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A novel chloroplastic isopentenyl diphosphate isomerase gene from *Jatropha curcas*: Cloning, characterization and subcellular localization

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ABSTRACT

Background: Jatropha curcas is a rich reservoir of pharmaceutically active terpenoids. More than 25 terpenoids have been isolated from this plant, and their activities are anti-bacterial, anti-fungal, anti-cancer, insecticidal, rodenticidal, cytotoxic and molluscicidal. But not much is known about the pathway involved in the biosynthesis of terpenoids. The present investigation describes the cloning, characterization and subcellular localization of isopentenyl diphosphate isomerase (IPI) gene from *J. curcas*. IPI is one of the rate limiting enzymes in the biosynthesis of terpenoids, catalyzing the crucial interconversion of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP).
Results: A full-length *JcIPI* cDNA consisting of 1355 bp was cloned. It encoded a protein of 305 amino acids. Analysis of deduced amino acid sequence predicted the presence of conserved active sites, metal binding sites and the NUDIX motif, which were consistent with other IPIs. Phylogenetic analysis indicated a significant evolutionary relatedness with *Ricinus communis*. Southern blot analysis showed the presence of an *IPI* multigene family in *J. curcas*. Comparative expression analysis of tissue specific *JcIPI* demonstrated the highest transcript level in flowers. Abiotic factors could induce the expression of *JcIPI*. Subcellular distribution showed that JcIPI was localized in chloroplasts.
Conclusion: This is the first report of cloning and characterization of IPI from *J. curcas*. Our study will be of significant interest to understanding the regulatory role of IPI in the biosynthesis of terpenoids, although its function still needs further confirmation.

1. Introduction

Terpenoids encompass an extraordinary variety of primary and secondary metabolites in bacteria, fungi, plants and animals. They play vital roles in the structure of cells, electron transport, photosynthesis, cell-to-cell signaling and interactions between organisms [1]. In addition, many terpenoids have important commercial values in medicine, industry and agriculture [2]. All terpenoids originate from the head-to-tail condensation of isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP). IPP and DMAPP are both deemed universal precursors of terpenoid biosynthesis (Fig. 1) [3]. Isopentenyl diphosphate isomerase (IPI, EC: 5.3.3.2) catalyzes the crucial interconversion of IPP and DMAPP, and thus regulates the IPP/DMAPP pool. An appropriate molar ratio of IPP to DMAPP is needed for synthesis of various terpenoid classes. So IPI is considered as a key enzyme in the regulation of terpenoid biosynthesis [4]. The characterization of this enzyme has been first reported in the baker's yeast by Agranoff et al. [5]. Since then, it has been found in most living systems. Kajiwara et al. [6] have reported that an *IPI* cDNA from *Haematococcus pluvialis* heterologously expressed in *Escherichia coli* could elevate contents of β-carotene and lycopene by 2.7 and 4.5 fold, respectively. Later, several similar results have been reported that overexpressions of plant *IPIs* in engineered *E. coli* lead to increased accumulation of β-carotene [7,8]. Now several *IPI* genes have been isolated from higher plants such as *Clarkia breweri* [9], *Melaleuca alternifolia* [10], *Daucus carota* [11], *Camptotheca acuminate* [12], and *Ipomoea batatas* [8].

Jatropha curcas belongs to the family Euphorbiaceae and is used in traditional folklore medicine to cure various ailments in Asia, Africa and Latin America [13]. It has been used to cure diseases like cancer, snake bites, paralysis, piles and dropsy [14]. Terpenoids are the main active secondary metabolites in *J. curcas*. Till now, at least 25 terpenoids have been isolated from this plant. They have shown a wide array of pharmacological properties including anti-bacterial, anti-cancer, insecticidal, rodenticidal, cytotoxic and molluscicidal [15]. However, some terpenoids (phorbol esters) in *J. curcas* are toxic to animals. They prevent seed cakes containing high content of proteins to be used as animal feed, which thus wastes most protein resource in the seeds [16]. Therefore, increasing the content of active terpenoids

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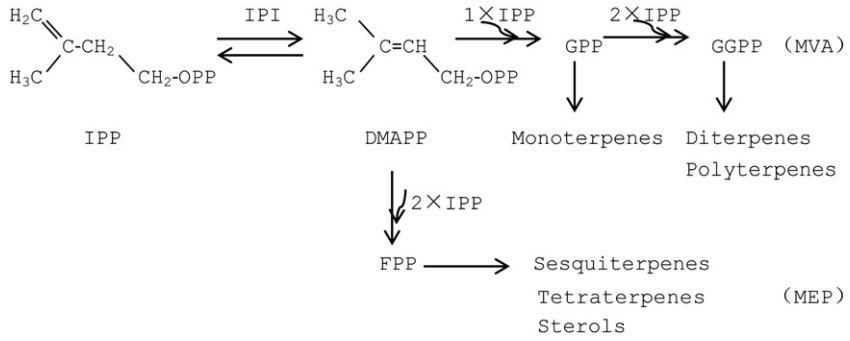

Fig. 1. Overview of terpenoid biosynthesis from IPP and DMAPP. It indicates that the IPP and DMAPP are required for various classes of terpenoids. FPP, farnesyl diphosphate; GPP, geranyl diphosphate; GGPP, geranylgeranyl diphosphate; MEP, methyl erythritol phosphate pathway; MVA, mevalonate pathway; OPP, diphosphate moiety. The block arrows denote multiple steps, and the double arrows reflect the expected equilibrium of the reversible IPP isomerase (IPI) reaction.

or decreasing the toxic ones could enhance the comprehensive utilization of *J. curcas*.

Recent interest generated in the regulation of terpenoid content has prompted many workers to elucidate the biosynthetic pathway. As the biogenesis of terpenoids is yet to be fully understood in *J. curcas*, it is imperative to identify, clone and characterize key pathway genes to understand the regulatory role of various enzymes. Up to now, several key genes involved in terpenoid biosynthesis pathway have been isolated and characterized from *J. curcas*. However, there is little information about the cloning and characterization of *Jatropha IPI*.

The present investigation was aimed at molecular characterization of IPI from *J. curcas*. Using degenerate primers and RACE PCR strategy, we have cloned a full-length cDNA encoding *JcIPI*. The gene was analyzed in detail, expression analysis was performed in specific tissues or under stress conditions, and southern-blot analysis was carried out. *JcIPI* heterologous expression in *E. coli* and subcellular localization in tobacco were also included in this study.

2. Materials and methods

2.1. Plant materials

The *J. curcas* mature seeds, roots, stems, leaves, flowers, barks and yellow fruits were collected from a five-year old plant in Sichuan Province, China, then snap frozen in liquid nitrogen and transferred to a -80°C freezer till further use for comparative tissue specific expression profile. Young seedlings of *J. curcas* germinated from the collected mature seeds were cultivated in a culture room maintained at 26 ± 2°C under a 16/8 h (light/dark) photoperiod. *Nicotiana tabacum* cultivar growing in soil was used for protoplast isolation and transient expression analysis.

2.2. RNA isolation and cDNA synthesis

Total RNA was isolated from the leaves of *J. curcas* using RNAprep pure Plant Kit (Tiangen, China). The quality and quantity of RNA were checked by agarose gel electrophoresis and by spectrophotometry (NanoVue ND-1000, China) respectively. RNA samples having A260/280 in the range of 1.8 to 2.0 and A260/230 > 2 were selected for cDNA synthesis and were stored at -80°C prior to RT-PCR and RACE analysis. First strand cDNA was synthesized using the Primescript™ RT reagent Kit (Takara, China).

2.3. Cloning of full length JcIPI

Two degenerate primers (DP1 and DP2, Table 1) designed on the basis of the conserved amino-acid regions of published IPIs from other plants were used for the amplification of the core cDNA fragment of *JcIPI*. Using cDNA as the template, PCR was performed under the following conditions: 95°C for 4 min, 35 cycles of 94°C for 30 s, 53.5°C for 30 s and 72°C for 50 s followed by a final extension of 72°C for 10 min. A 590 bp DNA fragment was recovered, cloned into the pMD19-T vector (Takara, China) and sequenced (Invitrogen, China). This fragment was subsequently used for designing gene specific primers for the cloning of 3′ and 5′ ends of *JcIPI* by RACE-PCR.

To obtain the 3′- and 5′-ends of *JcIPI*, the anchored-RACE extension method was used as described by Newton et al. [17]. The initial 3′ RACE PCR was carried out using 3P1 and P1 primers. The primary PCR product was used as the template for nested 3′ RACE PCR reaction in which 3P2 primer was used along with P2 primer. Both initial and nested PCR reactions were carried out under the following conditions: 4 min at 95°C, 35 cycles (30 s at 94°C, 30 s at 55°C and 1 min at 72°C) and 10 min at 72°C. For 5′ RACE PCR, a polyA tail was added to the 3′ end of the cDNA using the terminal deoxynucleotidyl transferase according to the manufacturer instructions (Takara, China). Then similar reactions were carried out for 5′ RACE-PCR in which 5P1 and P0 primers were used for the initial reaction while 5P2 and P2 primers for the nested PCR reaction. Thermo profile was as follows: 4 min at 95°C, 35 cycles (30 s at 94°C, 30 s at 59°C and 1 min at 72°C) and 10 min at 72°C. The two nested amplified fragments of both 3′ and 5′ RACE were cloned into pMD19-T vector and sequenced. After assembling the sequences of 3′ and 5′ RACE products, the full length cDNA sequence of *JcIPI* was obtained.

Table 1
Primers for cDNA cloning, quantitative real-time PCR and recombinant plasmid construction.

Primers	Sequence (5′–3′)
DP1	CGHCTYATGTTYGAVGAYGAATGYAT[a]
DP2	CCACCAYTTSA WCAARAARTTRTC[a]
P0	GCTGTCAACGATACGCTACGTAACGGCATGACAGTG$(T)_{18}$
P1	GTCAACGATACGCTACGTAACG
P2	TACGTAACGGCATGACAGTG
3P1	CACCTTCTGATGGAAAGTGGGG
3P2	AAGGAA AGCAGATGCTGGTGAGGA
5P1	TCCTCACCAGCAGCATCTGC TTTCCTT
5P2	CCCCACTTTC CATC AGAAGGTG
SP1	GCG***GGATCC***ATGTCTGTAGCC TCTCG
SP2	GCG***GAATTC***TTAAGTTAACTTGTG
QJcIPI1	GTAAATCCAAACCCTAATGA
QJcIPI2	GTTTGCATGTCAACCACA
18SrRNA1	CAACCATAAACGATGCCGACC
18SrRNA2	CAGCCTTGCGACCATACTCCC
SSP1	GC***TCTAGA***ATGTCTGTAGCCTCTCG
SSP2	CG***GGATCC***AGTTAACTTGTG

[a] H: A/T/C; Y: C/T; V: G/A/C; S: G/C; W: A/T; R: A/G. Emphatic sequence of ***GGATCC***, ***GAATTC*** and ***TCTAGA*** represented the restriction enzyme sites of *BamH I*, *EcoR I* and *Xba I* respectively.