ISOLATION, MOLECULAR CLONING AND CHARACTERIZATION OF PYK10 PROMOTER REGION FROM *ARABIDOPSIS THALIANA*

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Abstract
***Pyk*10 is a root and hypocotyl specific myrosinase from *Arabidopsis thaliana*. Myrosinase are a group of isoenzymes catalyzing the hydrolysis of glucosinolates. pyk10 is a root and hypocotyls specific myrosinase gene from *Arabidopsis thaliana*. In order to study the *pyk*10 promoter and the genomic structure of the gene, The promoter fragment of pyk10 was cloned by PCR method from Arabidopsis thaliana genomic DNA. Nucleotide sequence analysis showed that the fragment cloned was consist of 797bp. Comparison the sequence obtained with that reported (GenBank Accession no. AJ292756, CP002686, AC011436) showed that the nucleotide homology was as high as 100%. The pyk10 promoter contains regulatory sequence elements that have shown to be functional cis-sequences in other plant promoters, such as ACGT-, CANNTG-, GATA and I-box-cis-elements, which are binding sites for organ and tissue-specific transcription factors.**

Index Terms*- PYK 10, PCR-Polymerase Chain Reaction, cloning, promoter, *Arabidopsis thaliana

Introduction

Myrosinases (thioglucoside glucohydrolases) are a group of isoenzymes catalyzing the hydrolysis of glucosinolates, a class of sulfur and nitrogen containing plant secondary metabolites, present mainly in species of the *Brassicaceae* family. The degradation products are supposed to be involved in defence against pests and plant pathogens as well as in sulfur and nitrogen metabolism and growth regulation [1, 4, 5, 6, 7]. The activity and specificity of myrosinases are affected by interacting proteins, classified as myrosinase-binding proteins (MBPs), myrosinase-binding proteins related proteins (MBPRPs) and myrosinase-associated proteins (MyAPs) [2,3,10]. Studies of myrosinases in *Brassica napus* and *Sinapis alba* showed that they are encoded by large gene families, designated MA, MB and MC, with up to 15–20 genes in *B.napus* [2,9,11]. In *A. thaliana*, a small gene family consisting of only three genes (TGG1, TGG2,TGG3) was assumed [10]. However, the related promoter and genomic DNA sequences as well as expression profiles of myrosinase gene remain largely unexplored in *Arabidopsis thaliana* . In this study, the 797 bp genomic DNA sequences, designated as PYK10, was isolated ,cloned and sequenced from *Arabidopsis thaliana*.

2. Materials and methods

2.1Plant Materials

The plant material for this study was taken from Ajeet Seeds Pvt Ltd, Maharashtra. Fresh young leaves from all plants were collected for DNA extraction.

2.2 DNA isolation

Genomic DNA was isolated according to a modified CTAB method (Zhu et al., 2010)[15]. The concentration and quality of the obtained genomic DNA samples were estimated by measuring O.D. at 260/280 nm in UV spectrophotometer. Finally, all the genomic DNA samples were diluted to a final concentration of 40ng/µl with 1X TE buffer (10mM Tris-HCL; pH 8.0; 1mM EDTA). Intactness of genomic DNA was checked by agarose gel electrophoresis. DNA samples were stored at -20ºC for further use.

2.3Amplification of PYK 10 by PCR

The gene for *PYK 10* was amplified from DNA using *PYK 10* specific primers(Forward: 5' GAA TTC TTG ACC ACC ATA ACT GAT 3' & Reverse: 5' GGA TCC TTT TGT TTG TAA TTC TGA T 3').PCR reaction was performed using 40ng of DNA along with forward and reverse primers(10pmol each),200µM of dNTP's, 1.5mM Mgcl2 and 3U of high fidelity DNA polymerase(Promega, USA).The amplification cycle was initial 5 minutes denaturation, after that 35 cycles of denaturation at 94ºc for 20sec, annealing at 58ºc for 30sec and extension 72ºc for 1.30min.Final extension 72ºc for 7min.The PCR amplified product was analysed on 1% agarose gel along with DNA molecular weight marker.

2.4 Cloning and characterization of PYK 10 gene

The amplified *PYK* gene fragment was gel purified and ligated into pGEMT easy vector (Promega, USA).The recombinant plasmid *(pGEMT-PYK)* was characterized using E.coRI restriction digestion and analysed on 1% agarose gel for the insert. The gene in recombinant plasmid *pGEMT-PYK* was sequenced in Eurofins Genomics, Bangalore and sequence was blast in NCBI.

3. Results

3.1DNA isolation and amplification of PYK 10 by PCR

DNA isolated from leaf material of *Arabidopsis thaliana* presented in Figure 1. The gene for *PYK 10 was* amplified from DNA using *PYK 10* specific primer by using PCR in a reaction catalyzed by the enzyme Taq polymerase is presented in Figure2.

3.2 The construction and identification of PYK 10 gene.

The *PYK10* gene of the length of about 797 bp was amplified from DNA. The gene was then cloned in *pGEMT easy* vector (Promega, USA) which was confirmed by the restriction digestion experiment using *E.coRI* (Figure 3). The sequencing analysis confirmed the amplified gene to be 100 % identical to the target gene (GenBank Accession no. AJ292756, CP002686, AC011436). Sequence was deposited in the NCBI Gen Bank (ID KM236584).

Figures

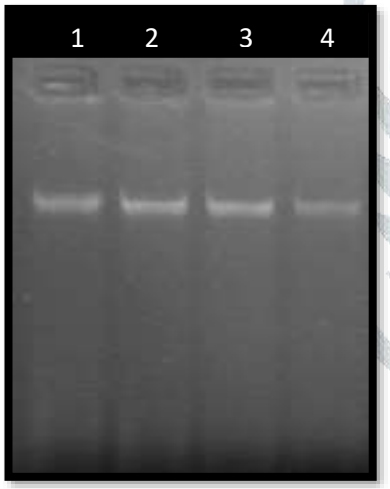

Fig1 Isoalation of DNA

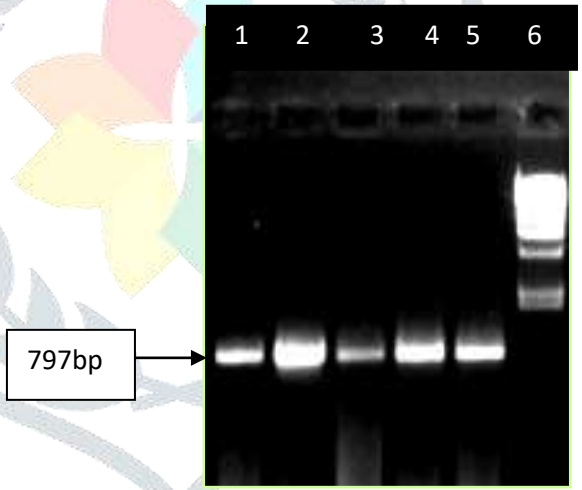

Fig2: PYK 10 amplification by PCR

Upon completion of DNA isolation , agarose gel (1%) was run at constant volt (100amp) for 1hr. **(Fig1)**Lane 1-4- Genomic DNA isolated from *Arabidopsis thaliana* leaf sample.**(Fig 2)**Lane 1-5- PYK 10 gene amplified from DNA, Lane 6- HindIII Maker (500bp- 10kb**).**

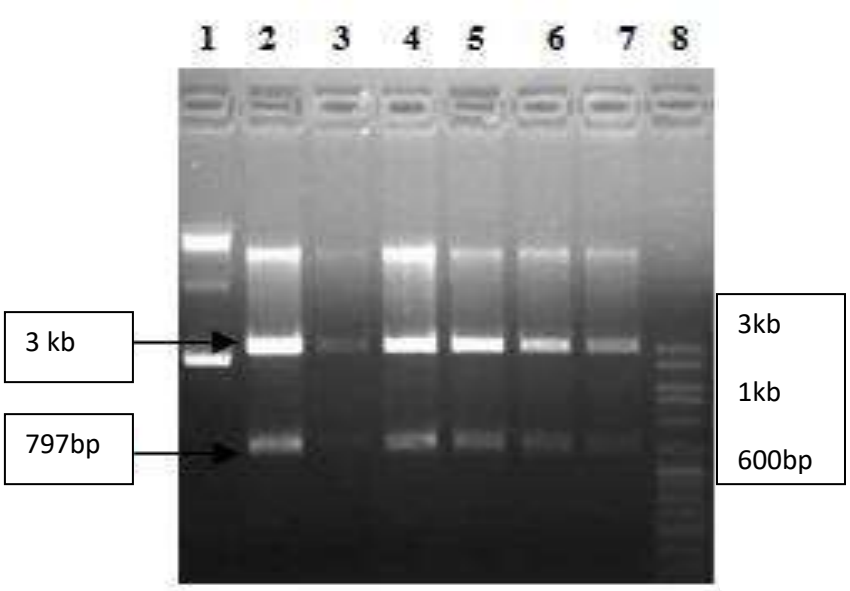

Fig 3. Characterization of recombinant plasmids.

Upon completion of restriction digestion, agarose gel (1%) was run at constant volt (100amp) for 1hr. (**A)** Restriction digestion of *pGEMT-PYK* by *E.coRI,* Lane 1-uncut plasmid, Lane 2-7-clone which contain *PYK 10* gene, Lane 8- 3kb Maker (100bp- 3kb**).**

Discussion

Pyk10 is a root and hypocotyls specific myrosinase gene from Arabidopsis thaliana. Myrosinase plays an important role in protecting plants against pathogens and insect pests by initiating breakdown of the secondary metabolites glucosinolates into toxic products (Textor and Gershenzon, 2009). In this report, we characterize the *pyk*10 gene with respect to its genomic structure and the promoter region responsible for root specific expression. These results could provide valuable information to elucidate the molecular characteristics of myrosinase gene and facilitate further investigation of the biological function of myrosinase in *Arabidopsis thaliana*.

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