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Promoter activity of a putative pollen monosaccharide transporter in *Petunia hybrida* and characterisation of a transposon insertion mutant

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Summary. For the growth of the male reproductive cells of plants, the pollen, the presence of sufficient sucrose or monosaccharides is of vital importance. From *Petunia hybrida* a pollen-specific putative monosaccharide transporter designated *PMT1* (for petunia monosaccharide transporter) has been identified previously. The present work provides an in-depth analysis and characterisation of *PMT1* in the context of pollen development with the *GUS* reporter gene and an insertion mutant. The promoter of the pollen-specific putative *PMT1* gene has been isolated by inverse PCR and sequenced. Analysis of plants transformed with the promoter-*GUS* fusion confirmed the specificity of this gene, belonging to the late pollen-specific expressed genes. GUS activity was detected even after 24 h of in vitro pollen germination, at the pollen tube tip. To elucidate the importance of *PMT1* for gametophyte development and fertilisation, we isolated a mutant plant containing a transposon insertion in the *PMT1* gene by the *dTph1* transposon-tagging PCR-based assay. The *PMT1* mutant contained a *dTph1* insertion in position 1474 bp of the transcribing part of the gene, before the last two transmembrane-spanning domains. Analysis of the progeny of the heterozygous mutant after selfing revealed no alterations in pollen viability and fertility. Mature pollen grains of a plant homozygous for the transposon insertion were able to germinate in vitro in a medium containing sucrose, glucose, or fructose, which indicates that *PMT1* is not essential for pollen survival. Several explanations for these results are discussed in the present work.

Keywords: Expression analysis; Monosaccharide transport; *Petunia hybrida*; Pollen; Transposon tagging.

Introduction

The interaction between the style and a developing pollen grain is a remarkable process which is only poorly understood and studied mainly from a nonmetabolic standpoint. The extraordinary speed of pollen tube growth and the extreme length of this single cell appendix require large amounts of metabolites for energy consumption and de novo cell wall biosynthesis. A vital compound for the development and growth is sucrose. During pollen grain development, soluble sugars and carbohydrates are transported by the tapetum and are taken up by the pollen. These sugars may be (1) metabolised immediately, (2) used as intine precursors, (3) converted into other molecules, or (4) stored as polysaccharide reserves (Pacini 1996). After germination of the pollen grain at the stigma, the pollen tube has to cross distances in the pistil often thousands of times the diameter of the pollen grains to deliver the male gametes to the embryo sac for fertilisation (Cheung 1996). Therefore, a great amount of energy is needed for the formation of the long pollen tube, requiring a high level of sugar import (Schlüpmann et al. 1994).

Free sugars, polysaccharides, glycolipids, and glycoproteins have been detected in rich amounts in the stigmatic exudate and the extracellular matrix of the style (Bell and Hicks 1976, Clarke et al. 1979, Cresti et al. 1986). In the stylar fluids of *Petunia hybrida*, mainly the free sugars sucrose, glucose, and fructose are available to the pollen tube (Konar and Linskens 1966).

The sugars' final destination, however, requires translocation from the anther, stigma, and style apoplast over the pollen tube membrane. It is known that usually the apoplastic unloading of sugars into the sink tissues involves a hydrolysis of sucrose into glucose and fructose by means of an acidic invertase, subsequently followed by an active uptake of these monosaccharides via monosaccharide-proton symporters (Roitsch and Tanner 1996). In terms of sink–

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source relationships, pollen and growing pollen tubes should be regarded as strong sinks.

The first cDNA encoding a plant hexose transporter was cloned from the green algae *Chlorella kessleri* (Sauer and Tanner 1989, Sauer et al. 1990a), after which many other sugar transporters have been identified in higher plants, e.g., in *Arabidopsis thaliana* (Sauer et al. 1990b), *Nicotiana tabacum* (Sauer and Stadler 1993), *Chenopodium rubrum* (Roitsch and Tanner 1994), *Ricinus communis* (Weig and Komor 1996), *Petunia hybrida* (Ylstra et al. 1998). In several species, these transporters are members of large gene families, and a sink-tissue-specific expression has been reported in *A. thaliana* (Sauer et al. 1990b, Truernit and Sauer 1995, Truernit et al. 1996, Scholz-Starke et al. 2003) and tobacco (Sauer and Stadler 1993).

In recent work, a putative monosaccharide transporter, *PMT1*, has been isolated from *Petunia hybrida*. The PMT1 protein shares high overall homology with earlier reported monosaccharide transporters and contains all conserved amino acids and motifs as well as the 12 putative transmembrane regions (Ylstra et al. 1998). This gene is expressed solely and specifically during pollen grain maturation and germination. It was shown that during in vitro pollen tube formation, most of the sugars are taken up from the medium in the form of hexoses. High invertase activity was detected in the extracellular medium of the anther (Ylstra et al. 1998). On the basis of these results, we here propose that during pollen grain germination in *P. hybrida* most of the sugars are taken up as glucose and fructose via the PMT1 protein. In *A. thaliana*, it has been shown that different monosaccharide transporter genes are transcribed during pollen development. One gene, *AtSTP2*, is expressed during the early stages of gametophyte development, and it may participate in the uptake of glucose units resulting from callose degradation during pollen maturation (Truernit et al. 1999). Other transporter genes were shown to be transcribed during the last stages of pollen development, *AtSTP4* (Truernit et al. 1996) and *AtSTP6* (Scholz-Starke et al. 2003).

In the present study, data are presented on the tissue-specific expression of the *PMT1* gene from *P. hybrida*. Using a gene construct consisting of a translational fusion of the *PMT1* promoter to the β-glucuronidase (*GUS*) reporter gene (Jefferson et al. 1987), we studied the expression pattern of the *PMT1* promoter–*GUS* fusion. The results confirm the specific expression of the *PMT1* gene during pollen grain formation and germination. The importance of this transporter for pollen viability and fertility was also investigated by mutating the *PMT1* gene by a technique involving transposon tagging and a PCR-based assay (Koes et al. 1995). The pollen viability and fertility from plants of the *PMT1* mutant suggest other mechanisms for sugar uptake in petunia pollen besides *PMT1*.

Material and methods

Plant material

Petunia hybrida cv. W138 and cv. W115 were grown under standard greenhouse conditions.

In vitro pollen tube growth

Pollen was collected from flowers at anthesis and germinated in medium (Jahnen et al. 1989) or in the same medium in which sucrose was replaced by D(+)-glucose monohydrate, extra pure D(−)-fructose (Merck, Darmstadt, Federal Republic of Germany), or D-mannitol (Janssen Chimica, Beerse, Belgium) at a concentration of 2% (w/w). Photographs of the cultures were made after 24 h.

Isolation of the PMT1 promoter by inverse PCR

First, 10 μg of DNA isolated from *P. hybrida* leaves was digested with *Eco*RI (a restriction site for *Eco*RI in the *PMT1* gene is shown in Fig. 1), extracted with 1 volume of phenol-chloroform, and ethanol precipitated. Then the pellet was resuspended in 80 μl of water. *Eco*RI-digested DNA (0.5 μg) was used for ligation (1 unit of ligase in a total volume of 350 μl, at 16 °C for 8 h). After ligation, PCR was performed using the primers prat73 (5′-GCTCTAGACCATGGCAGGAGGCTTTGCAGCTG) and PDG10 (5′-CGGGATCCATGGCATCTTGTTTGAACTGTTGATAGC). Amplification consisted of an initial denaturing step (3 min at 94 °C), followed by 35 PCR thermal cycles with 200 ng of each primer, 10 mM (each) deoxynucleotide triphosphate, and 5 U of Tag DNA polymerase (Gibco). Each cycle involved 1 min denaturing at 94 °C, 1 min annealing at 55 °C, and 1.5 min synthesis at 72 °C. Amplified DNA was fractionated on a 1% agarose gel. A clear fragment of 1.9 kb was cloned into pMOS*blue*-T vector (Amersham Life Science, RPN 1719). After sequencing this fragment, new primers were designed against the 5′ and 3′ regions of the promoter part of the sequence. A new PCR was performed using these new primers, PDG13 (5′-CGGATCCCCATGGCCATCTTG TTTGAACTGTTGATAGC) and PDG14 (5′-CAAGCTTGTATCTGTG CCATATCTATTGTAAAG) complementary to the 3′ and 5′ regions of the *PMT1* promoter and including a *Bam*HI and a *Hin*dIII site, respectively, which yielded a fragment with the expected size of approximately 1.4 kb. This fragment was ligated to pMOS*blue*-T and verified by sequence analysis.

Construction of binary vector and plant transformation

The promoter sequence was subcloned as a *Hin*dIII-*Bam*HI fragment into the pBI101.3 binary vector (Jefferson et al. 1987). The resulting plasmid pDOL11 was used to transform *Agrobacterium tumefaciens* LBA4404. Transformation and regeneration procedures were performed by a standard leaf disc transformation method (Horsch et al. 1985, van Tunen et al. 1990). A total of 20 kanamycine-resistant plants were grown under greenhouse conditions, and after flowering, different parts of the plants were analysed for GUS response.

Staining of transgenic petunia plants for GUS activity

Different tissues of petunia *PMT1-GUS* plants were submerged in a solution containing 0.05% X-Glc, 5 μM $K_3Fe(CN)_6$, 5 μM $K_4Fe(CN)_6$,

0.1% Triton X-100, 10 mM EDTA, 0.08% NaN_3, 0.1 M $NaPO_4$ buffer, pH 5, and incubated at 37 °C overnight. Afterwards, stained tissues were decoloured with 70% ethanol for 1 h.

DNA and RNA gel blot analyses

For DNA blot analysis, 10 μg of plant DNA was isolated from young leaves, digested with *Eco*RI and electrophoresed (Koes et al. 1986). The RNA was isolated from pollen essentially according to Verwoerd et al. (1989). Total RNA was denatured by glyoxal and dimethyl sulfoxide and electrophoresed on a 1.2% agarose gel in 15 mM sodium phosphate buffer, pH 6.5, according to Angenent et al. (1992). Gels were blotted onto Hybond N+ membranes and hybridized at 65 °C in 1 M NaCl, 1% sodium dodecyl sulfate, and 10% dextrane sulfate. The full-size cDNA-coding region of *PMT1* served as a probe. After overnight hybridisation, the membranes were washed in 0.1× SSC (0.015 M NaCl plus 0.0015 M sodium citrate) with 0.1% sodium dodecyl sulfate at 65 °C and an autoradiogram was made.

DNA sequencing analysis

The fragment corresponding to the *PMT1* promoter was sequenced in both directions, as well as the transposon insertion. Therefore dideoxy-chain termination was used for sequencing double-stranded pMOS-blue plasmid derivatives. Reactions were performed with either dyedeoxy or dye primer PRISM ready reaction-sequencing kits (Applied Biosystems, Foster City, Calif., U.S.A.) following manufacturers instructions.

Obtaining the mutant by transposon tagging using a PCR-based assay

The petunia line W138 was used for these experiments. This line contains about 200 copies of the nonautonomous transposable element *dTph1* (Gerats et al. 1990). Rare insertions of a transposable element into the *PMT1* gene were detected by PCR using a gene-specific and a transposon-specific primer, following the procedure of Koes et al. (1995). We screened 490 W138 plants for an insertion in the coding part of the *PMT1* gene. One leave was taken from each plant and pooled in 10 groups of 49 leaves. DNA was isolated from each pool and subjected to PCR using the primers *dTph1* element and the *PMT1* coding sequence. After amplification, an agarose gel was loaded with PCR products from individual blocks, rows, and columns, blotted onto Hybond N+ membranes, and hybridised at 65 °C using the coding part of *PMT1* as a probe.

Results

Isolation of the promoter

To isolate the *PMT1* promoter, we followed an IPCR strategy. Restriction analysis of the *PMT1* cDNA revealed an *Eco*RI restriction site near the 5′ part (Fig. 1A), leading us to use *Eco*RI for the IPCR technique. The result of IPCR was a 1.9 kb fragment of the *PMT1* promoter, which also contained part of the coding region of *PMT1* and a small intron of approximately 200 bp. It contained 1332 bp of the 5′ flanking sequence, which should be long enough to cover a substantial part of the *PMT1* promoter (Fig. 1A). With 68% AT and only 32% GC content (from 5′ to the start of the cDNA) this sequence is very AT rich as compared with 56% AT in the sequence of the *PMT1* open reading frame. Comparison with sequences of other pollen-specific promoters revealed no conserved boxes or sequences other than a number of potential TATA boxes. The

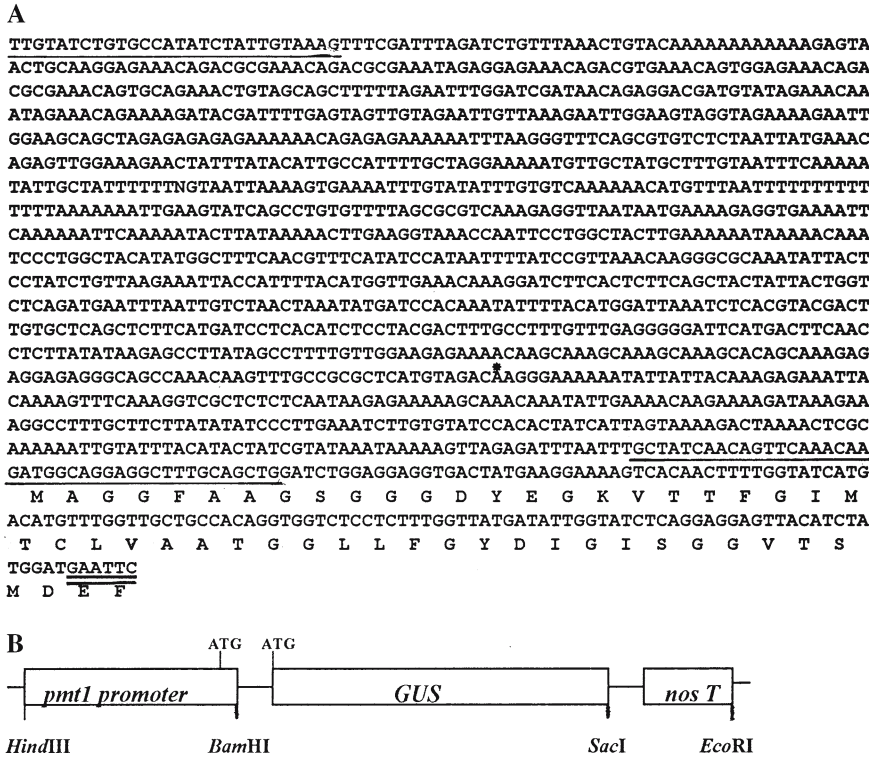

Fig. 1. A Sequence of the *PMT1* gene promoter. The sequence shown is the product obtained from *P. hybrida* genomic DNA by IPCR, used for the construction of the translational *PMT1-GUS* fusion, and a part of the cDNA up to the *Eco*RI site (double underlined). The sequence of the primers PDG14 and PDG13 used for cloning the promoter of the chimeric gene construct is single underlined. **B** Chimeric gene construct used for the transformation of *P. hybrida* via *A. tumefaciens* with its restriction map. The chimeric *PMT1-GUS* construct is a translational fusion between 1.3 kb of *PMT1* gene 5′-flanking sequences, including the first 52 amino acids, and the *GUS* gene "in frame" linked to the nopaline synthase terminator (*nosT*)

P. hybrida PMT1 promoter fragment isolated was used to determine the tissue specificity of the *PMT1* gene. Therefore, we generated a construct containing 1297 bp upstream of the start ATG plus the coding sequence for the first 52 amino acids was cloned in frame in front of the *GUS* gene (Fig. 1B) and used for transformation of *A. tumefaciens*. Recombinant *A. tumefaciens* cells were used for transformation of *P. hybrida* leaves.

Pollen-specific expression of PMT1-GUS

A total of 20 independent kanamycine-resistant petunia plants were generated. Six independent transformants were stained for GUS activity. All tested plants showed identical patterns of GUS staining; differences being detected only in the intensity of the staining.

When transgenic *PMT1-GUS* plants were analysed for GUS activity, strong staining was noted in the stamens (Fig. 2C) specifically present in pollen grains (Fig. 2D) (compare Fig. 2A–C with Fig. 2D). In contrast to this, no GUS activity was detected in petals (Fig. 2A), pistils (Fig. 2B), sepals (not shown), or leaves (not shown) or any tissues presented in Fig. 2 of nontransgenic control lines (not shown).

Expression of PMT1 gene during pollen development and in vitro pollen tube growth

Also, we investigated at what point during pollen grain formation the transcription of the *PMT1* promoter started. Male gametophytes were isolated at the stage of pollen mother cell, tetrads, microspore, bicellular pollen, late bicellular pollen, and mature pollen. As shown in Fig. 3, the first GUS-positive signals were found at the stage of bicellular pollen, after the first pollen mitosis (Fig. 3C). This signal gained in intensity with the maturation of the pollen, reaching its maximum in the mature pollen, after anther dehiscence (Fig. 3D). Due to the fact that the petunia line used for the experiments was a selfed line, only half of the pollen grains stained, as expected for a gametophyte-specific gene (data not shown).

The dry pollen from GUS-positive plants, when placed into germination medium, started to form the pollen tube. After 3 h of germination, the pollen and pollen tube were completely blue, whereas at 8 h, when the first callose plugs start to be produced and generative cells divide to produce sperm cells, the blue staining was more intense at the tube tip (Fig. 3E) and after 24 h of germination the signal was found only at the tube tip (Fig. 3F), where the protoplast is located.

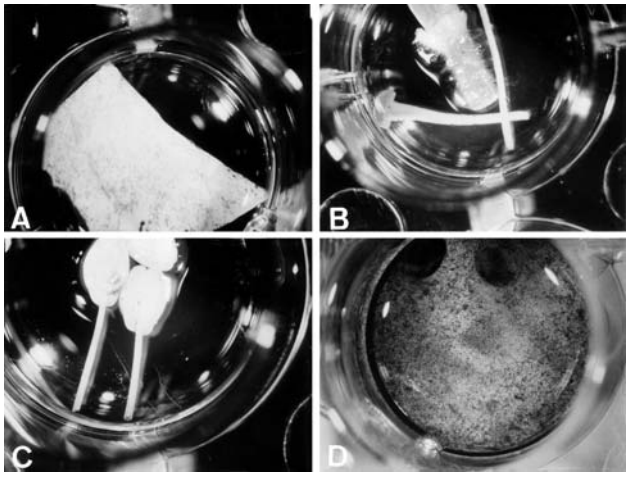

Fig. 2 A–D. Identification of *PMT1-GUS* activity in transgenic *P. hybrida* plants. The *PMT1-GUS* activity was visualized with the chromogenic substrate X-Gluc. **A** Section of a petal, **B** stigma, style and part of an ovary, **C** stamens with pollen, **D** pollen grains after anther dehiscence

Generation of a PMT1 mutant by transposon tagging

One set of 490 *P. hybrida* W138 plants were screened for insertions in the *PMT1* gene. The result of the PCR screening is shown in Fig. 4B. One strongly hybridising fragment was detected consistently in the three dimensions of the screen (block 10, row 1, and column 5). In addition, a number of weakly hybridising fragments appeared irregularly in the different lanes. These fragments probably derived from somatic insertion events giving rise to chimeric plants. PCR cloning and sequence analysis of DNA from the plant on position 10 1R 5C confirmed that it was heterozygous for a wild-type and a *dTph1* insertion allele of *PMT1*. After sequencing, it was confirmed that the insertion corresponded to the transposable element *dTph1* (Fig. 5A). Specifically, the transposon insertion was located at position 1474 of the coding part of the *PMT1* gene, before the two last transmembrane-spanning domains, as schematically shown in Fig. 5B. The W138 plant containing an insertion in the *PMT1* gene was selfed and seeds were sown to raise progeny plants. Total DNA was extracted from different progeny plants and Southern analysis was performed to study the segregation of the mutant allele in these plants. The results of the Southern analysis are shown in Fig. 6A. From a total of 24 plants, 13 contained both the *dTph1* insertion allele in the genome and the wild-type allele (1, 4, 5, 6, 7, 9, 13, 14, 15, 16, 17, 19, and 20), 6 were homozygous for the *dTph1* insertion (8, 10, 18, 21, 23, and 24), and 5 plants resembled the wild-type situation (2, 3, 11, 12, and 22). The segregation of the wild-type and *dTph1*

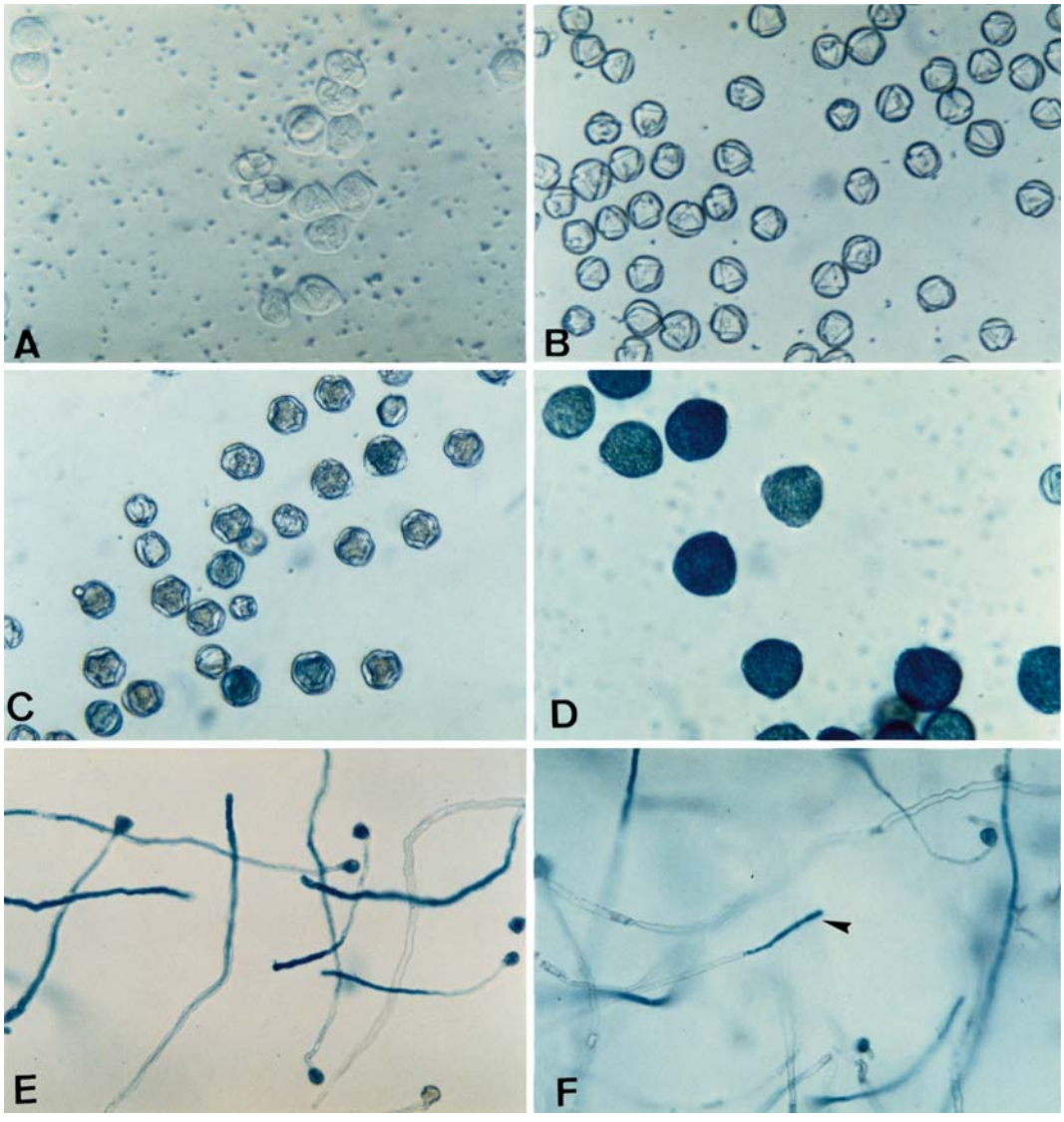

Fig. 3 A–F. Analysis of *PMT1-GUS* activity during pollen development, visualized with the chromogenic substrate X-gluc. **A** Pollen mother cells and tetrad stage; **B** microspore stage; **C** bicellular pollen, some blue staining is shown at this stage; **D** mature pollen; **E** mature pollen after 8 h of in vitro germination, showing elongating pollen tubes; **F** mature pollen after 24 h of in vitro germination, showing blue staining only at the tip of the long pollen tubes (arrowhead)

alleles from a heterozygous plant to the progeny correlates with the typical Mendelian segregation ratio of 1 : 2 : 1. The transcription of the mutant allele took place, and plants containing one or two copies of the mutated allele synthesised an mRNA transcript 284 bp longer than the wild-type mRNA, as shown by Northern blot (Fig. 6B).

Pollen germination of the PMT1 mutant

To determine whether a pollen homozygote for the mutated gene changes in growth speed, we performed pollen germination experiments for which plants homozygous for the *dTph1* insertion were chosen. Dry pollen from dehiscent anthers was germinated for 24 h in a medium containing sucrose, glucose, fructose, or mannitol as a carbon source. We were unable to detect changes in pollen germination and length of the pollen tube, etc., in the mutant (Fig. 7D–F) with respect to wild-type plants (Fig. 7A–C). Mannitol, a nonmetabolisable sugar in pollen, was used as a control of endogenous germination (Fig. 7C, F). The mutant plants were also able to set seeds in a number similar to that of the wild type (data not shown).

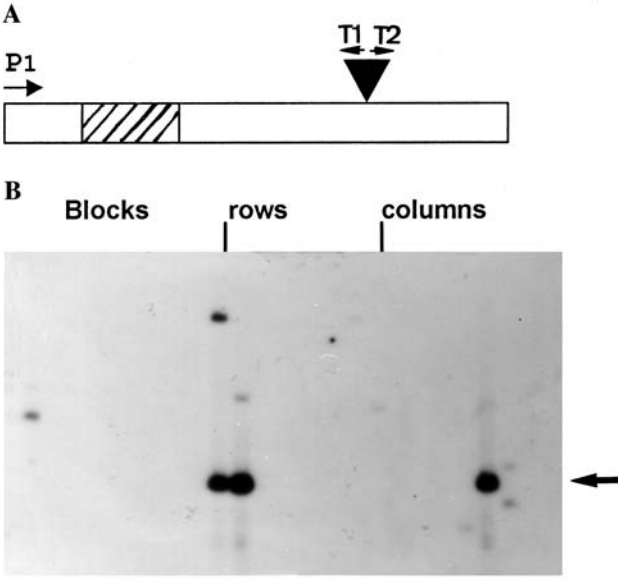

Fig. 4 A, B. Generation of *PMT1* transposon mutant. **A** Scheme of the screening for a *PMT1* insertion of the transposon *dTph1*. P1 corresponds to the primer of *PMT1* and T1 and T2 to the transposon primers (note that the transposon can integrate in 2 orientations). The black triangle outlines the transposon. **B** DNA gel blot analysis of the PCR products of plants used for the transposon screening. The arrow points to the signal found in the three dimensions of the screening (blocks, rows, and columns)

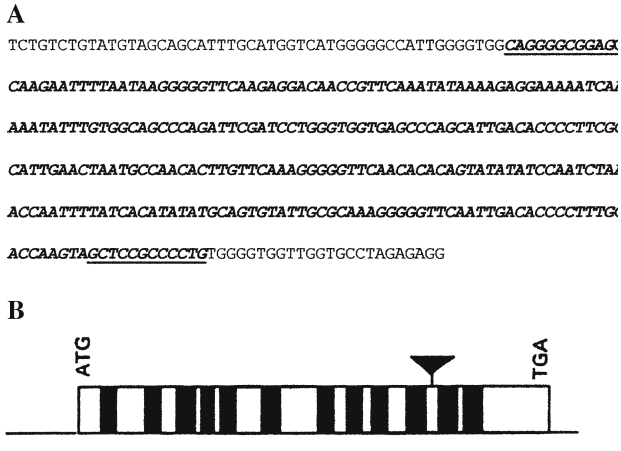

Fig. 5. **A** Sequence of a part of the *PMT1* gene with the transposon insertion. The sequence corresponding to the transposon is shown in italics, inverted repeats of *dTph*1 are underlined. It contains perfectly inverted repeats of 12 bp and is flanked by a duplication of target site sequences of 8 bp. **B** Scheme of the *PMT1* cDNA showing the site of the *dTph1* insertion (black triangle). Dark boxes indicate the transmembrane-spanning domains of the protein

Discussion

For pollen development and especially pollen tube growth, a high amount of sugars have to be transported from the apoplastic and stylar fluids. To date, metabolic requirements and molecular mechanisms involved have been investigated

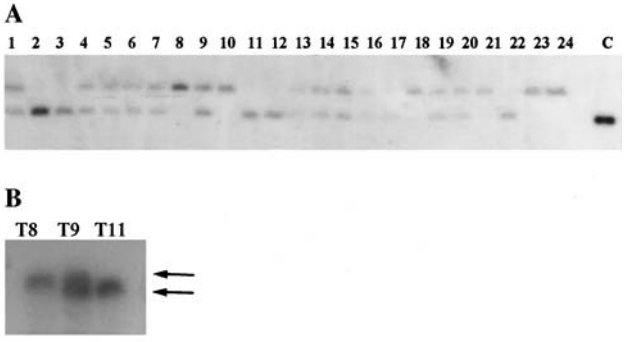

Fig. 6 A, B. Southern and Northern analysis of *dTph1* progeny. **A** DNA gel blot analysis of the progeny of the plant carrying a *dTph1* element in the *PMT1* gene; 10 μg of genomic DNA was digested with *Eco*RI, separated by electrophoresis on a 0.7% agarose gel, and transferred to a Hybond membrane. *C* Wild-type plant, *1–24* progeny. Plants with the upper band are homozygous only for the transposon insertion, with the lower band are wild-type-like, and with both bands are heterozygotic for the transposon insertion. **B** Northern blot analysis of progeny plants derived from the mutant. T8 is homozygotic, T9 heterozygotic, and T11 is wild-type-like

only in a limited way. Here, we describe the isolation and characterisation of a pollen-specific promoter from a gene encoding a putative monosaccharide transporter protein, as well as the isolation and analysis of a mutant for this gene.

The analysis of *PMT1* promoter::*GUS* plants clearly shows pollen-specific expression of *PMT1*. During pollen development, GUS activity was detected only after the first pollen mitosis, in bicellular pollen, and as it accumulates towards maturity. This type of expression thus characterises *PMT1* gene as a late pollen-specific-expressed gene (Mascarenhas 1990). In *A. thaliana*, it has been shown that different transporter genes are transcribed during pollen development (Scholz-Starke et al. 2003). In *P. hybrida*, starch reserves begin to accumulate after the first pollen mitosis (not shown), for which a great amount of sugars has to be transported to the cytoplasm of the pollen grain. This need of sugars could trigger *PMT1* transcription. Several other pollen-specific promoters have been isolated in different species. The promoter region of the *PMT1* gene does not appear to contain elements that direct pollen-specific gene expression in other plant species, as defined, for example, for the tomato *lat52* and *lat59* genes expressed in tomato pollen (Eyal et al. 1995, Twell et al. 1991).

Expression of the *PMT1* promoter–*GUS* translational fusion in *P. hybrida* provided clear indications that the *PMT1* monosaccharide transporter is expressed exclusively during pollen grain formation. This conclusion coincides with the expression data based on RNA blot analysis (Ylstra et al. 1998). After in vitro germination of pollen from transformed plants, GUS-positive staining in pollen tubes

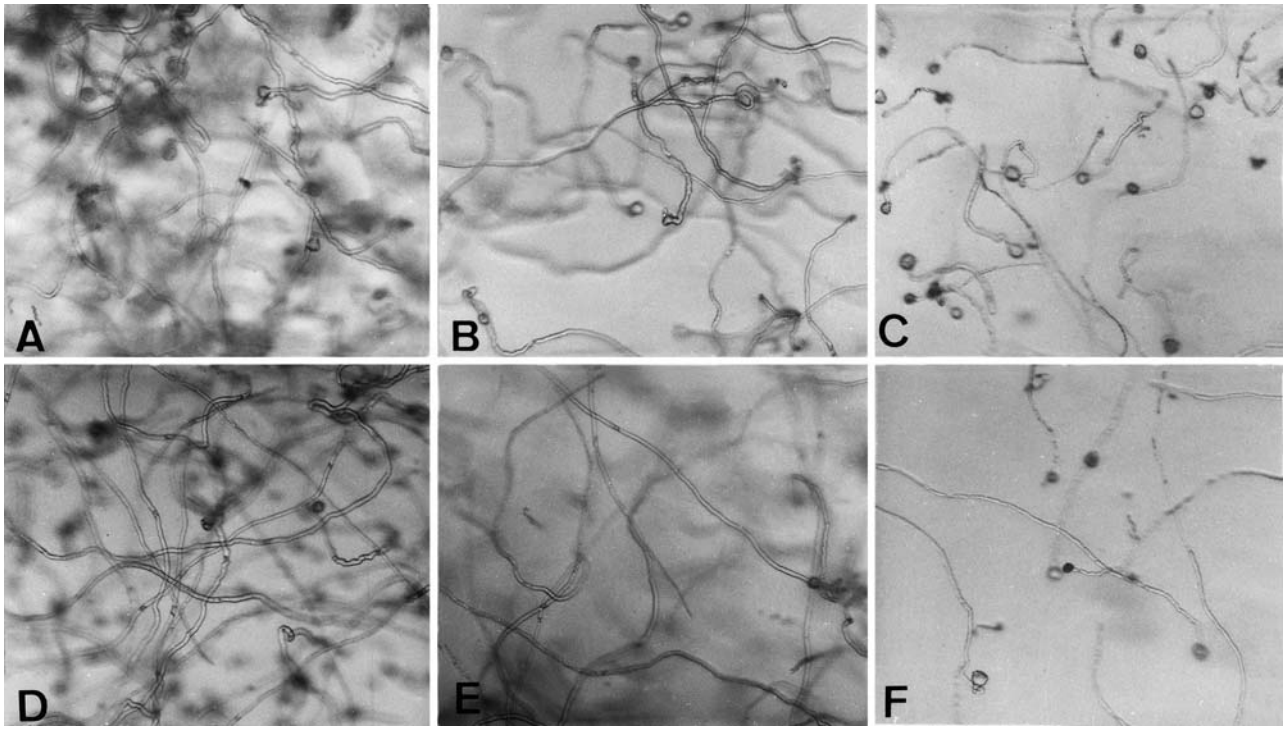

Fig. 7. In vitro pollen germination assay from wild-type plants (**A**–**C**) and from a plant which contained a *dTph1* insertion in both alleles of the *PMT1* gene (**D**–**F**). Pollen grains from dehiscent anthers were grown for 24 h on a medium which contained sucrose (**A** and **D**), glucose (**B** and **E**), or mannitol (**C** and **F**) as the carbon source

could be detected even after 24 h of germination. At this time the staining was found only at the pollen tube tip, where the protoplast is located. The importance of sugars for pollen development and germination has been demonstrated by several authors. In maize and rice, a water deficit during meiosis causes male sterility, the pollen being devoid of starch. This sterility is preceded by a decline in acid-invertase activity and changes in carbohydrate metabolism in the anthers (Dorion et al. 1996, Sheoran and Saini 1996). In petunia pollen during in vitro germination, a high invertase-like activity was measured, and in a germination medium which contained sucrose as the only carbon source, only the sugars glucose and fructose were detected after 13 h of germination (Ylstra et al. 1998). Wall-bound invertase activity was reported earlier to be present on lily pollen (Singh and Knox 1984), and more recently, other plants have been found to express genes or enzyme activity for soluble invertases in floral tissues, particularly in anthers and pollen (Miller and Ranwala 1994, Davies and Robinson 1996, Weber et al. 1996, Xu et al. 1996, Maddison et al. 1999, Goetz et al. 2001). The fact that pollen exhibits such invertase activity strongly suggests that pollen imports carbohydrates in the form of monosaccharides, and it was shown that the antisense repression of a tapetum- and pollen-specific extracellular invertase *Nin88* in tobacco induced male sterility (Goetz et al. 2001). Stigmatic tissues from pearl millet have been shown to attract pollen tubes (Reger et al. 1992a). It was shown that invertase activity is present in this tissue and converts sucrose in the medium into fructose and glucose, the latter having chemotropic activity on pearl millet pollen tubes in vitro (Reger et al. 1992b).

The finding of the pollen specificity for the *PMT1* monosaccharide transporter, as well as the fact that sucrose and glucose play an important role in pollen development and germination, made us think that we were dealing with a gene essential for male gametophytic development. To elucidate the importance of this gene, we generated a plant with the *PMT1* gene disrupted by a transposon. Sequence analysis revealed that the insertion was located in an exon of *PMT1*, specifically before the last two transmembrane-spanning domains (Fig. 5). The mutated gene generated an mRNA that gave a higher band in the gel after Northern blot analysis, thus indicating that the messenger contained the transposon within the transcript. Because *dTph1* contains in its sequence enough stop codons in all possible open reading frames, truncated protein-lacking domains XI and XII are produced in the mutants. For the *C. kessleri*

hexose transporter *HUP1* a series of *Km* mutants were generated by site-directed mutagenesis; mutations in amino acid in helix XI (V433L and N436Y) led to a dramatic increase in K_m values for glucose when expressed heterologously in *Schizosaccharomyces pombe* (Will et al. 1994, Büttner and Sauer 2000). In PMT1 of *P. hybrida* the residues V428 and N431 in helix XI correspond to the residues V433 and N436 of HUP1. Also in the carboxy-terminal part of PMT1 the motive LPETK427–476 is conserved when compared to earlier reported monosaccharide transporters, thus, this motive must be involved in the specific properties of the protein. This motive is absent in the *PMT1* mutant. For all the previous reasons we believed that the mutation would render a nonfunctional gene product. However, no effects on pollen fertility or competition were detected in the mutant plants: no differences in pollen germination and no differences appearing for pollen tube growth speed (e.g., Fig. 7) indicated also by the normal Mendelian segregation (e.g., Fig. 6A). One explanation for this absence of any phenotypic effect of the mutant could be redundancy for monosaccharide transport in pollen grains, and as soon as *PMT1* is not functional, another of these monosaccharide transporter genes could take over. By Southern blot analysis, it was shown that a family of four to five *PMT1*-related genes existed in the *P. hybrida* genome (Ylstra et al. 1998), and in *A. thaliana* at least three monosaccharide transporter genes were shown to be transcribed at the very end of pollen maturation (Scholz-Starke et al. 2003). In *Saccharomyces cerevisiae*, the hexose transporter family comprises 18 proteins, and only after knocking out all these proteins can glucose consumption and transport activity be completely eliminated (Wieczorke et al. 1999). After first submission of this paper, a similar result was found for the monosaccharide transporter gene *AtSTP6* in pollen of *A. thaliana*. Plants homozygous for a mutant containing a transposon in the *AtSTP6* gene showed no differences with the wild-type plants with respect to pollen viability, pollen germination, fertilisation, and seed production (Scholz-Starke et al. 2001), possibly because of the existence of other pollen monosaccharide transporters. Another possible explanation for our result is that the uptake of monosaccharides in pollen can share both saturable and nonsaturable components, as in the case of sucrose. A sucrose-binding protein has been characterised in soybean (Overvoorde et al. 1996) that mediates nonsaturable sucrose uptake during heterologous expression in *S. cerevisiae*. Given the importance of sugars for pollen development and germination, we expect that future experiments will shed more light on this undervalued area.

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