Differential expression of four members of the H⁺-ATPase gene family during dormancy of vegetative buds of peach trees

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Abstract. Vegetative-bud dormancy in peach (Prunus persica L. Batsch) trees is known to be correlated, at least partially, with properties of the underlying bud tissues during winter. Variations in the activity and amount of plasma-membrane H⁺-ATPase were observed. A full-length cDNA, PPA2 (Prunus persica H⁺-ATPase 2) and three partial cDNAs (PPA1, PPA3 and PPA4) for the plasma-membrane H⁺-ATPase from peach trees were isolated by reverse transcription (RT)-coupled rapid amplification of cDNA ends (RACE) polymerase chain reaction (PCR). The accumulation of plasma membrane H⁺-ATPase transcripts was then studied in vegetative buds during dormancy and breaking of dormancy. Competitive RT-PCR analysis revealed that, during dormancy, the plasma membrane H⁺-ATPase transcripts were higher in the tissues underlying the buds than in the buds themselves. After dormancy release, the level of PPA1, 2, 3 mRNA increased, whereas the level of PPA4 decreased in the buds. When trees were kept in a greenhouse (i.e. sheltered from chilling), no accumulation of PPA mRNA could be detected. These results suggest that there is a differential accumulation of H⁺-ATPase mRNA between the bud and the underlying bud tissues during dormancy, and that chilling could act as a decisive factor.

Key words: Bud dormancy – Chilling (bud dormancy) – Gene expression (bud dormancy) – H⁺-ATPase – Prunus (bud dormancy) – Tree dormancy

Introduction

Woody perennial plants of temperate areas are subjected to seasonal variations. Dormancy processes allow plants (and plant organs) to survive under adverse winter conditions. Dormant organs can resume their growth when environmental and endogenous conditions become propitious again. This developmental plasticity is critical for the survival of plants and it is therefore essential to determine the mechanism of growth inhibition during dormancy. The dormancy of vegetative buds could have three different origins (Lang et al. 1987): (i) unsuitable environmental factors (ecodormancy); (ii) endogenous factors within the affected structure (endodormancy); and (iii) physiological factors outside the affected structure (paradormancy or correlative inhibition). In order to study paradormancy in peach tree buds, we investigated the short-distance relationships between the bud and the underlying bud tissues.

Previously it was shown that bud dormancy in Jerusalem artichoke was partially correlated with the properties of the underlying tissues. The underlying parenchyma of dormant buds showed higher sucrose absorption (Gendraud and Laffleuriel 1983) and had a higher cytoplasmic pH (Gendraud 1981) than non-dormant structures. The higher cytoplasmic pH of underlying tissue cells was related to a higher plasma membrane (PM) H⁺-ATPase activity in vitro (Pétel et al. 1992). It appears that buds will grow only when the nutrient-absorption capacities of the underlying tissues are low.

Similarly, variation in the active sucrose absorption of buds or their underlying tissues was observed in peach trees (Prunus persica L. Batsch cv. Redhaven) during winter dormancy. From November to December, the underlying bud tissues accumulated more nutrients than the bud (Marquat et al. 1996). The active absorption of sucrose in buds was lowest in December, while later in January an important increase was observed. The active sucrose absorption could be explained by an increase in H⁺/sucrose co-transport. In fact, the PM H⁺-ATPase generates a chemiosmotic H⁺ gradient across the plant.
PM. This gradient is used to drive solute uptake through 
H\(^+\)-coupled symporters (Delrot 1989; Bush 1993). 
Recently, PM H\(^+\)-ATPase activity was measured in 
PM-enriched fractions isolated from peach tree buds or 
their underlying tissues (Aue et al. 1999). Plasma-membrane 
H\(^+\)-ATPase activity was higher in the tissues 
underlying the buds than in buds from October to 
November and was correlated, as shown by western 
blotting, with a lower amount of PM H\(^+\)-ATPase in 
buds than in their underlying tissues.

It is well known that plant H\(^+\)-ATPases are encoded 
by a multigene family (Ewing and Bennett 1994; Suss-
mann 1994; Michelet and Boutry 1995) and the differential 
transcriptional regulation of the H\(^+\)-ATPase genes 
among various organs has been demonstrated (DeWitt 
et al. 1991; Perez et al. 1992; Ewing and Bennett 1994; 
Harper 1994; Houl
c
ne and Boutry 1994; Michelet et al. 
1994; Moriau et al. 1999; Oufa
ttole et al. 2000).

In order to understand the molecular mechanisms of 
bud growth inhibition during dormancy, we looked for a 
relationship between H\(^+\)-ATPase transcript accumula-
tion and the morphogenetic poten
tialities of peach tree 
buds. We report the first isolation and characterization 
of cDNA clones encoding for a PM H\(^+\)-ATPase in a 
woody plant (i.e. P. persica). We analyzed the transcrip-
tional expression of four H\(^+\)-ATPase genes in buds and 
their underlying tissues, both harvested from trees 
maintained in natural conditions or in a greenhouse 
(deprived of chilling).

Materials and methods

Plant material

Four-year-old peach trees (Prunus persica L. Batsch cv. Red
haven) graft
d
ed on cultivar GF 305 were grown outdoors, in natural 
conditions (seasonal climatic variations of France). Some trees, 
cultivated in 200-l containers, were transferred to a greenhouse and 
maintained at temperatures higher than 15 °C from October 1997 
to April 1998, in order to deprive the trees of winter chilling. 
Organ
c
s were harvested from both tree samples and frozen 
immediately in liquid nitrogen.

Extraction of RNA

Total RNA was extracted from several organs (roots, stems, tissues 
underlying the buds, vegetative buds, leaves) according to the 
method recently described (Gévau
tant et al. 1999). The RNA was 
quantified spectrophotometrically and its quality and integrity 
was checked by gel electrophoresis. 

Aliquots of RNA were treated with 2 units per µg RNA of 
RNase-free DNase I (Promega), to remove template DNA, and 
diluted to 10 ng µl\(^{-1}\) for use in competitive reverse transcription-polymerase 
chain reaction (RT-PCR). Messenger RNA was purified using the PolyATract mRNA-isolation system (Promega).

Isolation of an H\(^+\)-ATPase cDNA clone

The highly conserved ATP-binding domain was chosen in known 
PM H\(^+\)-ATPase sequences to design two degenerate oligonucleo-
tides for PCR (Ewing and Bennett 1994). A 23-mer, designated as 
P1 [5’TTC(T,A)CCA(T,A)GAG(A)CACAAA(G)TAT(C) 
GAG(A)AT3’], and an antisense 23-mer, designated as P2 [5’GC 
A(T,G)TA(T,G)AT(A,C,T)GT(A,G)TT(C,T)TCTAT3’], 
corresponding to amino acids (FPEHKYEE) and (MKNYTIY), 
respectively, were synthesized.

The first-strand cDNAs were produced with 2 µg of total RNA 
using the avian myeloblastosis virus (AMV) reverse transcriptase from 
the First Strand Synthesis kit (Amersham), following 
the manufacturer’s instructions. Amplification by PCR was carried out in 
25 µl total volume, using 0.5 units of Taq polymerase (Appli-
gen), 200 µM deoxyribonucleotide triphosphates and 0.8 µM of primers (P1 and P2). Diluted cDNA was used as the template, and 
PCR amplification was performed during 40 cycles at 94 °C (30 s), 
45 °C (30 s), and 72 °C (1 min). The resulting 254-bp DNA 
fragments were cloned into the pCR II T-vector (Invitrogen).

Isolation of the full-length H\(^+\)-ATPase cDNA (PPA2)

The 5’ end and 3’ end of a cDNA were isolated using the RACE 
method (Marathon cDNA amplification Kit; Clontech Laborato-
ries, Palo Alto, Calif., USA), following the manufacturer’s 
instructions. The PCR amplification was carried out using a 
PPA2-specific primer (R5’)[5’GCC AGA AGA AGA CAA CTG 
TCA TTA GC3’] for the 5’ end and (R3’) [5’GCT ACA GGA 
GAG GAA GCA CAT CTG 3’] for the 3’ end.

Finally, the full-length cDNA clone was obtained by hot-start 
PCR using two cDNA-specific primers based on the sequences of 
the 5’ and 3’ ends: A250 primer [5’ CGA TTC GGC TTA CCT 
CAT CCT CAT CC3’] and an antisense primer A230 [5’ GCC 
TAC AAA AGA GAC ATC CCC TGC C3’].

Identification and characterization of cDNA clones

The cDNA clones were partially sequenced using the dideoxy chain 
termination method with the T7-polymerase (Pharmacia). The complete sequencing was performed with an automated sequencer 
(Genome Express, Grenoble, France).

Sequence alignment was performed and similarity scores were 
calculated using the CLUSTAL-W 1.8 program (Gibson et al. 
1994).

Preparation of standard RNA

Competitive PCR for the quantification of H\(^+\)-ATPase mRNA 
was conducted according to the method described by Siebert 
and Larrick (1992). Standard RNA was prepared as follows. The 
cDNA clone APR3 was modified by insertion of a 518-bp 
het
erologous DNA fragment (an Aul-HiscII fragment from 
nucleotides 331 to 849 of the cDNA for the adenine nucleotide 
translocator of Solanum tuberosum; EMBL accession number 
X57557) within the region amplified by the primer pair.

The RNA standard was obtained by in vitro transcription with T7 RNA polymerase of the linearized plasmid. After treatment 
with RNase-free DNase I (Promega), the mixture was extracted 
with phenol/chloroform and precipitated with ethanol. The trans-
script was resuspended in diethylypyrocarbonate (DEPC)-treated 
water, quantified spectrometrically and its integrity was determined 
by gel electrophoresis.

Quantification of H\(^+\)-ATPase mRNA by competitive RT-PCR

The RT-PCR was performed using the Titan one Tube RT-PCR 
System (Boehringer Mannheim) according to the manufacturer’s 
specifications, with the following modifications: 25 µl of standard 
transcript was added to 10 ng of plant RNA, and 25 µM 
downstream and upstream primers (P1, P2 described above).