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Functional assessment of *CODM* gene in different cultivar of Papaveraceous species via *in silico* analysis

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ABSTRACT

Medicinal use of the opium poppy (*Papaver somniferum* L) has ancient history, but the isolation of morphine was not described until the early nineteenth century. Morphine is the most important alkaloid of opium poppy in the last 50 years. In the morphine pathway has been reported to generate morphine in this species, *CODM* has a crucial role as the gene coding the enzyme responsible for demethylation of codeine to morphine. In this study we extract of *CODM* gene sequence from *Papaver fogax* and *Papaver oriental*. The sequence of this gene via *in silico* technique converted to the protein sequence. After this step we use the bioinformatics tools for analysis of these sequences.

Keywords: *CODM* gene, *Papaveraceous* species, Homology modeling

INTRODUCTION

Medicinal plants play a crucial role in the human life, particularly within the developing countries due to their cost effectiveness. However, Plants are used medicinally in different countries and are a source of many potent and powerful drugs In addition, medicinal plants are harvested, transported, and traded in both rural and urban markets, generating economic opportunities for vulnerable groups, especially women and farmers facing decreasing agricultural income Medicinal use of the opium poppy (*Papaver somniferum* L) has ancient history, but the isolation of morphine was not described until the early nineteenth century Morphine is the most important alkaloid of opium poppy in the last 50 years Poppy (*Papaver somniferum* L.), is a plant of the dicot family *Papaveraceae*, cultivated for seed, oil and opium. It is an erect, annual herb, 30–170 cm tall; flower buds are ovate, dropping before anthesis (hook stage); the fruit is a capsule that usually contains a high number of very small seeds. Some cultivars contain up to 50% oil in seed, mainly in the form of oleic and linoleic acids and may be used as a source of linoleic acid. The oil is of high quality for human consumption because of its high amount of polyunsaturated fatty acids [1]. In the pathway has been reported to generate morphine in this species, CODM has a crucial role as the gene coding the enzyme responsible for demethylation of codeine to morphine. Global licit cultivated area of opium poppy was about

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175,000 ha. Opium poppy is one of the most important crops for the pharmaceutical industry for the production of natural opiate alkaloids, mainly morphine, codeine and thebaine that are extracted mainly from the crushed dried capsules emptied of seeds. In Spain, the annual morphine demand of the pharmaceutical industry in 2000 was 5500 kg which was met by 5700 ha of cultivated opium poppy with more than 7000 kg of total morphine production [1,4].

MATERIALS AND MTHODS

DNA purification

DNA was extracted from *Papaver fogax* and *Papaver oriental* by using the CTAB protocol.

Custom Primers

Custom primers with similar melting points were designed to amplify and sequence the *CODM* gene using Primer 3 program, http://biotools.unmassmed.edu/bioapps/primer3_www.cgi. Delta G values for homodimer, heterodimer, and hairpin loops were determined using OligoAnalyzer 4.0, http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/, by Integrated DNA Technologies. Custom primers (Table 1) were designed to amplify segment of the *CODM* CDs 1200 bp.

Table 1: Sequence of PCR primers

Forward	*5'- ATGGAGACACCAATACTTATCA -3'*
Reverse	*5'- CACATCCTCATGTA**G**TCGAG -3'*

Polymerase Chain Reaction (PCR) Amplification of CODM gene

PCR reagents and protocol were used at listed concentrations in 25µL reactions. A magnesium curve from 2mM to 4mM was performed to identify optimal reaction conditions for High Fidelity *Pfu* Polymerase. 1µL of 575ng/µL genomic template DNA was used in each reaction. A negative control without template DNA was performed in order to identify any potential errors. PCR was performed under varying conditions until successful. PCR amplification of the *CODM* gene was accomplished using the following program:

- ✓ Initiation Denaturing Temperature at 94°C for 4 minutes.
  - o For 35 cycle:
    - Denaturing Temperature at 94°C for 30 seconds.
    - Annealing Temperature at 60°C for 60 seconds.

- Extension Temperature at 72°C for 90 seconds.

✓ Final Extension Temperature at 72°C for 10 minutes.

✓ Sample hold at 4°C.

Gel Electrophoresis

Gel electrophoresis was used to visually confirm the presence of PCR products. 1% agarose gel (Sigma-Aldrich) was cast using 0.5X TBE buffer and Ethidium Bromide (EB) (Sigma-Aldrich, CAS Number 1239-45-8) 1.5mL/45mL gel was added to stain the gel. 6X loading dye were added to each sample at appropriate dilutions. Banding lengths were determined by comparison to the 1Kb plus Ladder by Invitrogen® (Carlsbad, California, USA). Gels were typically run at 110V, until banding patterns were evident.

Sequence Analysis of CODM gene

Isolated DNA from *Papaver fogax* and *Papaver oriental* was sequenced at BIONEER (in Korean) CO, using M13 Forward and Reverse primers, as well as designed custom sequencing primers. The PCR product with the fewest number of mutations furthest away from the start codon was selected for further experimentation. After the direction of the sequence to analysis the *CODM* gene we used the NCBI database.

Data sets

The peptide sequence from *CODM* gene generated by ExPasy database, http//:web.expasy.org/translate. Structurally homologous subsets of the experimentally determined 3D structures of the LEA proteins were retrieved from PDB and Phyre 2 and HMMER databases.

RESULTS

DNA Extraction

Extracted DNA from *Papaver fogax* and *Papaver oriental* leaves was quantified at 575ng/µL and confirmed by the presence of a band through gel electrophoresis.

Polymerase Chain Reaction (PCR) Amplification of *CODM* gene

PCR was performed using custom designed primers to amplify the DNA sequence of the *CODM* gene (figure 1).

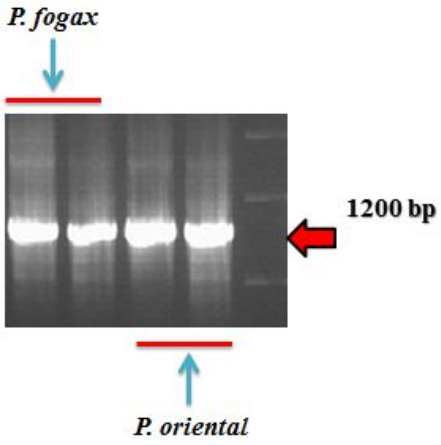

Figure 1. PCR result.

Sequence Analysis of CODM gene

All samples were sent for sequencing with M13 Forward and Reverse primers and custom designed sequencing primers. Sequence analysis revealed minor mutations in all samples. Each sequence was analyzed individually using Chromas Sequence Chromatogram Viewer (Figure 2). When sequenced using M13 primers by employing primer walking technique revealed 100% similarity with the reported sequence in NCBI [5]. The sequence of this gene *via in silico* technique converted to the protein sequence and peptide sequence used for generated 3D protein structure.

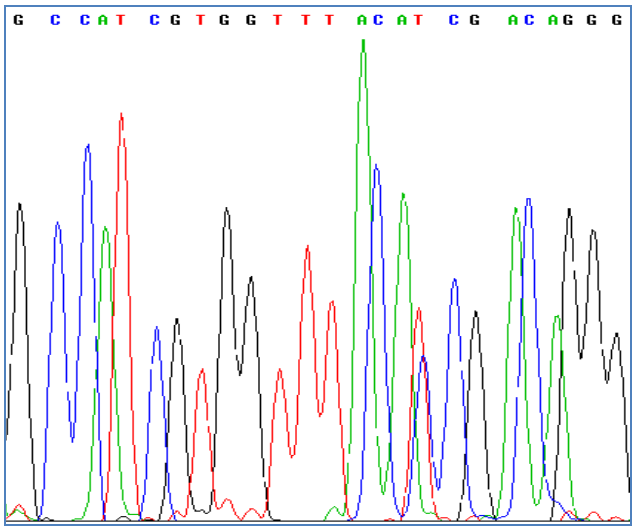

Figure 2: Chromatograms showing minor mutations occurring in the *CODM* gene.

Prediction 3-dimensional structure of CODM

Comparative modeling to build 3D structure of the CODM protein was made based on the experimentally solved structural homologous. The amino acid sequences of CODM protein in *Papaver fogax* and *Papaver oriental* were submitted to Phyre 2 server (Figure 3). The atomic coordinates for the proteins were generated based on Hidden Markov Model in the HMMER database.

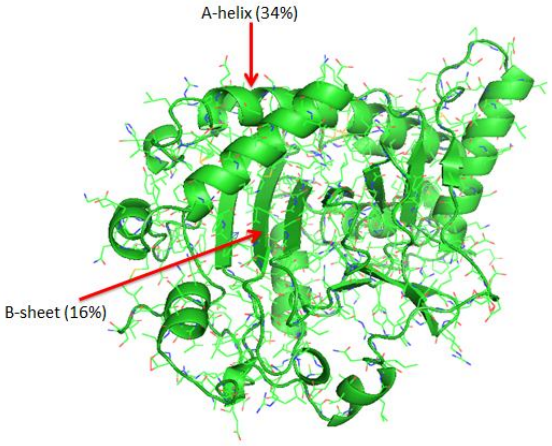

Figure 3: Predicted 3-Dimensional Structure of the CODM protein

Identification of the conserved domain

This computational analysis focuses on identified a number of conserved domain structure and conserved sequence in *CODM* gene. *In silico* analysis provides an efficient way to indicating conserved sequence and conserved domains in genome [2,3]. However, by using the *in silico* analysis we can predicating and identified the conserved domain structure and conserved sequence in gene sequence. In this study a total of 3 conserved domain structure and 2 conserved sequence identified in *CODM* gene sequence (Figure 4).

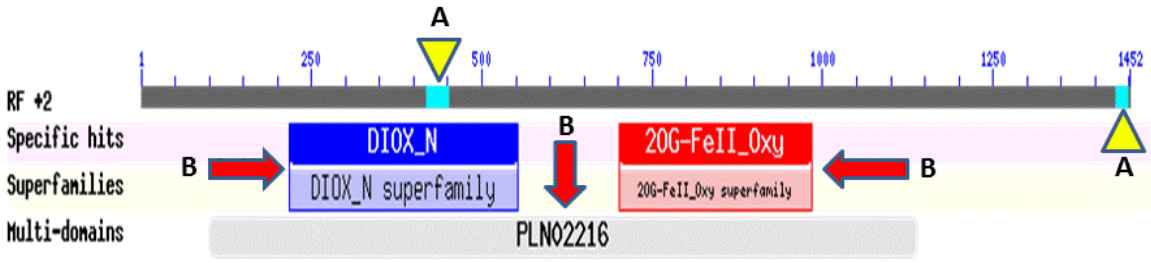

Figure 4: Conserved sequence (A) and conserved domain (B) in *CODM* gene.

Identification of DNA binding-site:

To identify DNA binding-site, we used the BindN database. Our result showed in the CODM protein sequence have been a total of 60 DNA binding-site.

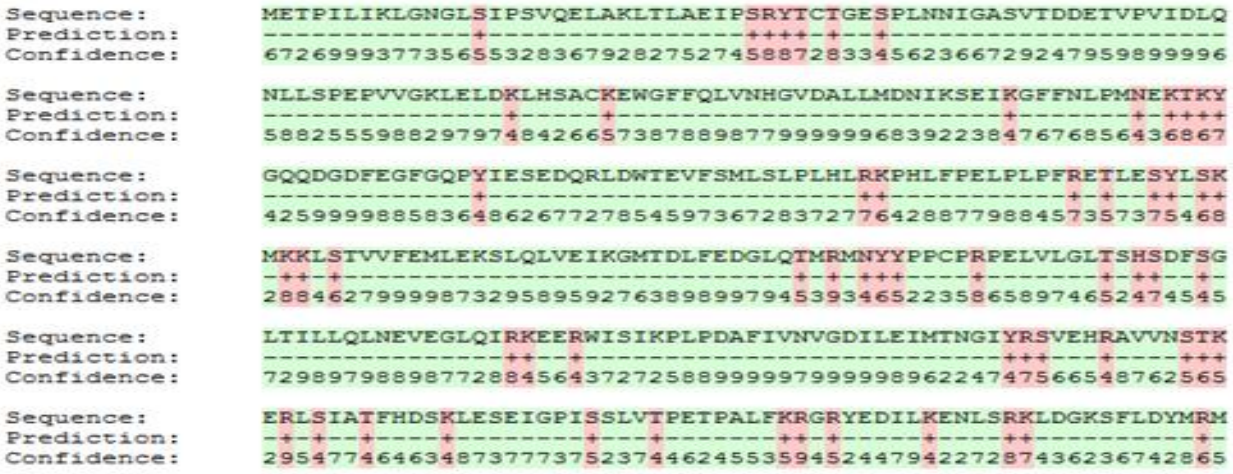

Figure 5: Binding residue in CODM protein sequence. Binding residues are labeled with"+ and in red. Non-binding residues are labeled with "-"and in green.

DISCUSSION

Prediction of 3D structure of a protein molecule signifies an important step towards understanding the structure–function relationships in the concerned protein family [2] In the present study, model of CODM protein of *Papaver fogax* and *Papaver oriental* was generated from the Phyre 2 server, based on the Structural homologues derived from the HMMER and protein databanks. The generated model could be helpful in understanding functional characteristics of this important class of protein. The fast and reliable method to obtain the full-length genomic sequence of *CODM* gene might be from genome sequencing. Prediction of 3D structure of a protein molecule signifies an important step towards understanding the structure–function relationships in the concerned protein family.

Conflict of interest statement

The authors have no conflict of interest related to this article to declare.

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