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Isolation and sequencing of *Myo*-Inositol Phosphate Synthase cDNA from common bean (*Phaseolus vulgaris* L.)

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Abstract

Myo-Inositol Phosphate Synthase (MIPS; EC 5.5.1.4) catalyses the first committed step in inositol biosynthesis. The enzyme converts D-glucose 6-phosphate, an intermediate of the glycolytic pathway, to *myo*-inositol phosphate (Ins phosphate), which is subsequently converted to *myo*-inositol by the action of inositol monophosphatase [3]. It has been demonstrated that MIPS is a key enzyme during the early stages of common bean embryo development [2]. Its activity correlates positively with the relative growth rate of common bean in early embryo development such as globular and cotyledonary stage and during postembryonic development which show that its expression is temporally and spatially regulated [1], [2].

To gain a better understanding of the role and regulation of MIPS in common bean embryo development, we isolated a MIPS cDNA clone, from a cDNA library prepared from mRNA of common bean (*Phaseolus vulgaris* cv. BAT93) seed 15 days after anthesis (DAA). This was followed by 5'-3' rapid amplification of cDNA ends (RACE). The cDNA is 1872 bp long and contains an open reading frame encoding a protein of 510 amino acids with ATG at position 82 and the stop codon TGA at position 1609. The predicted amino acid sequence is very similar to the MIPS sequences of other plants (Figure 1). It shows 88% similarity to MIPS nucleotide sequences of *Glycine max* (GenBank AY038802), 87% to *Phaseolus vulgaris* (GenBank AJ853494), 86% to *Medicago sativa* (GenBank EF408869), 80% to *Nocotina tabacum*, 79% to *Arabidopsis thaliana* (Genbank NM120143), 78% to *Triticum aestivum* (GenBank AF120148), and 77% to *Zea mays* (GenBank AF056326) respectively. The PvMIPS polypeptide contains four highly conserved motifs found in other plant species: GWGGNNG (domain 1), VLWTANTER (domain 2), NGSPQNTFVPGL (domain 3) and SYNHLGNNDG (domain 4). These 4 domains are involved in MIPS protein binding and are essential for MIPS functions such as cofactor NAD^+ binding and reaction. RT-PCR expression analyses were carried out to detect endogenous common bean MIPS transcripts in different common bean organs and developing seeds. The results revealed the presence of common bean MIPS transcripts in ovules, cotyledons, leaves, and flowers while no signal could be observed in stems or roots. Common bean MIPS transcripts were also observed in the different

seed development stages: globular, heart, torpedo, and cotyledonary stages. Genomic Southern analysis was carried out to determine the complexity of the MIPS encoding gene(s). Five different restriction enzymes were used, yielding DNA fragments of various sizes. These banding patterns suggest that at least 3 copies of common bean MIPS are present in the *Phaseolus vulgaris* genome. This is the first report of a full-length cDNA of MIPS from *Phaseolus vulgaris* cv. BAT93.

Materials and Methods

3'and 5' RACE and the full-length cDNA cloning of MIPS

Full-length cDNA clones were generated by RACE (rapid amplification of cDNA ends) technology. 5'- or 3'-RACE was performed with the SMART™ RACE cDNA Amplification Kit (Clontech) as described by the supplier. First-strand cDNA synthesis was performed on 500 ng of poly(A)$^+$ RNA isolated from seed (15 DAP), the 5'/3' cDNA synthesis primer, SMART II™ oligonucleotide, and Power-Script™ reverse transcriptase. This cDNA was then used in a PCR using universal primer mix and a 5' RACE gene-specific primer, 5'-AGGTTTGAGGGGCTGAA-3', or a 3' RACE gene-specific primer, 5'-ATCTGGCTATCAGGAGG-3'. After electrophoretic separation on a 1% agarose gel, the PCR product was diluted and subject to a second round of PCR using the nested universal amplification primer, 5'-AAGCAGTGGTATCAACGCAGAGT-3'. Amplification were performed with initial denaturation step at 94°C for 3min, 25 cycles of 94°C for 30 s, 60°C for 45 s and 72°C for 3min, and a final elongation step at 72°C for 10 min in the Gene Cycler™ (Bio-Rad). PCRs were performed with Platinum *Tag* High-Fidelity DNA polymerase (Invitrogen). Products were purified with the Gene Elute Gel Extraction Kit (Sigma) and subsequently cloned into pJET1.2/blunt vector using the CloneJET™ PCR Cloning Kit (Fermentas).

MIPS sequencing, detection of homology and sequence alignment

Plasmid MIPS was prepared from positive clones with the Qiagen Mini Prep Kit and sequencing with the Thermo Sequenase™ DYEnamic™ Direct Cycle Sequencing Kit (GE Healthcare, UK). MIPS sequencing was performed on an LI-COR sequencer (Westburg). Sequence data were compiled and analyzed with the e-seqV2.0. Homology between the predicted amino acid sequences and those present in the GenBank database was detected by using BLAST 2.0, provided by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST/.).

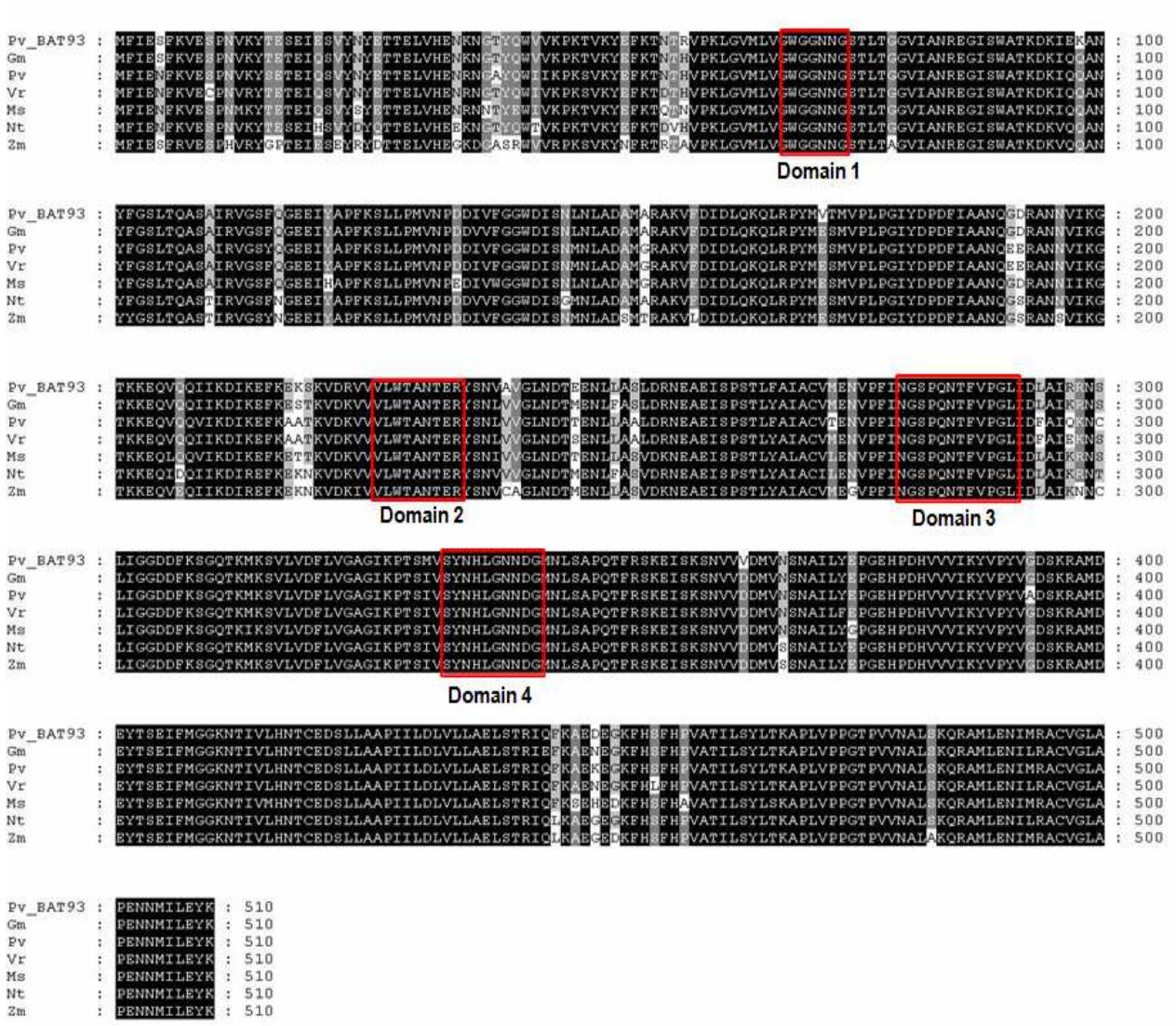

Figure 1. Comparison of the deduced amino acid sequences, showing the presence of conserved amino acid residues in the *Phaseolus vulgaris* cultivar BAT 93 (Pv_BAT93) translated sequence, when compared with other known eukaryotic MIPS sequences such as *Glycine max* (Gm; GenBank ABC55421), *Medicago sativa* (Ms; GenBank ABO77439), *Phaseolus vulgaris* (Pv; GenBank CAH68559), *Vigna radiata* (Vr; GenBank ABW99093), *Nicotiana tabacum* (Nt; GenBank BAA95788) and *Zea mays* (Zm; GenBank ACG33827).

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