International Journal of Chemical and Biochemical Sciences

Journal Home page: www.iscientific.org/Journal.html

Isolation of Actin and GAPDH partial genes from Black Cumin (*Nigella sativa* L.) seeds

Iram Zovia[1], Muhammad Javid Iqbal[2] and [3]Amer Jamil

[1]*Department of Chemistry, Government College University, Faisalabad*
[2]*COMSATS Institute of Information Technology, Islamabad-44000, Pakistan*
[3]*Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad*

Abstract

Black Cumin (*Nigella sativa* L.) is a medicinal plant extensively use in variety of ailments as medicine. *Actin* and *GAPDH* are the two important housekeeping genes were isolated and used to investigate gene expression levels in plant cells by using PCR technique. DNA was extracted from the black cumin seeds by CTAB method and PCR was performed for the amplifications of the genes from genomic DNA by using designed primers. Successful amplifications of the *Actin* and *GAPDH* genes were achieved at 10 ρmoles primer and 2 mM $MgCl_2$ concentration on 100 bp each. The isolated genes may be utilized in further gene expression studies for other medicinal plants.

Key words: Actin, GAPDH, *Nigella sativa*, housekeeping genes, gene expression

Full length article Received: 29-12-2012 Revised: 03-01-2013 Accepted: 05-01-2013 Available online: 31-01-2013
**Corresponding Author*, e-mail: imjavid@gmail.com

1. Introduction

Plants have always been a common source of medicaments, either in the form of traditional preparations or as pure active principles and the value of medicinal plants to human livelihoods is essentially countless [1]. Medicinal plants have contributed hugely towards medicine field, through providing ingredients for drugs or having played central roles in drug discovery. Some drugs, having botanical origins, are still extracted directly from plants; others are made through transformation of chemicals found within them. A larger number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials [2]. Seeds of *Nigella sativa* have been employed for thousands of years for the medicinal purposes due to anticancer, antidiabetic, antiradical, analgesic, antimicrobial, anti-inflammatory, bronchodilator, antihypertensive and renal protective properties. Akerele (1993) suggested that plant-derived drugs have an important place in both traditional and modern medicine. For this reason a special effort to maintain the great diversity of plant species would undoubtedly help to alleviate human suffering in the long term. Proven agro-industrial technologies should be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines. About 80% of the world's people depend largely on traditional plant-derived drugs for their primary health care (PHC). Medicinal plants served as sources of direct therapeutic agents and raw materials for the manufacture of more complex compounds, as models for new synthetic products, and as taxonomic markers. Some essential plant-derived drugs were atropine, codeine, morphine, digitoxin/digoxin, and quinine/artemisinin [3]. The black cumin (*Nigella sativa*) seeds and its crude or essential oils have been widely used in traditional nutritional and medicinal applications and has been extensively studied for its nutritional value and biological activities. The black cumin oil have been shown to be anticancer, antidiabetic, antiradical and immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, antihypertensive and renal protective. Moreover, black cumin seeds have many antioxidative properties and activities. *N. sativa* seeds revealed promising narcotic analgesic activity mediated possibly through opioid receptors. The oil from the seeds exhibited CNS depressant and potent analgesic effects. It was also found to potentiate pentobarbitone-induced sleeping time [4]. Various gene expression techniques are in use to study the expression levels in different plant cells. Housekeeping genes are essentially used as reference genes for data normalization in all such gene expression studies including quantitative RT-PCR (qRT-PCR). The most common strategy is to use a reference gene as an internal standard that is assumed to

remain constant between experimental groups [5]. In plants, *Actin* cytoskeleton participates in the definition of cell polarity and orientation of cell division, cell elongation, cell wall development, transport processes, positioning of membrane receptors, and in programmed cell death. Recent studies have shown that *GAPDH* exhibits a number of activities including phosphotransfease activity, nuclear RNA export, DNA replication and DNA repair. *Actin* is a ubiquitous protein in eukaryotic cells. Based on the observations of tissue localization and a variety of physiological approaches, a number of functions have been proposed for *Actin* in higher plants. Multiple genes encoding actin isoforms, which are more or less specifically expressed in particular tissues or organs, have appeared during plant evolution [6]. *Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* is a key enzyme in glycolysis where it catalyzes the reversible inter-conversion between glyceraldehyde-3-phosphate and 1,3-diphosphoglycerate [7]. As a membrane protein, *GAPDH* functions in endocytosis; in the cytoplasm, it is involved in the translational control of gene expression; in the nucleus, it functions in nuclear tRNA export, in DNA replication, and in DNA repair. Moreover, *GAPDH* was detected as an essential part of gene expression observed in apoptosis, as part of the cellular phenotype of age-related neuronal disorders like Alzheimer's disease and CAG triplet repeat disorders [8].

The reported partial gene sequences of *Actin* and *GAPDH*, isolated from *Nigella sativa*, will highly be functional in gene expression studies of medicinal plants in future.

2. Materials and methods

The presented research work was carried out in Molecular Biochemistry Lab, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad, Pakistan. Leaf tissue was taken 150-200 mg and ground in liquid nitrogen with pestle and mortar to make fine powder and transferred in a microcentrifuge tube. The powdered tissue was mixed with 700 µL pre-warmed CTAB buffer and incubated at 65ºC for 30 minutes, then bring to normal at room temperature. An equal volume of chloroform: isoamyl-alcohol (24:1) was mixed well and centrifuged at 13000 rpm for 10 minutes at room temperature. Upper DNA containing layer was separated and transferred into a new separate microcentrifuge tube and added 0.6 volumes Isopropanol to precipitate the DNA and kept at -80ºC for 30 minutes for further precipitation. This DNA was centrifuged at 13000 rpm for 10 minutes to get compact pellet which was washed with 70% ethanol and then air dried. Finally the pellet was dissolved in 50 µL of sterile distilled water. Polymerase Chain Reaction master mix was used for the amplification of *Actin* and *GAPDH* genes from *Nigella sativa* [9]. Concentration of the isolated DNA was determined on GeneQuant pro (Amersham) at 260 nm absorbance. The DNA amplified samples were run on agarose gel (0.8%) electrophoresis [10] and documented on gel documentation analysis (Gene Eye) system. *Actin* and *GAPDH* genes were amplified from the genomic DNA by using the primers which were designed from available sequences for the genes from other plants on genome database and obtained from GeneLink, USA.

3. Results and Discussion

The study was focused on isolation of two important genes for gene expression studies from *Nigella stavia*. DNA from the plant leaves was isolated by CTAB method that yielded sufficient amount of good quality DNA as shown in Fig. 1. Concentration of the DNA was found to be in the range 65 µg/mL. Analysis of vascular plant *Actin* gene sequences suggested that an even greater diversity should exist within the plant *Actin* protein families, but previous studies on plant proteins had not demonstrated the presence of multiple *Actin* isovariants. Antibodies recognizing a conserved amino-terminal plant *Actin* peptide, a family of plant *Actin* peptides from a variable region, and two monoclonal antibodies to conserved epitopes within animal *Actin* were used to identify isovariants of soybean *Actin* resolved by two-dimensional isoelectric focusing (IEF) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Approximately six to eight *Actin* isovariants with π values ranging from 5.1 to 5.8 had been identified from soybean hypocotyls. Stems, leaves, and roots with varying amounts of most isovariants present in all four organs. Acidic isovariants were present in much higher levels in leaves and stems [11]. *Actin* is an extremely abundant protein that comprises a dynamic polymeric network present in all eukaryotic cells, known as the Actin cytoskeleton. The structure and function of the *actin* cytoskeleton, which is modulated by a plethora of *actin*-binding proteins, performs a diverse range of cellular roles. The major functions for *Actin* include providing the molecular tracks for cytoplasmic streaming and organelle movements [12]. The chloroplast glyceraldehyde-3-phosphate dehydrogenase *(GAPDH)* is composed of two different subunits, GapA and GapB. cDNA clones containing the entire coding sequences of the cytosolic precursors for GapA from pea and for GapB from pea and spinach had been identified, sequenced and the derived amino acid sequences had been compared to the corresponding sequences from tobacco, maize and mustard. These comparisons showed that GapB differs from GapA in about 20% of its amino acid residues and by the presence of a flexible and negatively charged C-terminal extension, possibly responsible for the observed association of the enzyme with chloroplast envelopes in vitro [13].

Well documented housekeeping genes are present in all the plants and a variety of housekeeping genes were often used as endogenous controls in gene expression experiments. Quantitative gene expression data were often normalized to the expression levels of control or so-called housekeeping genes. An inherent assumption in the use of housekeeping genes is that expression of the genes remains constant in the cells or tissues under investigation. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is one of the most commonly used housekeeping genes used in comparisons of gene expression data [14]. The use of reverse transcription-polymerase chain reaction (RT-PCR) to measure mRNA levels has led to the common use of *β-actin* and *GAPDH* housekeeping genes as denominators for comparison of samples. Expression of these genes was assumed to remain constant, so normalizing for variations in processing and signal quantization. However, it was well documented that *β-actin* and *GAPDH* expression is up-

regulated with proliferation, activation, and differentiation. They hypothesized that samples which differ in their cellular profiles and activation status had different levels of expression of *GAPDH* and *β-actin* [15].

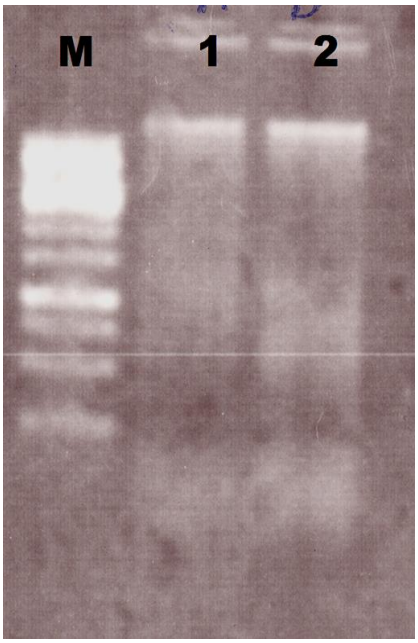
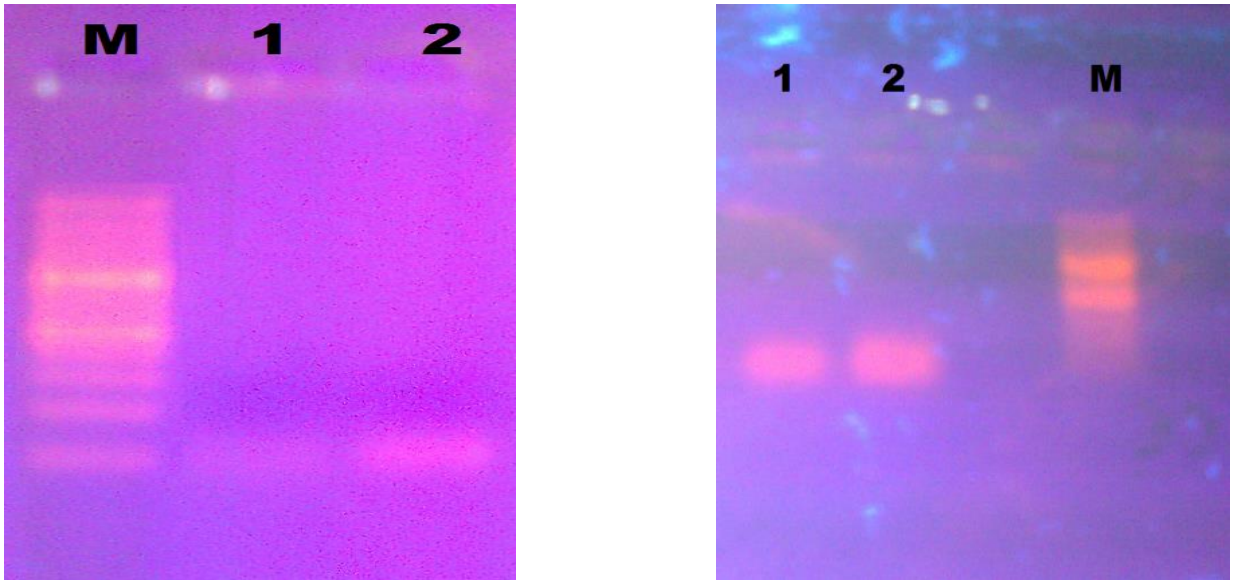


Fig. 1. DNA extracted from *Nigella sativa* by CTAB method



(A) **(B)**

Fig. 2. PCR amplification of *Actin* (A) and *GAPDH* (B) genes from *Nigella sativa*.

4. Conclusions

The isolation of *Actin* and *GAPDH* partial genes proved their presence in *Nigella sativa* and likely to be stable under all prevailing conditions. These housekeeping genes were isolated successfully by using PCR and could be employed in future gene expression studies.

References

[1] A.C. Hamilton. (2004). Medicinal Plants, conservation and livelihoods. Biodiversity and Conservation. 13: 1477-1517.

[2] M.L. Salem. (2005). Immunomodulatory and therapeutic properties of *Nigella sativa* L. seeds. International Immunopharmacology. 5: 1749-1770.

[3] O. Akerele. (1993). Nature's Medicinal Bounty: Don't throw it away. World Health Forum, 14(4): 390-395.

[4] M.F. Ramadan. (2007). Nutritional value, functional properties and nutraceutical applications of Black Cumin (*Nigella sativa* L.): An Overview. International Journal of Food Science and Technology. 42: 1208-1218.

[5] K. Dheda, J.F. Huggett, S.A. Bustin, M.A. Johnson, G. Rook and A. Zumla. (2004). Validation of housekeeping genes for normalizing RNA expression in Real-time PCR. BioTechniques. 37: 112-119.

[6] K. Schwarzerova, Z. Vondrakova, L. Fischer, P. Borikova, E. Bellinvia, K. Eliasova, L. Havelkova, J. Fiserova, M. Vanger and Z. Opatrny. (2010). The role of *Actin* isoforms in somatic embryogenesis in Norway spruce. BMC Plant Biology. 10 (89): 1471_2229.

[7] T.K. Moe, J. Ziliang, A. Barathi and R.W. Beuerman. (2001). Differential expression of Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) β-actin and Hypoxanthine phosphoribosyltransferase (HPRT) in postnatal rabbit sclera. Current Eye Research. 23(1):44-50.

[8] M.A. Sirover. (1997). Role of the Glycolytic protein, Glyceraldehyde-3-Phosphate Dehydrogenase, in normal cell function and in cell pathology. Journal of Cellular Biochemistry. 66: 133-140.

[9] J.J. Doyle and J.L. Doyle. (1990). Isolation of plant DNA from fresh tissues. Focus. 12: 13-15.

[10] J. Sambrook and D.W. Russell. (2001). Molecular Cloning, A laboratory manual, (3rd Ed). Cold Spring Harbor, New York, USA.

[11] B.G. Mclean, S. Huang, E.C. Mckinney and R.B. Meagher. (1999). Plants contain highly divergent *Actin* isovariants. Cell Motility and Cytoskeleton. 17: 276-290.

[12] C.J. Staiger, F. Baluska, D. Volamann and P. Barlow. (2000). *Actin*: A dynamic framework for multiple plant cell functions. Developments in Plant and Soil Sciences, 89: 676.

[13] H. Brinkmann, R. Cerff, M. Salomon and J. Soll. (1989). Cloning and sequence analysis of cDNAs encoding the cytosolic precursors of subunits Gap A and Gap B of chloroplast Glyceraldehyde-3-Phosphate Dehydrogenase from pea and spinach. Plant Molecular Biology, 13(1): 81-94.

[14] R.D. Barber**,** D.W. Harmer**,** R.A. Coleman and B.J. Clark. (2005). *GAPDH* as a housekeeping gene: analysis of *GAPDH* mRNA expression in a panel of 72 human tissues. Physiological Genomics. 21: 389-395.

[15] E. Glare, M. Divjak, M. Bailey and E. Walters. (2002). ß-Actin and *GAPDH* housekeeping gene expression in asthmatic airways as variable and not suitable for normalizing mRNA levels. Thorax. 57(9): 765-770.