

Identification and cloning of putative water clarification genes of *Moringa peregrina* (Forssk.) Fiori in *E. coli* XI₁ blue cells

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Abstract

Background: Water purification processes include the use of chemical compounds despite the concern that they may induce diseases. An ecological solution to this dilemma can come from the use of plant seeds for this purpose. *Moringa peregrina* (Forssk.) Fiori seeds have water clarification ability. Therefore, the aim of this work was to look for certain water clarification genes in *M. peregrina*.

Materials and Methods: After preparation of *M. peregrina* callus, mRNA was extracted from these cells. After application of reverse transcriptase, the obtained cDNA (s) were used for PCR amplification of the desired genes using primers based on MO_{2,1} gene of *Moringa oleifera*. DNA amplification products were cloned in *E. coli* XI₁ blue cells and DNA sequences were compared with Mo_{1,2} gene in *M. oleifera*.

Results: We obtained 3 PCR products (approximately 200, 300, and 400 bps).

Conclusion: After comparison of the sequences of 300bp band obtained from *M. peregrina* with Mo_{1,2} gene in *M. oleifera*, it seems that 300bp band is a good candidate to investigate regarding its potential flocculent activity.

Key Words: Flocculent activity, genes cloning, *Moringa peregrina*

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INTRODUCTION

More than 3.5 million people die each year from water-related diseases; 84 percent of them are children and 98 percent of the total deaths occur in

the developing world.^[1] Nowadays a variety of water purification processes exist including the building of complex engineering solutions that typically require the use of a chemical purification process. The most common type of water treatment systems used in drinking water facilities is the processes of coagulation and flocculation to remove the organic and inorganic particles from water before the disinfection process.^[2] Modern systems typically use chemical compounds like alum and ferric salts to change the water from a liquid to a semi-solid in a process called coagulation.^[3] However, the use of these chemicals may induce Alzheimer or other diseases.^[4,5]

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An ecological, economical, and sustainable solution to this dilemma can come from the seeds of a *Moringa* species commonly known as the miracle tree. *Moringa* is the sole genus in the flowering plant family Moringaceae. The 13 species it contains are from tropical and subtropical climates and range in size from tiny herbs to massive trees.^[6] The *Moringa* tree has a wide range of use in the areas of agriculture, health, and industry for developing countries. *Moringa* serves as a medicinal plant, animal fodder, and a food source for humans. However, the *Moringa* tree is most praised for its nutritional abilities. Just about every part of the *Moringa* can be consumed and it contains some concentrations of vitamins and minerals.^[7,8] The most widely known species is *Moringa oleifera*, a multi-purpose tree native to the foothills of the Himalayas in northwestern India.^[9] *M. peregrina* (Forssk.) Fiori is also widely grown, but to a much lesser extent than *M. oleifera* in Saudi Arabia, India and south of Iran.^[10]

Moringa oleifera contains small storage proteins capable of flocculating particles in suspension in water that can be used for water treatment.^[11] Preliminary studies have suggested that the active components are water-soluble cationic peptides with molecular masses ranging from 6 to 16 kDa. The coagulating activity associated with *M. oleifera* Lam. seed extracts had indicated the activity of low molecular weight proteins. The sequence of one of these proteins has been determined and shown to be a positively charged 6-kDa polypeptide (around 200bp), called MO_{2.1}. It acts as a water clarification agent.^[12,13] Flo is also an antibacterial peptide isolated from seeds of a tropical tree (*Moringa oleifera* Lam.). The amino acid sequence of Flo and its sub-fragments were based on *Moringa oleifera* Lam. seed component MO_{2.1}. Flo has antibiotic activity that leads to growth inhibition and killing of bacteria, including antibiotic-resistant human pathogens. (Swiss-Prot primary accession number: P24303). *Moringa peregrina* seeds also have shown coagulating activity^[14] and antioxidant activity.^[15] Its antioxidant activity has been reported to be related to flavonoid and glucosinolates of the plant^[16] but seed's protein seems to be responsible for coagulating activity.

In the present study, the presence of MO_{2.1} protein in *Moringa peregrina* was studied. Also, the DNA sequences of *M. peregrina*, using primers based on MO_{2.1} of *M. oleifera*, were amplified, and the DNA amplified products were cloned in *E. coli* XL1 blue cells and DNA sequences were compared with *M. oleifera*.

MATERIALS AND METHODS

The cDNA(s) coding for putative water clarification genes were amplified by RT-PCR from poly-A RNAs

purified from callus of *M. peregrina*. *M. peregrina* seeds were provided by Kerman Jahad Keshavarzi Research Center. The fresh callus of *Moringa peregrina* was obtained using seedling on Murashige and Skoog media^[17] Callus was crushed in liquid nitrogen and mRNA was extracted using the "PLANT RNeasy kit"(QIAGEN, Germany) and cDNA was prepared by "RevertAid™ First strand cDNA synthesis kit"(Fermentas, Poland). Primers were designed based on the sequence of MO_{2.1} protein in NCBI. The sequences of forward and reverse primers were 5'-CAG GGA CCT GGT CGG CAG CCG GAC TTT CAG-3' and 5'-TTA GGT GCT AGG TAT ATT GGA TGC CAC TCG GTA-3', respectively. PCR conditions were as follows: 94°C, 30 s; 36°C, 30s; 72°C, min; 30, cycle. Three DNA fragments of around 200 bp, 300bp, and 400bp were amplified. After extraction of these fragments from agarose gel, they were cloned in pTZ57R/T vector using "InstAclone™ PCR cloning kit"(Fermentas, Poland. Subsequently, the prepared plasmids by "Gene JET™ plasmid, miniprepkit"(Fermentas, Poland) were sent for sequencing (Kowsar Biotech, Iran).

RESULTS

mRNA was isolated from *Moringa peregrina* [Figure 1] and after preparation of cDNA, PCR amplification of putative water clarification genes was performed using specific primers as described in Materials and Methods. As presented in Figure 2 three bands of approximately 200, 300, and 400 bps can clearly be seen. After cloning of these fragments into pTZ57R plasmid, their presence was confirmed by double digestion with *sacI* and *smaI* restriction enzymes [Figures 3 and 4].

After sequencing these fragments, only 300bp band showed some homology with MO_{2.1} sequence (60% homology) while 200 and 400bp bands did not show any similarities with MO_{2.1} sequence MO_{1.2} sequence:

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CAGGGACCTGGTTCGGCAGCCGGACTTTTCAGCGT
TGCTGCCAACAGCT GCGGAACATATCTCCTCCT
TGCAGGTGCCATCACTCAGGCAAGCAGTACAG
TTGACACACCAGCAGCAGGGACAGGTGGGTCTCCT
CAGCAGGTAAGGCAGATGGTACCGAGTGGCATC
CAATATACCTAGCACCTAA.
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Cloned sequence

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CAGGGACCTGGTTCGGCAGCCGGACTTTTCAGTGA
NNNNNNNNNNATAGCATCTGAATGATGCCACA
GGGTTTTGAGAACANATTCACCTTCTTCACTGCT
GGATTTTAGCAACTCCACACCCAAGTAACATCTG
CAAAATATGTAAAAAATGATCTTTTCCAACCATGA
AGGAAAATAAACCTGCAGTTTTTCTTTCACAAGCA
ACAAATAAAGGAAAGAGTCTCTAGCATTCTCTAAA
GTACAAGTAGATTATAAAGTGCCACGACCCTTC
```

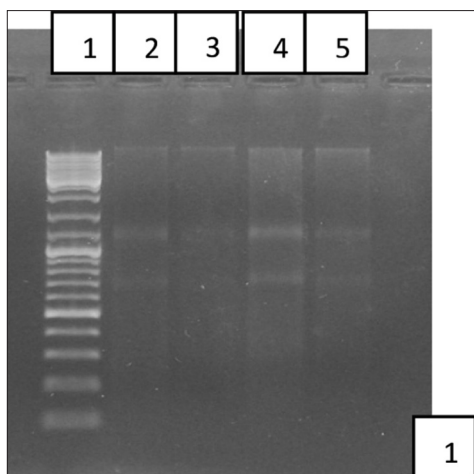


Figure 1: Electrophoresis of the prepared mRNA from f *Moringa peregrina*. 5 μ l of the product was electrophoresed on 1% agarose gel. Lane 1: DNA size marker. Lane 2, 3, 4, 5: mRNA samples

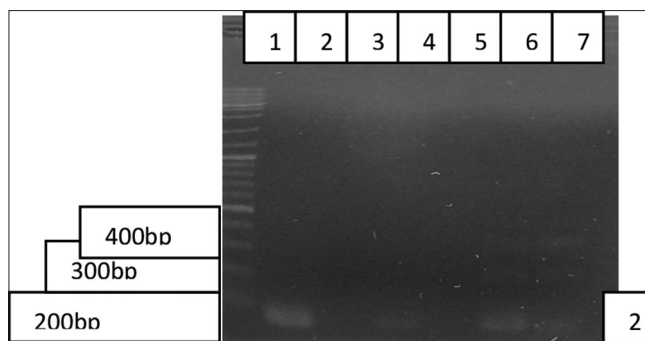


Figure 2: Electrophoresis of the obtained PCR products. 3 μ l of the product was electrophoresed on 0.8% agarose gel. In lanes 6 and 7 three bands of approximately 200, 300, and 400 bp bands can be observed. Lane 1: DNA size marker. Lanes 2-7: PCR product sample. Lane 1: DNA size marker. Lanes 2-7: PCR product sample

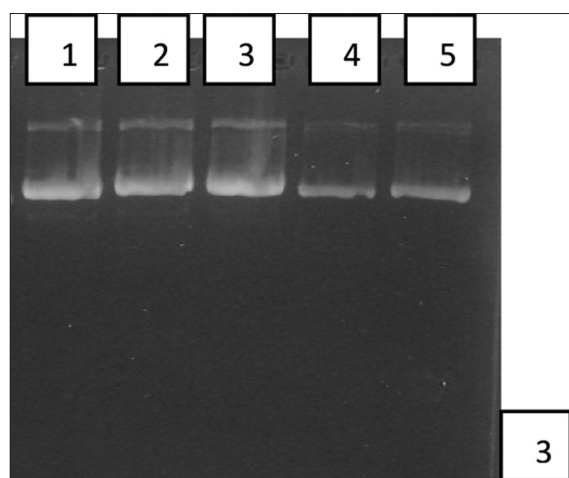


Figure 3: pTZ57R plasmids obtained after ligation with the obtained PCR fragments (lanes 1-5). 5 μ l of each plasmid was loaded on 0.8% agarose gel and electrophoresed

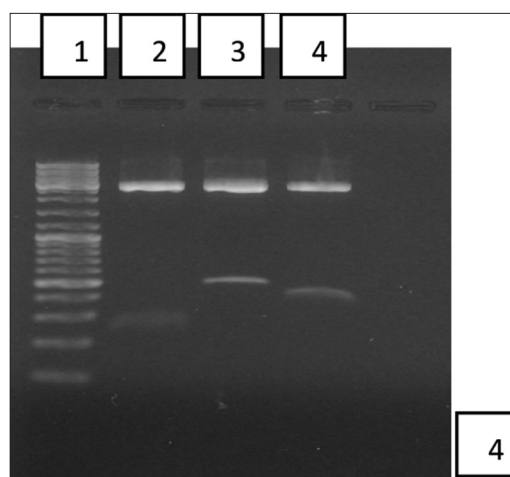


Figure 4: Double digestion of the recombinant pTZ57R plasmids with *sacI* and *smaI* restriction enzymes. 5 μ l of each sample was loaded on 0.8% agarose gel and electrophoresed. Three bands of about 200 bp and 300bp and a 400bp were observed after digestion. Lane 1: DNA size marker. Lane 2, 3 and 4: Recombinant pTZ57R plasmids Digested with *SacI* and *SmaI* restriction enzymes

TCAAGGTCAGATATCCATAAGCTTTTGTGTGATT
ACTTGTAATAGGTGTATCATGTGAACAAAGAAA
ATTAANAAAAAAGAAGGTGCTATAACCGATAGCTC
TGGCAAATCCAACGAGCAAGTGTCTGAGCTTCA
GGAGTACCGAGTGGCATCCAATATACCTAGCACC
TAA.

DISCUSSION

Moringa is the sole genus in the Moringaceae plant family and contains several species including *M. Olifera* and *M. Peregrina*. A protein with high flocculant activity was identified by Gassenschmit *et al.*, 1995. Several proteins fraction with flocculant activity were identified but only one of them, Mo_{1,2}, was entirely sequenced.^[18] Short amino acid sequences of two other proteins, Mo_{1,3} and mo_{1,4}, were also determined. Broin *et al.*, cloned and expressed the recombinant protein MO (2.1), in *Escherichia coli*.^[13] This protein, MO (2.1)

had a flocculant activity as assessed by different methods. This suggests that several protein families with flocculant activity can be identified in *Moringa* genus.^[19]

In the present study we decided to search for a similar protein in *M. Peregrina*. We obtained three PCR products from *Moringa peregrina* and compared their sequences with the flocculant protein, Mo_{1,2}, in *Moringa oleifera*. One of the open reading frames (ORF) of the 300bp band seemed to have approximately 60% homology with Mo_{1,2}, sequence. Other ORFs for the 300 and well as 200 and 400 kb bands did not lead to a meaningful coding sequence.

CONCLUSION

It can be concluded from this study that based on the homology found between the 300bp band and Mo_{1,2}, it is possible that this fragment codes for a protein

with flocculent activity in *Moringa peregrina*. Further experiments are required to examine this finding.

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