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BRIEF COMMUNICATION

Molecular cloning of a phosphoenolpyruvate carboxylase cDNA from tropical epiphytic CAM orchid

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Abstract

A full-length cDNA encoding phosphoenolpyruvate carboxylase (PEPC) was isolated from tropical epiphytic CAM orchid *Mokara* Yellow. The cDNA designated as *Mpepc1* is 3 450 bp in length with an open reading frame of 2 862 bp encoding 954 amino acids. The deduced amino acid sequence of *Mpepc1* shows 83 % identity with *pepc2* of sorghum, 82 % with *pepc1* and *pepc2* of maize and 81 % with *pepc* of *Arabidopsis thaliana*. RT-PCR analysis showed that *Mpepc1* was expressed in mature leaves, immature leaves, and aerial roots of *M.* Yellow. No expression was detected in the flower.

Additional key words: amino acid sequence, *Mokara* Yellow, RACE.

Phosphoenolpyruvate carboxylase (PEPC) catalyses the carboxlylation of phosphoenolpyruvate to oxaloacetate and Pi, the first step in the photosynthetic assimilation of CO_2 in CAM and C_4 plants. It has been proposed that plant PEPCs are encoded by a small multigene family and each member within a gene family encodes a distinct isoform which is associated with specific physiological functions (Lepiniec *et al.* 1994). In maize and sorghum, three PEPC isoforms were identified (Yanagisawa *et al.* 1988, Cretin *et al.* 1991, Kawamura *et al.* 1992, Lepiniec *et al.* 1993). So far most of the molecular studies on PEPC had been carried out on C_3 or C_4 plants. Here we report the cloning of a full-length cDNA encoding PEPC from a tropical epiphytic CAM orchid hybrid *Mokara* Yellow (*Arachnis hookeriana* × *Ascocenda* Madame Kenny).

Plants of *M.* Yellow were obtained from a local nursery. They were planted in pots of sand in growth chambers with 150 - 200 μmol m^{-2} s^{-1} of photosynthetically active radiation at leaf height (*Thorlux* lamp, 400 W, 240 V, 50 Hz; Redditch, UK), 70 - 80 % relative humidity, 30/25°C day/night temperature and 12-h photoperiod. Plants were watered daily with half strength Hoagland's solution B.

Total RNA was isolated from different tissues at different developmental stages using NucleoSpin RNA plant kit (*Macherey-Nagel*, Düren, Germany). Based on the available published sequences, the following primers were synthesized: sense primer P1: 5'-TCT TGG ATG GGT GGT GAT CGT GAT GG-3' and antisense primer P2: 5'-AAA GAT CCA AGG GAT TGC ACG GAG TGA-3'. RT-PCR was performed with one-step RT-PCR kit (*BD Biosciences*, Palo Alto, CA, USA) according to the manufacturer's instructions. The amplified 1 475 bp fragment was cloned into the pTZ57R vector with InsT/Aclone PCR Cloning Kit (*Fermentas*, Vilnius, Lithuania). The identity of the amplified product was confirmed by sequencing.

The following gene specific primers were designed from the sequence of the 1 475 bp fragment to perform 5' and 3' RACE using the SMART RACE cDNA kit (*BD Biosciences*). GSP1: 5'-GGT GGA ACC TGT TGG AAG AGG AGG CGG TCC-3' and GSP2: 5'-GCA CGC CAT TCT GGC TTA GGA GAG ATT GGG-3'. The 5' RACE and 3' RACE products were cloned into the pTZ57R vector and sequenced. To generate full-length cDNA, primers were designed from the 5' end of the 5' RACE product sequence and the 3' end of the

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Abbreviations: PEPC - phosphoenolpyruvate carboxylase; RACE - rapid amplification of cDNA ends; RT-PCR - reverse transcriptase polymerase chain reaction.

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3' RACE product sequence. The template for this final PCR reaction was from the original first-strand RT-PCR step.

Results showed that the full-length cDNA encoding PEPC from *M.* Yellow is 3 450 bp in length with an open reading frame of 2 862 bp encoding 954 amino acids. The deduced amino acid sequence of *Mpepc1* shared 83 % identity with that of *pepc2* from sorghum, 82 % identity with those of *pepc1* and *pepc2* from maize, *pepc1* from sorghum, *pepc* from rice and sugarcane, 81 % with those of *pepc* from *Arabidopsis thaliana* and wheat (Table 1).

Table 1. Amino acid sequence identity of *Mpepc1* (accession number AF530570) with PEPC from other plant species calculated by *Align X* program of sequence analysis software *Vector NTI* (*InforMax*, Bethesda, Maryland, USA).

Gene name	Accession number	Identity [%]
Sorghum-*pepc2*	X59925	83
Sorghum-*pepc1*	X55664	82
Maize-*pepc1*	AB012228	82
Maize-*pepc2*	X61489	82
Rice-*pepc*	AF271995	82
Sugarcane-*pepc*	M86661	82
Vanilla-*pepc*	X87148	82
Arabidopsis thaliana-*pepc*	AY074346	81
Wheat-*pepc*	AJ007705	81

RT-PCR cannot be used to quantify the level of gene expression. However, in this study, equal amounts of RNA were used for each RT-PCR reaction using the same RT-PCR kit and equal amounts of RT-PCR product were loaded on agarose gels. The relative amounts of RT-PCR product (as shown by the intensity of signal on agarose gel) might be a good indicator for the relative levels of mRNA expression in different tissues of plants. The highest level of *Mpepc1* expression was found in mature leaves, although it was detectable in immature leaves and aerial roots of *M.* Yellow (Fig. 1). No expression was detected in the flowers. There is evidence that in all plants a constitutively expressed PEPC isoform (C_3 isoform, which catalyses mainly anaplerotic reactions) exists (Lepiniec *et al.* 1991) and in CAM plants a CAM-specific PEPC isoform is expressed which is responsible for primary CO_2 fixation of this photosynthetic pathway (Cushman *et al.* 1989). The strong expression of *Mpepc1* in the leaves (CAM-performing organ) suggests that *Mpepc1* might be the CAM-specific PEPC isoform in the constitutive CAM plant *M.* Yellow. Further study is needed on other PEPC isoforms in this plant.

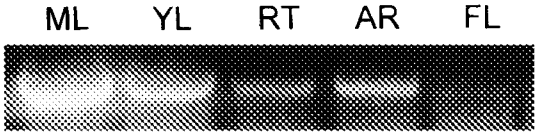

Fig. 1. RT-PCR analysis of *Mpepc1* expression in different tissues of *M.* Yellow. ML - mature leaves, YL - young leaves, RT - aerial root tips, AR - aerial roots without root tips, FL - flower. About 0.2 μg of total RNA was used for each reaction. 0.01 cm^3 RT-PCR product was loaded on each lane of 1 % agarose gel. The following primers were used for the RT-PCR reaction: RT1: 5'-GAG GCA GCC CCT GCA GCT GTG G-3' and RT2: 5'-TCA TAC AGA GCA GCG ATT CCT GAG TTG CCT-3'.

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