of gene during reproductive development thus remain less characterized (Qin *et al.*, 2014). There are many isoforms of *actin* gene which are all less than 3kb in size independently scattered in the genome (McKinney & Meagher, 1998). A specific *actin* gene has an approximate structure of 100-nucleotides 5' untranslated region (UTR), a translated region of 1200-nucleotides and 3'UTR of about 200-nucleotides. There are six introns in *actin* gene in well characterized nineteen locations. Coding sequence of *actin* genes is broken up by three small introns each of 90bp in length (Shah *et al.*, 1982).

The *actin* genes of monocot and dicot plants are more strongly linked than intragenic copies, representing that *actin* gene is purely from gene family of ancient times. Therefore identifying more novel reference genes will be highly valuable for normalization purposes. The use of single reference gene for data normalization is considered to be no longer acceptable (Bustin and Nolan, 2004; Dheda *et al.*, 2004; An *et al.*, 2012) and it is highly recommended that multiple reference genes should be used for normalization studies (Vandesompele *et al.*, 2002; Remans *et al.*, 2008). Additionally, the identification of novel reference gene will also help the researcher to choose the best internal control with stable expression for the expression analysis under particular conditions. So improved options for reference gene will be available and probability of having the best suitable gene will be increased. *Actin* gene fulfill the criteria of suitable reference gene as shown in recent studies of quantitative gene expression (Pinto *et al.*, 2012). It is necessary to assess a gene for validation before using it for normalization (Zhang *et al.*, 2005; Chang *et al.*, 2012). Validation of genes makes the data more reliable (Dong *et al.*, 2012). Whenever an experiment is run under new conditions, validation of reference genes will help to avoid many misinterpretations in data analysis (Majerowicz *et al.*, 2011). Using a poor reference gene will ultimately cause misleading interpretation of data (Wan *et al.*, 2010).

Up till now most of information about genomes is limited to model plants and some cultivated crops only. None of such informative data exist regarding economically or medicinally important plant species both in wild and even in cultivated plants. Thus, for the present work, we selected saline, cold and drought tolerant wild medicinal plant species from cold, hot and semi-arid areas of Pakistan. The main purpose of this study was to identify and sequence novel orthologues of *actin* gene from selected plants for their use as internal control genes for expression studies in future.

Materials and Methods

Plants collection: We selected seven different wild plants of economic and medicinal importance namely; *Rhazya stricta*, *Fagonia indica*, *Parthenium hysterophorus*, *Whithania coagulans*, *Capparis decidua*, *Verbena officinalis* and *Aerva javanica*. The decoction of *Rhazya stricta* is used in treatment of various illness including fever, inflammatory disorders, throat infections and diabetes mellitus (Al-Gonemi, 1992). The leaves extract is reputed as curative agent in syphilis. It is reported that extract fractions exhibits sufficient anti-depressant effect also (Ali *et al.*, 1998). It is a rich source of antispasmodic action (Tanira *et al.*, 1996). *Fagonia indica* is reported to inhibit cancerous cell growth. This plant is considered to be a remedy in tumors of rats (Bhandari, 1990; Ahmad & Basha, 2000). There are useful phyto-chemicals in it and thus help in easy urination, cures the asthma, as blood purifier and the aerial parts are of it are highly antioxidant in nature (Ali *et al.*, 2008). The decoction of *Parthenium hysterophorus* is conventionally used in treatment of several diseases including dysentery, diarrhea, malaria and in urinary tract infections (Surib *et al.*, 1996). It is reported that *P. hysterophorus* has anti-tumor constituents

(Das *et al.*, 2007). *Whithania coagulans* is used in treatment of insomnia, impotence, asthma, liver diseases and in diabetes (Bown, 1995). It also has antitumor, antimicrobial, cardiovascular and free radical scavenging effect (Maurya & Akansha, 2010). Important constituent the Whithanoloids possessing anti-inflammatory and anti-angiogenic effects, thus represents development of potent anti-cancer drugs.

Similarly, *Verbena officinalis* has antibacterial, anti-rheumatics and efficient expectorants (Chevallier, 1996; Guarrera *et al.*, 2005). It is concluded that they have great potential to act against microbial diseases (Hernández *et al.*, 2000), against cancer cell lines (Dudai *et al.*, 2005), as anti-inflammatory and potent antioxidant as well as in gastroprotective (Speroni *et al.*, 2007). It has been used traditionally as medicine as liqueur or tea in treatment of fever (Yuan and Olmstead, 2008). *Aerva javanica* is useful in treatment of diabetes mellitus, removal of inflammations, and is diuretic and demulcent as well. The seeds extract is used in relieving headache and rheumatism (Srinivas *et al.*, 2009). It shows significant resistance against certain bacteria and fungal strains (Mufti *et al.*, 2012). It also helps in diarrheal infections thus encompasses effect on intestinal tract (Joanofarc & Vamsadharaz, 2003). Berries of *Capparis decidua* have anti-diabetic effect in the body. An alkaloid the Capparisidine extracted from leaves acts as an anti-depressant that normalizes the heart attacks. Its bark has been used in cure of asthma, coughs and inflammation. While, roots are useful in fever and buds for the cure of boils. Bark is used as anthelmintic whereas shoot is utilized as tonic for fertility and purgative. The wood is beneficial in muscles disorders (Chopra *et al.*, 1999; Sharma, 2003).

Extraction of genomic DNA: Genomic DNA was isolated from fresh leaves of young plants by Cetyl Trimethyl Ammonium Bromide (CTAB) method with necessary changes (Richards, 1997). 2×CTAB buffer solutions were prepared by mixing 100 mM Tris HCl (pH 8), 20 mM ethylenediaminetetraacetic acid (EDTA) (pH 8), 1 M Sodium chloride (NaCl) and 1% merceptoethanol reagent. Leaf sample weighing (~0.3 g) of each above mentioned plants was taken separately, washing and sterilization was done with distil water followed by 70% ethanol. The leaf sample was crushed and homogenized in preheated (65°C) 2×CTAB buffer with the help of chilled mortar and pestle. The homogenized mixture of leaf tissue was then transferred to 1.5 ml eppendorf tubes and kept for incubation at 65°C for 40-45 minutes while inverting the tubes every ten minutes. This homogenized solution was centrifuged at 10,000 rpm for 10 minutes; 750 µl supernatant was collected from the top of tube and transferred to new eppendorf tubes. Equivalent volume of chloroform-isoamylalcohol (24:1) was added to supernatant and centrifuged again at 10,000 rpm for 10 minutes; 600 µl supernatant was collected in new autoclaved tubes and 480 µl chilled isopropanol was added, tubes were incubated at 4°C for 10 minutes and 12 µl of 1 M Sodium acetate was added to the supernatant. Mixture was placed at -20°C for 30 minutes for further DNA precipitation then centrifugation was done at 12,000 rpm at 4°C for 10 minutes. Washing of DNA pellet was done with 70% chilled ethanol and was air dried at room temperature followed by

re-dissolving in 40 to 45 μ l mixture of Tris EDTA buffer containing 2 μ l (10 μ g/ μ l) of RNase. The resuspended samples of DNA were incubated at 37°C for 25-30 minutes to completely remove RNA impurities. Finally, purified samples were stored at -20°C for further use.

Quantitative and qualitative analysis of DNA samples:

Quantity and quality of DNA was checked with the help of NanoDrop 1000 spectrophotometer (ND/-1000 V3.7.1, ThermoScientific). The optical density at OD260/280 nm absorbance of DNA was equivalent to standard ratio 1.8 for genomic DNA. The presence of extracted DNA and its quality was further analyzed by running samples on 1% agarose gel stained with ethidium bromide. For this DNA samples (5 μ l) were loaded in each well along with 3 μ l of Bromophenol blue loading dye. This was visualized under UV light by Dolphin Doc^{plus} gel documentation system (Wealtec).

Primer designing and polymerase chain reaction (PCR) of *Actin* gene:

Actin gene sequences from different plants were retrieved from NCBI database and aligned by using clustalW multiple alignment *Actin* primers were designed from conserved regions of the aligned *actin* genes. We used forward primer sequence (5' TCCATAATGAAGTGTGATGT `3) and reverse primer sequence (3' GACCTGACTCGTCATACTC `5). Amplification of *actin* gene was done by PCR using Go Taq[®] Green Master Mix of Promega, USA (Cat. # M7122) mixture. Initial denaturation was done at 95°C for 5 min followed by 35 cycles of denaturation for 45 sec at 94°C, annealing temperature of 55°C for 1 min and extension duration of 1 min at 72°C. Final extension was carried out at 72°C for 10 minutes. PCR products were observed on 1% agarose gel by loading 4 μ l of sample and 1 kb DNA ladder and visualized under UV light.

PCR Products purification: Amplified products of PCR were purified by using PureLink[®] PCR Purification Kit (Cat. # K3100-01). As prescribed by the manufacturer instructions, four volumes of Binding buffer (B2) added in one volume of PCR sample and mixed well. This mixture was loaded in Spin column in Collection tube and centrifuged the column at >10,000 \times g for 1 min. The filtrate was removed after centrifugation and washed with 650 μ l ethanol mixed Wash Buffer (W1). Centrifuge the column at >10,000 \times g for 1 minute. Discarded the flow through and placed it in Collection Tube and re-centrifuge at highest speed >13,000 rpm for 2-3 minutes. Finally, transfer the Spin Column into clean 1.7 mL Elution Tube (supplied with the kit) and added 25 μ l Elution Buffer to centre of column. Column was incubated at room temperature for 1 minute and centrifuged at maximum speed for 2 minutes. Purified PCR products were stored at -20°C for further use.

Sequencing of partial *Actin* gene: Sequencing was done by using sequencer of Beckman CEQ 8800 model following Sanger Dideoxy Chain termination method. Sequencing PCR reaction mixture was made by adding RRv3.1 master mix (enzyme, dNTPs, MgCl₂, 5 \times sequencing buffer), forward primer (1 μ l), template (1 μ l) and water (6 μ l). First denaturing temperature was 95°C

for 1 min, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C (*actin*) for 30 seconds, and extension at 72°C for 4 minutes, followed by final extension at 72°C for 10min.

Bioinformatics analysis of gene sequences: Analysis of sequences was done by BioEdit. Confirmation of newly sequenced partial sequences of *actin* gene was done by BLAST by using "Somewhat similar sequences (blastn)" options. Several *actin* gene sequences retrieved from NCBI by using BLAST were then aligned in BioEdit software and unrooted phylogenetic trees (UPGMA trees) were constructed by ClustalW multiple alignments while surfing through Website: www.genome.jp/tools/clustalw/ [accessed Sep 10, 2014].

Results and Discussion

Species specific sequences of several housekeeping plant genes are still not known and for gene expression studies these sequences are essential for a given plant. Similarly, the species specific sequences of *Actin* gene of several important medicinal plants were not known. Therefore, we isolated and sequenced partial sequences of *actin* gene from *F. indica*, *R. stricta*, *P. hysterothorus*, *W. coagulans*, *C. decidua*, *V. officinalis* and *A. javanica* for their potential use as internal control genes for gene expression investigation after appropriate validation. The sequences were deposited in GenBank with accession numbers [*Parthenium hysterothorus* (KJ774023), *Fagonia indica* (KJ774024), *Rhazya stricta* (KJ774025), *Whithania coagulans* (KJ774026), *Capparis decidua* (KJ774027), *Verbena officinalis* (KJ774028) and *Aerva javanica* (KJ774029)]. All selected plants were collected from saline, cold, drought and arid areas showing resistance towards environmental stresses and possesses wide range of medicinal uses in treatment of different diseases as given in material and methods.

High quality genomic DNA extraction is prerequisite for the process of PCR and genes identification techniques. Thus, magnitude of quality and quantity of extracted DNA was calculated by NanoDrop (ND/-1000 V3.7.1). The DNA from whole genome was isolated from leaves of target plants by using modified (CTAB) method (Richards, 1997) as described in material and methods section. *Chenopodium album* was taken as positive control sample in current study. Genomic DNA was examined on 1% agarose gel which showed the sharp bands of high molecular weight DNA with minimum degradations of all the plant DNA samples as shown in (Fig. 1A). We designed gene specific primer of *actin* gene by aligning several known orthologs or homologues of plant *actin* gene. The amplification of all candidate genes produced a single band of approximate size of 371 bp (Fig. 1B). Isolated genomic DNA of selected plants was used as a template for polymerase chain reaction (PCR) to amplify *actin* gene fragment by using gene specific primer. Including *Chenopodium album* treated as positive control. Approximately 371 bp sized products were amplified from each selected plant species as well as in positive control as shown in (Fig. 1B). There was no amplification of *actin* gene observed in non-template controls as expected.

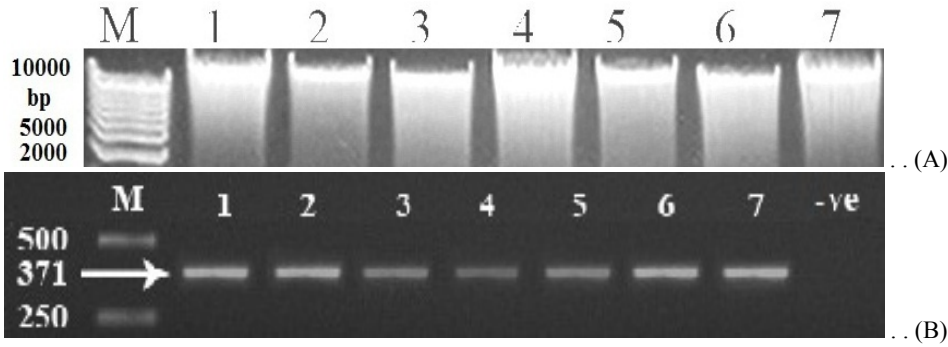


Fig. 1.(A) Genomic DNA extraction of selected medicinal plants in respective lanes 1-7 (*R. stricta*, *F. indica*, *P. hysterophorus*, *C. decidua*, *W. coagulans*, *V. officinalis* and *A. javanica*) showed good quality DNA. (B) PCR amplified products of partial fragment of *actin* gene from selected medicinal plants lanes 1-7 (*R. stricta*, *F. indica*, *P. hysterophorus*, *C. decidua*, *W. coagulans*, *V. officinalis* and *A. javanica*) using gene specific primers. Nothing was amplified in negative control samples (-ve) as expected.

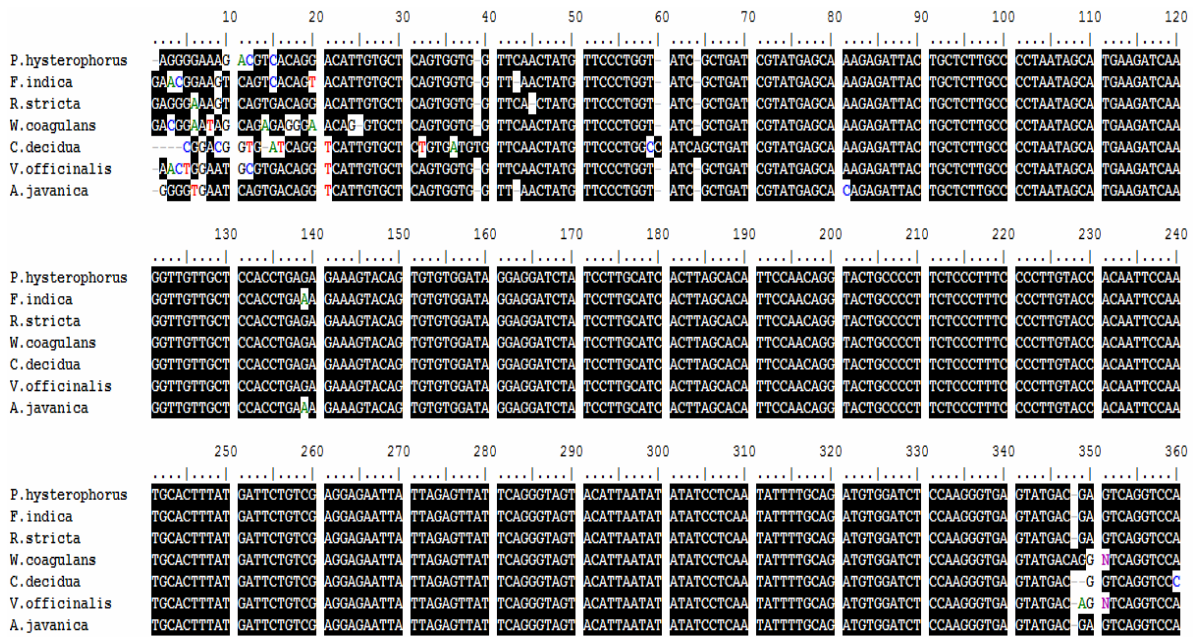


Fig. 2. Alignments of novel genomic DNA sequences of partial sequences of *Actin* gene from *R. stricta*, *F. indica*, *P. hysterophorus*, *C. decidua*, *W. coagulans*, *V. officinalis* and *A. javanica* by using ClustalW Multiple Alignment. The alignment shows that this specific fragment of *actin* gene is highly conserved among these plants.

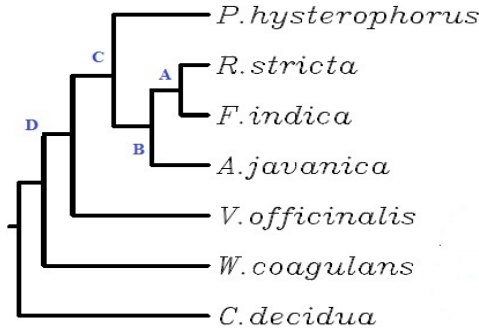


Fig. 3. Phylogenetic tree of partial *actin* gene sequences isolated from *P. hysterophorus*, *R. stricta*, *F. indica*, *A. javanica*, *V. officinalis*, *W. coagulans*, and *C. decidua* by using UPGMA software. The tree shows that the *actin* gene of these plants have close evolutionary relatedness.

Amplified product of each selected plant was purified by using PureLink® PCR Purification Kit and sequenced. New isolated partial sequences of *actin* gene from selected target plants were primarily analyzed by BLAST to verify the similarities with known *Actin* genes downloaded from NCBI. All newly sequenced partial *Actin* sequences showed maximum identity of ~95% with orthologs available in the database like *Pyrus pyrifolia*, *Eriobotrya japonica*, *Malus domestica* and *Zea mays* etc. Plants used for this study are of immense medicinal and economical values. We have identified partial *actin* gene for its use as housekeeping gene and sequence it among selected group of medicinal wild plants. BLAST results of the nucleotide sequences of selected plants yielded similarities to the *actin* gene sequence of other plants like *Camellia sinensis*, *Armoracia rusticana*, *Arabidopsis thaliana*, *Beta vulgaris*,

Vaccaria hispanica, *Gossypium hirsutum*, *Thellungiella halophila*, *Sorghum bicolor* hypothetical protein, *Hordeum vulgare*, *Zea mays*, *Nicotiana tabacum*, *Populus tomentosa*, *Chenopodium rubrum*, *Vaccaria hispanica*, *Helianthus annuus*, *Capsella rubella*, *Celosia argentea*, *Brassica rapa*, *Gossypium hirsutum*, *Setaria italica*, *Glycine max*, *Litchi chinensis*, *Solanum tuberosum*, *Solanum lycopersicum*, *Jatropha curcas*, *Armoracia rusticana*, *Brachypodium distachyon*, *Celosia argentea*, *Thellungiella halophila*, *Armoracia rusticana*, *Thellungiella halophila*, *Armoracia rusticana*, *Brachypodium distachyon*, *Celosia argentea*, *Thellungiella halophila*, *Armoracia rusticana*, *Musa acuminata*, *Populus trichocarpa*, *Eriobotrya japonica*, *Malus x domestica*, *Karenia brevis*, *Nitraria sibirica*, *Citrus sinensis*, *Cicer arietinum*, *Trifolium pratense*, *Hevea brasiliensis*, *Morella rubra*, *P. sativum*. In all these plants the gene sequence identity was not below 80% leading to ~95% similarity index showing that *actin* can be used as a potent reference gene, because it has remained more or less conserved throughout its evolution. Results of BLAST pointed out *Eriobotrya japonica* (JX089586.1) and *Pyrus pyrifolia* var. *culta* *actin* (AB826125.1) having 95% highest of all similarities, ACT3 of *Camellia sinensis* (FJ355923.1) with 92% identity. Whereas, *Tom51 actin* in *Solanum lycopersicum* (U60481.1) and *Jatropha curcas* (HM044307.1) possesses 91% do have higher similar index among all BLASTn calculations. In fact, *Populus tomentosa* (JX986590.1) with lowest percentage yielding 74% and Soybean (V00450.1) with 76% lower similarity in ancestral match. The data generated here suggested that, *actin* being most conserved sequence in some species throughout evolution also showed some of non conserved match as well.

Several conserved patterns were observed among these sequences when we aligned all the newly sequenced *actin* sequences as shown in (Fig. 2). This data suggests that this specific fragment of *actin* gene is highly conserved among these and several other known plants. Based on these results we assumed that these primers can be used to identify *actin* gene from wide range of plants. The data generated after multiple alignment was showing sufficient conserved region among all observed plants. Using ClustalW, we constructed a phylogenetic tree to check the relation of these plants based on this gene. Phylogeny results illustrated extensive conservation in all related novel segments of the *actin* gene. This showed that certain species are highly similar in evolutionary relatedness but few species are showing a distant evolution pattern. Like, *F. indica* with *R. stricta* (A-group) is said to be more closest group, *A. javanica* with the A-group species making a separate (B-group) said to be closer group, where *P. hysterophorus* making (C-group) which is only a close connection between both groups AB and the rest three species i.e. *V. officinalis*, *W. coagulans* and *C. decidua* are distantly connected with the mentioned species of all other groups (Fig. 3). Thus all such type of information in UPGMA tree on basis of *actin* gene ancestral relatedness confirmed the distant relationship

of one another. Although, these distantly related species possesses an evolved conservation with other groups from outside grouping which proved the *actin* gene conservation among different species. *Actin*. Therefore it is considered of significant importance for normalization studies. In fact, *actin* gene is a potential reference gene under different conditions in particular organism (Zhang *et al.*, 2005). We identified and sequenced partial portion of *actin* gene from selected plants. These plants exhibit great economical and medical values, so it can be used as reference gene after proper validations in future. Plants collected in this study were highly medicinal, salt and drought tolerant, so there is vast interest for research purposes in identification of internal control genes. Growth of plant is generally affected by certain abiotic stresses like drought, metal, heat or cold etc. Even a single or combination of abiotic stress, in very short span of time can cause alteration in gene expression. Studies relating expression of genes can unwind the central elements and their activities in various metabolic pathways of plants. Thus normalization of reference gene towards a target gene is the key component in this regard (Cortleven *et al.*, 2009). Due to this reason, it is necessary to validate a reference gene cautiously before using any housekeeping gene. These types of tasks can be accomplished easily for model plants or other economically important plants on the basis of sequencing data. Due to lack of sufficient information about wild plants, we characterized and identified seven medicinally important plants for assessment of internal controls in scrutiny of gene expression.

Actin gene was isolated from chickpea and designated as CarACT1 residing almost in all organs including stems, roots, flowers, seeds, leaves and in growing tissues do contribute in gene expression studies (Peng *et al.*, 2010). Similarly, *actin* alpha (*Act-α*) has been reported and validated as suitable internal control gene in cold stress as well as good to use in normalizing drought stress studies in *Ficus carica* and *C. album* (Aman *et al.*, 2013). Same results were validated by using geNorm that proved that *actin* alpha (*Act-α*) can be used in different abiotic stresses (heat, drought, cold and salt) as an efficient internal control gene in *Chenopodium album* (Haq *et al.*, 2012). *Actin* alpha (*Act-α*) and *actin* beta (*Act-β*) were characterized as reference gene in normalization in *Arabidopsis thaliana* (Zhang *et al.*, 2010). Our sequences generated in current study showing sufficient identical homology which is correlated with their research, so *actin* gene could serve as a reference gene. So far, *actin* has been isolated in different plant species. (Peng *et al.*, 2010) isolated this gene from chickpea suggesting that CarACT1 is present in cytoplasm with most similarity index with other actins namely AtACT11, OsACT1 and StACT3 (Reece *et al.*, 1990; Huang *et al.*, 1997). Further, other transcripts are surprisingly gathered at all stages in almost all organs of chickpea plant especially in developmental organs thus confirming that *actin* is involved in physiological development of organs.

Conclusion

The purpose of this study was to characterize and identify the partial portion of *actin* gene, sequence the target gene, analyze the sequenced data by using bioinformatics tools (BLAST) and compare its homology with other known *actin* gene. Phylogenetic relationships of different plants were checked based on these partial *actin* gene sequences. Our results illustrated highly conserved nature of *actin* gene among these selected plants. These novel partial fragments of *actin* genes from these wild medicinal plants can be used as internal controls for future gene expression studies of these important plants after precise validations of their stable expression in such plants. This is the first report on identification and characterization of such internal control gene for expression studies among variety of wild plants that possesses economical and medicinal values.

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