BIOLOGIA PLANTARUM 47 (4): 613-615, 2003/4

BRIEF COMMUNICATION

Molecular cloning of ADP-glucose pyrophosphorylase large subunit cDNA from *Oncidium*

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Abstract

A full-length cDNA for ADP-glucose pyrophosphorylase large subunit (AGPL) was isolated from tropical epiphytic orchid *Oncidium* hybrid Goldiana. The cDNA was 1754 bp in length with an open reading frame of 1551 bp encoding 517 amino acids. The deduced amino acid sequence showed 73 % identity with those of potato isoform 3 (AGPL3) and *Arabidopsis thaliana* isoform 1 (AGPL1), 71 % identity with that of barley isoform BLPL. RT-PCR analysis showed that AGPL was expressed in mature leaf, immature leaf, developing inflorescence and flower of *Oncidium*. No expression was detected in roots.

Additional key words: barley isoform BLPL, hybrid Goldiana, potato isoform AGPL3, RACE.

ADP-glucose pyrophosphorylase (AGPase) plays a central role in starch biosynthesis in both photosynthetic and nonphotosynthetic plant tissues as a regulatory enzyme. It catalyses the first step of starch biosynthesis generating ADP-glucose and pyrophosphate from glucose 1-phosphate and ATP. Plant AGPase is a heterotetrameric enzyme composed of two small and two large subunits (Morell *et al.* 1987, Okita *et al.* 1990); the presence of both subunits is essential for normal enzymatic function.

AGPase genes have been isolated from starch accumulating plants such as potato (Muller-Rober *et al.* 1990, Nakata *et al.* 1991, La Cognata *et al.* 1995), barley (Villand *et al.* 1992a,b), wheat (Olive *et al.* 1989, Ainsworth *et al.* 1993), rice (Anderson *et al.* 1989), maize (Bae *et al.* 1990, Bhave *et al.* 1990, Giroux *et al.* 1995), from sucrose accumulating plants such as tomato (Park and Chung 1998) and from model plant *Arabidopsis thaliana* (Villand *et al.* 1993). So far there is no report on the AGPase gene from ornamental plants. Here we report the first isolation of a full-length cDNA for AGPase large subunit from a tropical epiphytic C_3 orchid *Oncidium*.

Adult mericloned plants of *Oncidium* hybrid Goldiana were obtained from a local nursery. The plants were planted into pots with sand and half strength Hoagland's solution B, and maintained in controlled environment growth chambers [150 - 200 μmol m^{-2} s^{-1} of photosynthetically active radiation at leaf height (*Thorlux* lamp, 400 W, 240 V, 50 Hz), 70 - 80 % relative humidity, 30/25 °C day/night temperature and 12-h photoperiod].

Total RNA was isolated from leaves 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' CGTTTTCCCACTGCAAATGATTTTGGCTCTG 3'
and antisense primer P2:
5' TCTCCCCGATCCCAATTGGAACCTTTCCTT 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 fragment of 400 bp 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.

Gene specific primers were designed from the sequence of the 400 bp fragment to perform 5' and 3' RACE using the *SMART RACE* cDNA kit (*BD Biosciences*). The 5' RACE and 3' RACE products were

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Received 17 December 2002, *accepted* 16 May 2003.

Abbreviations: AGPase - ADP-glucose pyrophosphorylase; AGPL - ADP-glucose pyrophosphorylase large subunit.

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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 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 for AGPase large subunit from *Oncidium* is 1754 bp in length with an open reading frame of 1551 bp encoding 517 amino acids (Fig. 1). The deduced amino acid sequence of AGPase large subunit from *Oncidium* shared 74 % identity with that of AGPL3 from tomato, 73 % identity with those of AGPL3 from potato and AGPL1 from *Arabidopsis thaliana*, 51 and 52 % identity with those of AGPL1 and AGPL2 of maize, respectively (Table 1).

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                            M  E  L  C  M  K  S  Q  I  A  L  S  A  A  V  L  Q  P  H  A  G  S  G  R  F  S
   1 GAAAACACCTTGTAAGCCATTCATGGAGCTGTGCATGAAGAGCCAAATCGCCCTCTCGGCCGCGGTGCTTCAGCCGCACGCCGGCTCAGGGAGGTTTTCC
      D  G  E  T  M  G  Q  R  L  D  F  S  S  F  R  R  A  N  E  Q  I  C  R  R  R  R  A  S  L  L  R  M  S
 101 GATGGAGAGACAATGGGACAGCGGCTTGATTTCTCGAGCTTTCGCCGTGCGAATGAGCAGATTTGCCGCCGGCGGAGGGCATCGTTGTTGCGGATGTCTT
     F  A  T  T  E  L  V  A  E  G  K  L  R  D  L  E  L  E  G  R  D  P  R  T  V  V  A  V  I  L  G  G  G  A
 201 TCGCCACTACTGAACTTGTAGCTGAGGGAAAGTTGCGGGATTTGGAGTTGGAGGGAAGAGATCCTCGGACAGTTGTGGCAGTCATTTTAGGAGGTGGAGC
       G  T  R  L  F  P  L  T  R  Q  R  A  K  P  A  V  P  I  G  G  A  Y  R  L  I  D  V  P  M  S  N  C  I
 301 GGGAACCCGCCTCTTTCCTCTCACAAGGCAAAGGGCCAAACCTGCGGTGCCCATAGGGGGGCATATAGATTGATCGATGTTCCAATGAGCAACTGTATT
      N  S  G  I  N  K  V  Y  I  L  T  Q  F  N  S  A  S  L  N  R  H  L  L  R  A  Y  N  F  S  N  G  I  G
 401 AATAGTGGAATCAATAAGGTGTATATTCTCACCCAGTTCAACTCTGCATCACTCAACAGACATCTTTTGAGGGCGTACAATTTCAGCAATGGCATAGGCT
     F  G  D  G  F  V  E  V  L  A  A  T  Q  T  P  G  E  A  G  K  K  W  F  Q  G  T  A  D  A  V  R  Q  F  H
 501 TCGGGGATGGTTTTGTCGAGGTATTGGCGGCCACTCAAACTCCAGGTGAGGCAGGAAAGAAATGGTTTCAAGGCACGGCCGATGCCGTTCGTCAATTCCA
       W  L  F  E  D  A  K  G  K  E  I  E  D  V  L  I  L  S  G  D  H  L  Y  R  M  D  Y  M  D  F  V  Q  S
 601 TTGGCTATTTGAGGATGCCAAAGGCAAGGAAATTGAGGATGTACTCATCCTCTCTGGAGATCATCTTTACCGTATGGACTACATGGACTTTGTTCAGAGC
      H  R  Q  S  G  A  D  I  T  I  S  C  V  P  M  D  V  S  R  A  S  D  F  G  L  M  K  I  D  N  N  G  R
 701 CATAGACAAAGTGGTGCAGACATCACAATCTCCTGTGTGCCTATGGATGTCAGCCGCGCATCAGATTTTGGTCTAATGAAGATAGACAACAATGGAAGGG
     V  L  S  F  S  E  K  P  K  G  Q  E  L  K  A  M  E  V  D  T  S  V  L  G  L  S  R  E  Q  A  K  K  T  P
 801 TTCTTTCATTCAGTGAAAAACCTAAAGGTCAAGAATTAAAGGCGATGGAAGTGGATACTTCTGTCCTTGGTTTGTCGCGAGAACAGGCAAAGAAGACTCC
       F  I  A  S  M  G  V  Y  V  F  K  K  E  I  L  L  N  L  L  R  W  R  F  P  T  A  N  D  F  G  S  E  I
 901 ATTCATTGCTTCAATGGGGGTTTATGTTTTCAAGAAGGAGATACTATTAAATCTTCTAAGATGGCGTTTTCCCACTGCGAATGACTTTGGCTCTGAAATA
      I  P  A  S  A  K  E  L  F  V  K  A  Y  L  F  N  D  Y  W  E  D  I  G  T  I  K  S  F  C  E  A  N  L
1001 ATTCCTGCGTCAGCTAAAGAACTATTTGTCAAGGCATACCTTTTTAATGATTATTGGGAAGATATTGGGACAATAAAATCCTTTTGTGAGGCAAATCTTT
     S  L  T  R  H  P  P  N  F  S  F  Y  D  A  T  K  P  I  Y  T  S  R  R  N  L  P  P  T  A  I  N  N  S  K
1101 CTCTCACCCGACATCCACCCAATTTCAGCTTTTACGACGCAACAAAACCAATATATACATCACGAAGAAATTTACCTCCAACGGCGATCAACAATAGCAA
       I  V  D  S  I  I  S  H  G  S  L  L  S  N  C  L  I  E  H  S  V  V  G  I  R  S  R  I  N  D  N  V  H
1201 GATTGTTGATTCTATTATATCCCATGGAAGTCTCTTGAGTAACTGCTTGATAGAACACAGTGTCGTCGGCATTCGATCCAGAATAAACGATAACGTCCAC
      L  K  D  T  V  M  L  G  A  D  L  Y  E  T  D  A  E  I  A  A  L  L  A  E  G  R  V  P  V  G  I  G  E
1301 TTGAAGGATACTGTGATGCTCGGTGCCGACCTCTACGAAACTGATGCAGAAATAGCAGCATTGCTTGCTGAAGGAAGAGTTCCTGTTGGAATAGGAGAGA
     N  T  K  I  K  D  C  I  I  D  K  N  A  R  I  G  K  N  A  T  I  S  N  V  D  G  V  Q  E  A  D  R  S  A
1401 ACACAAAGATCAAGGATTGTATCATTGATAAGAATGCGAGAATCGGAAAGAATGCAACAATCTCAAACGTTGACGGTGTGCAAGAAGCAGACCGGAGTGC
       E  G  F  Y  T  R  S  G  I  T  V  I  L  K  N  S  T  I  P  D  G  F  A  I
1501 TGAAGGGTTCTACACTCGTTCAGGGATTACAGTTATACTAAAGAATTCAACAATACCTGATGGATTTGCCATATGAAGCTTAGTCTTTATAGCCTAGGGT
1601 TAGTTATTATAGCAAGTGATATTCAATGTTTAGCCACAGCTACATCATGAATAAAATTATAATGTTCTGCTGCTGTTAAAGTCTATTTTTCGCTTCCTT
1701 GTACAATAATGATTTTATTAATATTTTTCTCATCAAAAAAAAAAAAAAAAAAAA
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Fig. 1. Nucleotide sequence and deduced amino acid sequence of *Oncidium* ADP-glucose pyrophosphorylase large subunit cDNA. The start and stop codons are underlined.

Table 1. Amino acid sequence identity [%] of AGPLs calculated by *Align X* program of sequence analysis software *Vector NTI* and *Fasta33* network service (EMBL). EMBL database accession numbers of the displayed AGPLs are as follows: AGPL1-arab (*Arabidopsis thaliana*), U72290; AGPL2-arab, X73366; BLPL-barley, U66876; AGPL1-maize, S48563; AGPL2-maize, Z38111; AGPL-oncidium, AF530569; AGPL1-potato, X61187; AGPL2-potato, X74982; AGPL3-potato, X76136; AGPL-rice, U70541; AGPL3-tomato, U85497; AGPL2-wheat, X14349.

	AGPL2-arab	AGPL2-maize	AGPL2-potato	AGPL2-wheat	AGPL3-potato	AGPL3-tomato	AGPL1-arab	AGPL-rice	BLPL-barley	AGPL1-potato	AGPL-oncidium
AGPL1-maize	55	67	55	70	50	51	50	77	51	56	51
AGPL2-arab		58	70	59	61	61	60	56	62	65	60
AGPL2-maize			58	83	50	50	51	70	52	58	52
AGPL2-potato				60	58	59	59	56	58	64	60
AGPL2-wheat					51	53	53	72	53	61	53
AGPL3-potato						90	74	51	73	65	73
AGPL3-tomato							78	52	72	60	74
AGPL1-arab								51	71	57	73
AGPL-rice									53	57	54
BLPL-barley										62	71
AGPL1-potato											60

Most plants so far studied have multiple isoforms of AGPL that have different patterns of expression in plants (Olive *et al.* 1989, Villand *et al.* 1992a, La Cognata *et al.* 1995, Park and Chung 1998, Harn *et al* 2000). In the present study, AGPL from *Oncidium* also showed an organ-specific expression pattern. AGPL mRNA was detectable in mature leaves, immature leaves, developing inflorescences, and flowers of *Oncidium* (Fig. 2). However, no expression was detected in the roots. These results indicated that there might be a different isoform of AGPL in the roots of *Oncidium*. Further work is needed to verify this point.

Fig. 2. RT-PCR analysis of AGPL expression in different organs of *Oncidium*. ML - recently matured leaves, YL - young leaves, DI - developing inflorescences, RT - roots, FL - flowers. About 0.2 µg total RNA was used for each reaction.

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