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Actin and GAPDH partial genes from green pepper (*Capsicum annum* L.)

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Abstract

Capsicum annum L. is a medicinal plant and is a good source of phenolics, antioxidants, carotenoids, etc. The plant also exhibits antimicrobial activities due to antimicrobial compounds including bioactive peptides. Expression of bioactive genes from the medicinal plants is gaining attention due to its potential against microbial resistance. In this regard analysis of gene expression is important in determining differential gene expression. We report here isolation of two housekeeping genes viz *Actin* and *GAPDH* from capsicum. Isolation of DNA was done by CTAB method while amplification of the genes was done by PCR. Electrophoresis showed amplified *Actin* gene of 375 bp and *GAPDH* of 100 bp that will be helpful in future gene expression studies.

Key words: Actin, GAPDH, *Capsicum annum*, gene expression, housekeeping genes

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1. Introduction

Sweet pepper (*Capsicum annum* L.) is one of the most important commercial perishable vegetable with a short shelf life and high susceptibility to fungal diseases [1]. Its fruits have different forms and size, and several colors, ranging from yellow to red, from intense purple to dark green to black, depending on the genotype or the seasonal period of breeding [2]. Pepper fruits are high source of anti-oxidants; they have vitamin C and E in higher amounts as well as carotenoids and xanthophylls. Analysis of gene expression is of great importance and this analysis is helpful in determining differential gene expression between normal and diseased or stressed cell, therefore, helpful in determining the mechanism of diseases and to determine therapeutics for the treatment of diseases [3]. To study gene expression levels there are many methods. RT-PCR is a sensitive method for quantification of expression of genes; however, there may be chances of error due to variation in samples' material. These errors can be removed by use of internal references genes against which the values for mRNA level expression can be normalized [4]. Plants undergo several responses due to environmental changes such as stress, heat shock etc and those genes are used as references or housekeeping genes which are unaffected by these changes at all stages of cell development, therefore, helpful in normalizing gene expression. Expression of the housekeeping genes will remain the same under various conditions, hence may be used for normalization and do not need experimental evidences for their stability [5]. Most commonly used reference genes are *GAPDH*, *Actin*, ribosomal genes, cyclophilin and elongation factor 1α-, adenine phosphoribosyl transferase and tubulin. We selected *actin* and *GAPDH* as reference genes from capsicum.

Actin is a ubiquitous protein which is involved in several processes as cell elongation, cytoplasmic organization, cold acclimation, and maintenance of cell polarity, polar tip growth, helping defense response, intracellular trafficking and cytokinesis [6]. *Glyceraldehyde-3-phosphate dehydrogenase* (GAPDH) is thought as a classical glycolytic protein for its pivotal role in energy production. GAPDH displays a number of diverse activities such as apoptosis and assembly of microtubules [7]. *GAPDH* gene controlled by an active promoter [8], is highly expressive in the form of protein and forms 5% of total content of soluble cellular proteins in eukaryotes.

We report here isolation of partial gene sequences of *Actin* and *GAPDH* from *Capsicum annum* that will be

highly useful in gene expression studies of the medicinal plants stratum.

2. Materials and methods

CTAB method and Polymerase Chain Reaction were used for the isolation and amplification of *Actin* and *GAPDH* from *Capsicum annum* respectively. Green peppers were ground to a fine powder in liquid nitrogen mixed in a microcentrifuge tube with 700 µL pre-warmed CTAB buffer and incubated it at 65º C for 30 minutes [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 system. *Actin* and *GAPDH* genes were amplified by PCR from the genomic DNA by using the primers 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 key genes for gene expression studies from *Capsicum annum* L. DNA from the plant was isolated by CTAB method that yielded sufficient amount of good quality DNA as shown in Fig. 1. The genes were successfully amplified as shown in Figs. 2 and 3.

Quantitative gene expression data are 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. Although exceptions to this assumption are well documented, housekeeping genes are of value in fully characterized systems. *GAPDH* is one of the most commonly used housekeeping genes used in comparisons of gene expression data. Comparative levels of expression can be used to add value to gene expression data in which *GAPDH* is used as the internal control [11]. Similarly, actins are ubiquitous and highly conserved proteins that play key roles in cell formation and cellular activities. An *actin* gene isolated from chickpea and designated as CarACT1 was found to be ubiquitously accumulated in all major organs, such as seedling roots, stems, leaves, flowers, young pods, and seeds, as well as in diverse developmental stages, such as leaf senescence, seed development and germination. Therefore, the *actin* gene with could be served as a potential reference gene for transcription level of interesting genes in chickpea [12].

In view of the above discussion the *GAPDH* and *actin* genes isolated from *Capsicum annum* might contribute significantly in gene expression studies in *Capsicum* species due to high medicinal potential of the plant. A survey of the Mayan pharmacopoeia revealed that tissues of *Capsicum* species (Solanaceae) are included in a number of herbal remedies for a variety of ailments of probable microbial origin. Many of the Capsicum species exhibited antimicrobial effects against fifteen bacterial and yeast species [13]. Similarly, antioxidant potential of the plant has also been shown [14].

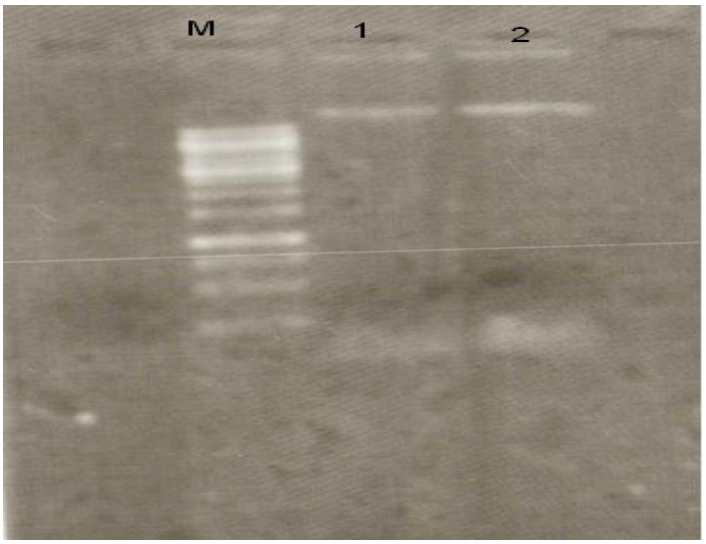

Fig. 1. DNA extracted from *Capsicum annum* by CTAB method.

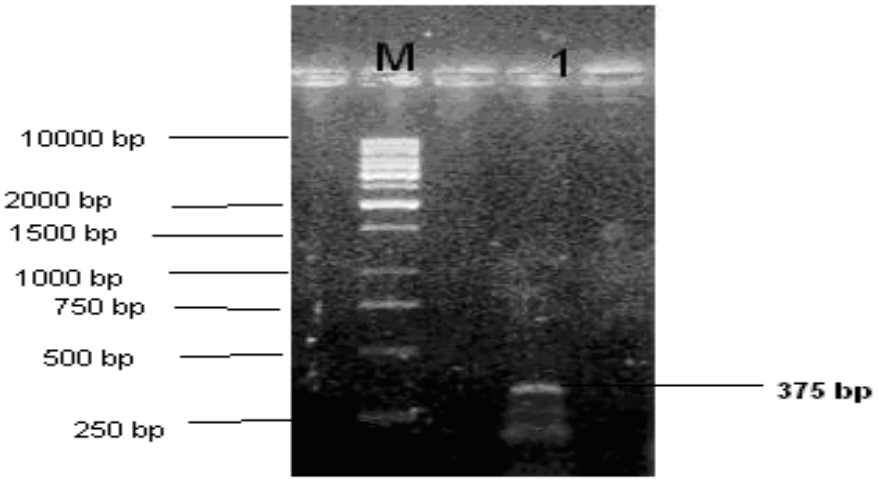

Fig. 2. Amplification of Actin gene

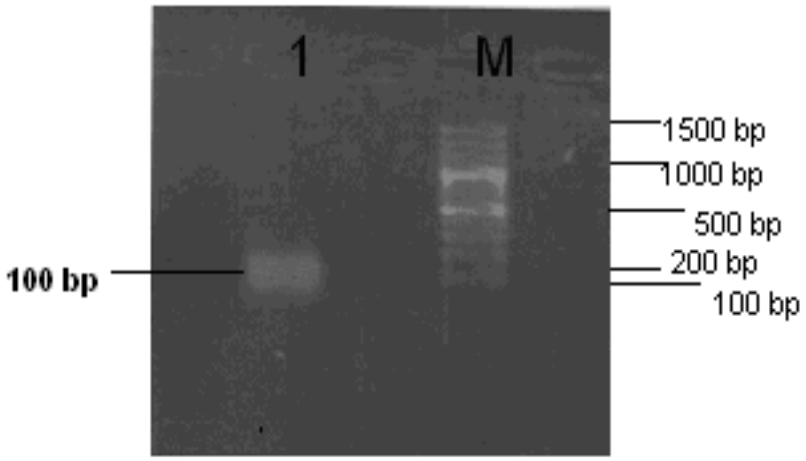

Fig. 3. Amplification of *GAPDH* gene

4. Conclusion

Isolation of *Actin* and *GAPDH* partial genes proved their presence in *Capsicum annum* L. likely to be stable under all conditions. These housekeeping genes were isolated successfully by using PCR and could be employed in gene expression studies.

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