Sex Plant Reprod (1995) 8:173–179

ORIGINAL PAPER

Gerben J. van Eldik · Wim H. Vriezen · Miriam Wingens
Rene K. Ruiter · Marinus M.A. van Herpen
Jan A.M. Schrauwen · George J. Wüllems

A pistil-specific gene of *Solanum tuberosum* is predominantly expressed in the stylar cortex

Received: 18 July 1994 / Revision accepted: 22 September 1994

Abstract In a program aimed at studying genes expressed in pistils, the cDNA clone STS15 was isolated from a cDNA library of pollinated pistils of *Solanum tuberosum* and was found to be expressed only in pistils. During development of the pistil, the accumulation of STS15 transcripts, which are 0.7 kb long, reached a maximum just before anthesis and declined in fully open flowers. Southern blot analysis revealed that *sts15* was present as a small gene family in dihaploid potato. In situ hybridization experiments indicated that STS15 was strongly expressed in the cortex of the style and at a low level in the stigma. No hybridization signal was observed in the transmitting tissue. The temporal and spatial expression patterns of STS15 indicate that the gene products of the *sts15* gene might be involved in the function of the stylar cortex or in making the pistil competent for pollination.

Key words Pistil-specific gene · Stylar cortex · STS15 cDNA · *Solanum tuberosum*

Introduction

In flowering plants, the pollen, i.e. the male gametophytes, and the pistil, which contains the female gametophytes (Esau 1977), come into close contact at the onset of pollination. During this interaction, the genetic background of both tissues determines whether or not successful fertilization will take place. Although rejection of the pollen, or self-incompatibility, has been extensively investigated at the molecular level (Sims 1993), relatively few data have been obtained that shed light on the molecular basis of a successful pollen-pistil interaction. Such an interaction is characterized by germination and undisturbed growth of the pollen tubes that ultimately leads to fertilization of the egg cell in the embryo sac (Lord and Sanders 1992).

The process of pollination starts when mature pollen, developed in the pollen sacs of the anther (Mascarenhas 1990), is shed and transferred to the mature stigma of a receptive pistil. After landing, recognition processes initiate the cascade of events that lead to fertilization (Mascarenhas 1990). This recognition implies that pistils are able to discriminate between the different types of pollen they receive and to determine whether they will accept or reject the pollen (Knox 1984). According to the species, pollen acceptance or rejection may take place at two distinct moments after pollination, either when the pollen has landed on the stigma or when the pollen tubes are in the pistil. After the pollen has been accepted on the stigma, it germinates and produces a pollen tube. This pollen tube penetrates the stigmatic tissue (Van Went and Willemse 1984) and grows into the intercellular matrix of the stylar transmitting tissue. In some species, the choice between acceptance and rejection is delayed until the pollen tube has travelled one third of the length of the style (gametophytic self-incompatibility, such as in the Solanaceae). After the pollen or the pollen tubes are accepted, successful growth depends on the qualitative and quantitative composition of the nutrients in the stylar transmitting tissue. The intercellular matrix of the transmitting tissue is filled with proteinaceous substances and low-molecular-weight compounds like glucose and galactose (Herrero and Dickinson 1979; Sedgley et al. 1985; Konar and Linskens 1966). Its mass is influenced before and during pollination by temperature and other environmental factors (Van Herpen 1984). Compounds in the tissue may affect the growth of the pollen tubes (Herrero and Arbelo 1989; Kroh et al. 1971; Ichimura and Yamamoto 1992), because substances from the intercellular matrix are taken up by the pollen tubes (Kroh et al. 1970; Labarca and Loewus 1973). In the So-

The nucleotide sequence data reported will appear in the EMBL, DDBJ and GenBank nucleotide sequence databases under the accession number X80472.

G.J. van Eldik · W.H. Vriezen · M. Wingens · R.K. Ruiter
M.M.A. van Herpen · J. A.M. Schrauwen (✉) · G.J. Wullems
Department of Experimental Botany, University of Nijmegen,
Toernooiveld 1, 6525 ED Nijmegen, The Netherlands;
FAX no: +31-80-553450; e-mail: U624003@ vm. uci. kun. nl

lanaceae, for example, the S-RNases are taken up in vitro by the pollen and pollen tube (Gray et al. 1991) and determine whether pollen tube growth will be arrested or will proceed (Lee et al. 1994; Murfett et al. 1994).

To understand more about the genes involved in the acceptance or rejection of the pollen and pollen tubes in the different tissues, several pistil genes have been isolated and are being investigated. These genes, predominantly expressed in the pistil, can be divided into two major groups (Gasser and Robinson-Beers 1993). The first group contains the S genes involved in self-incompatibility; these are all expressed in the pistil and thoroughly described (Sims 1993; Newbigin et al. 1993; Nasrallah and Nasrallah 1993). The second group comprises pistil genes that are not involved in self-incompatibility. This group includes genes expressed in the stylar transmitting tissue of tomato (Gasser et al. 1989; Budelier et al. 1990) and tobacco (Ori et al. 1990). Several genes in this group encode proline-rich proteins. Some of them are extensin-like (Goldman et al. 1992; Chen et al. 1992; Baldwin et al. 1992), whereas others are characterized only by a high proline content (Chen et al. 1993; Cheung et al. 1993). Other pistil-specific genes are a proteinase inhibitor precursor of *Nicotiana alata* (Atkinson et al. 1993) and STIG1, a tobacco gene that is specifically expressed in the stigmatic secretory zone (Goldman et al. 1994). None of the genes described so far have been identified as specific for compatible pollination; however, some extensin-like genes have a modulated expression upon pollination (Goldman et al. 1992; Wang et al. 1993). Also, nothing is known about the effect of the pollen gene products on the expression of these pistil-specific genes.

The aim of our research was to isolate and characterize genes expressed in pollinated pistils and establish their functional role during pollen tube-pistil interaction. To achieve these goals, we differentially screened a cDNA library of pollinated pistils of *Solanum tuberosum* and isolated several pistil-specific cDNAs. The temporal and tissue-specific expression and localization of the transcripts of one of these genes and its putative function are described.

Materials and methods

Plant material

Plants of self-incompatible dihaploid (2n=2x=24) *Solanum tuberosum* clones heterozygous with respect to the S-alleles (S_1S_3 and S_2S_4) were supplied by Professor E. Jacobsen (Wageningen Agricultural University, The Netherlands). Cuttings were grown in climate chambers at 20° C under a light/dark regime of 16/8 h.

Pistils (without the ovary) were collected from flowers at anthesis. Mature pollen was collected at anthesis and dried at room temperature for 48 h in open petri dishes (Van Herpen 1984). Pollinations were carried out just before anthesis, and pistils were collected after 24 h. All tissues were frozen in liquid nitrogen and stored at –80° C.

Development of potato flowers was divided into four stages according to Kaufmann et al. (1991). Stage 1 is characterized by a closed green bud (3–4 mm). Stage 2 is a flower bud (5–6 mm), still closed, in which the anthers are turning yellow. Stage 3 is a partly opened flower bud (8–9 mm) in which the petals and anthers are colored. Stage 4 corresponds to flowers at anthesis (>10 mm).

RNA and DNA extraction

Total RNA was isolated using the method of Frankis and Mascarenhas (1980) with slight modifications according to Goldberg et al. (1981). Plant tissue frozen in liquid nitrogen was homogenized in 4 ml RNA extraction buffer [100 mM TRIS-HCl pH 8.0, 50 mM EDTA, 1% SDS, 0.1 mM NaCl, 50 mM β-mercaptoethanol and 1% Tri-isopropylnapthalene sodium salt (Kodak)] and an equal volume of phenol, and incubated at 60° C for 10 min. Additional phenol/chloroform/isoamylalcohol (25:24:1) extractions were performed until no interface remained. RNA was obtained after successive ethanol and 2 M LiC precipitations.

The same procedure, omitting β-mercaptoethanol, was used to extract high-molecular-weight DNA from young leaves.

cDNA library construction and differential screening

Cross-pollinated ($S_1S_3 \times S_2S_4$) pistils were harvested 24 h after pollination. Poly(A)$^+$ RNA was isolated using a PolyATtract poly(A)$^+$ mRNA isolation kit (Promega). cDNAs were synthesized from poly(A)$^+$ RNA, using a Uni-ZAP XR cDNA synthesis kit (Stratagene), according to the protocols of the manufacturer. The library was packaged using Gigapack II gold packaging extracts (Stratagene). The cDNA library was differentially screened on nitrocellulose with ^{32}P-labelled single-stranded cDNA probes. The cDNA probes were prepared from either cross-pollinated pistil poly(A)$^+$ RNA or unpollinated pistil poly(A)$^+$ RNA (Sambrook et al. 1989). The ExAssist/SOLR in vivo excision system (Stratagene) was used for automatic excision of the positive cDNA clones from lambda ZAP II to yield the pBluescript II SK(-) vector in XL-1 Blue *E. coli* cells (Stratagene).

The isolated STS15 cDNA clone was not full-sized. Therefore, the 5'-Amplifinder RACE kit (Clontech) was used to isolate the 5' end of the STS15 cDNA clone. Two nested internal primers were designed (VE5, TCGAAATCATTCCAAGGCCACGGAG; VE6, GCACATTGACTCGCGGAGCAGCTTG). The primer VE5 was used to synthesize cDNA and the primer VE6 was used for PCR amplification of the 5' cDNA end according to the protocols of the manufacturer. The PCR fragments were cloned with the TA Cloning System (Invitrogen) into the pCRII vector.

DNA manipulations and sequence analysis

Plasmid DNA was prepared as outlined in Sambrook et al. (1989). Radioactively labelled probes were prepared from cDNA fragments in low melting point agarose using the random-primer labelling system (Church and Gilbert 1984). Nucleotide sequence analysis was carried out by the dideoxynucleotide chain termination method (Sanger et al. 1977) using the T7 DNA polymerase sequencing system of Pharmacia. Both nucleotide and deduced protein sequences were analyzed using the University of Wisconsin Computer Group programs (Devereux et al. 1984).

Northern blot analysis

Equal amounts of total RNA (10 µg) from different tissues were electrophoretically separated on 1.5% agarose gels and transferred to Hybond-N (Amersham) according to Sambrook et al. (1989). The STS15 cDNA was used as a probe. Hybridization was performed for 20 h at 55° C in 6× SETS (1× SETS is 0.15 M NaCl, 0.02 M TRIS-HCl pH 7.8, 1 mM EDTA), 5× Denhardt's (1× Denhardt's is 0.02% Ficoll, 0.02% PVP and 0.02% BSA), 0.1% SDS and 75 µg/ml denatured herring sperm DNA. Washing was carried out at 55° C in 1× SSC, 0.1% SDS or at 55° C 4× SSC, 0.1% SDS.

The filters were exposed to Valca HPX44 X-ray films with an intensifying screen at −80° C.

Southern hybridization of genomic DNA

Genomic DNA (30 µg) was digested with *Eco*RI or *Hin*dIII and electrophoretically separated in 0.7% agarose gels. Southern blotting and hybridization were performed on Hybond-N according to the protocol of Amersham. The STS15 cDNA was used as a probe.

In situ hybridization

In situ hybridization was performed according to Reijnen et al. (1991). Pistils at stage 3 were collected and fixed in GPMP buffer (2% glutaraldehyde, 2% paraformaldehyde, 50 mM mannitol, 18.5 mM K_2HPO_4 and 10.5 mM KH_2PO_4, pH 7.0). After dehydration, the pistils were embedded in paraffin (Paraclean, Klinipath), cut in 10-µm-thick sections, and mounted on slides (Angerer et al. 1987). The mounted slides were deparaffinized, hydrated and (pre)hybridized according to Cox et al. (1984) and Hanson et al. (1989) with slight modifications (Reijnen et al. 1991). The probes used in the in situ hybridization were ^{3}H-labelled antisense and sense RNA, with an activity of 2×10^6 cpm. The probes were synthesized by in vitro transcription of STS15 cDNA cloned in the pBluescript II SK(-) vector. The samples were immersed in a light-sensitive emulsion (Ilford L4). After exposure for 21 days at 4° C the developed silver grains were detected in the emulsion by measuring the reflectance with the BioRad MRC-600 Confocal Laser Scanning Microscope.

Results

Identification of a gene predominantly expressed in pistils

To isolate genes expressed in pollinated pistils during compatible pollination, a cDNA library of cross-pollinated potato pistils was constructed and differentially screened against unpollinated pistils. Although we expected to identify pollination-induced genes, by this protocol we isolated two recombinant clones which proved to be only pistil-specific and were not pollination-induced. One of them, STS15, was chosen for further analyses.

The tissue-specific expression pattern of the *sts15* gene was determined by northern blot hybridizations of total RNA of several tissues (Fig. 1). The STS15 cDNA

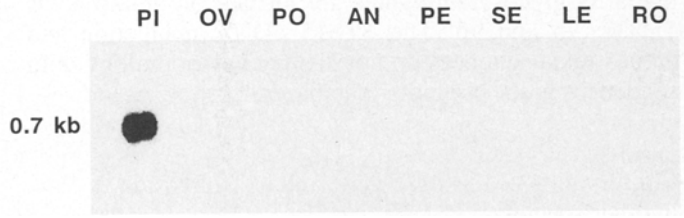

Fig. 1 Tissue-specific expression of the pistil-specific *sts15* gene. Equal amounts of total RNA from different tissues were probed with ^{32}P-labelled STS15 cDNA. *PI* Pistils, *OV* ovary, *PO* pollen, *AN* anther, *PE* petals, *SE* sepals, *LE* leaf, *RO* root. Following hybridization the blot was washed in 1× SSC, 0.1% SDS at 55° C and used for autoradiography. The size of the RNA transcripts is indicated at the left

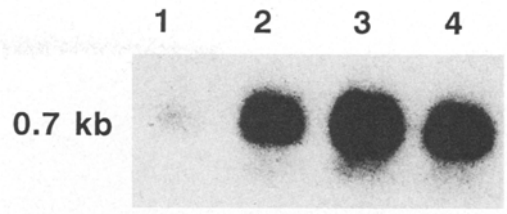

Fig. 2 Expression of the *sts15* gene during pistil development. Lanes 1 to 4 correspond to the four stages of flower development: *1* early green bud, *2* closed bud and anthers turning yellow, *3* partly opened bud, *4* flower at anthesis. Total RNA was isolated from pistils of the different developing stages and equal amounts of RNA were probed with the STS15 cDNA. The size of the RNA transcripts is indicated at the left. Following hybridization the blot was washed in 1× SSC, 0.1% SDS at 55° C and used for autoradiography

strongly hybridized to a single mRNA transcript of 0.7 kb present in complete pistils. At lower stringency, a very weak signal could be observed in ovaries and sepals; the signal was the same size as in pistils (data not shown). STS15 transcripts were not detected in any other tissue even after extended exposure.

To detect STS15 transcripts during pistil development, RNA extracted at four stages (see Materials and methods) of flower development (Kaufmann et al. 1991) was probed with STS15 cDNA. The STS15 mRNA was already present at stage 2, then increased and reached a maximum at stage 3. At anthesis, the STS15 mRNA level decreased (Fig. 2).

Localization of STS15 spatial expression

The expression of STS15 in the different pistil tissues was localized by in situ hybridization carried out on longitudinal sections of pistils at stage 3. Figure 3 shows representative results of the hybridizations. An overview of a complete pistil with bright-field illumination is given in Fig. 3A. Using an antisense RNA probe of STS15 and detection of the hybridizing signal with confocal laser scanning microscopy (CLSM), a very strong signal was detected in the cortex of the style (Fig. 3B). Whether the STS15 mRNA is also present in the epidermis could not be distinguished with this approach (Fig. 3B). No hybridization signal was observed in the transmitting tissue, the stylar part of the pistil surrounded by the cortex (Fig. 3C). The hybridization level in the stigma was much lower than in the cortex of the style (Fig. 3D). Occasionally, hybridization was observed in the papillae. The sense STS15 cDNA probe showed no hybridization in any tissue examined (Fig. 3E, F).

Sequence analysis of STS15 cDNA

Sequence analysis of STS15 cDNA revealed one open reading frame and a deduced protein of 183 amino acids (Fig. 4), resulting in a predicted molecular mass of 20.4

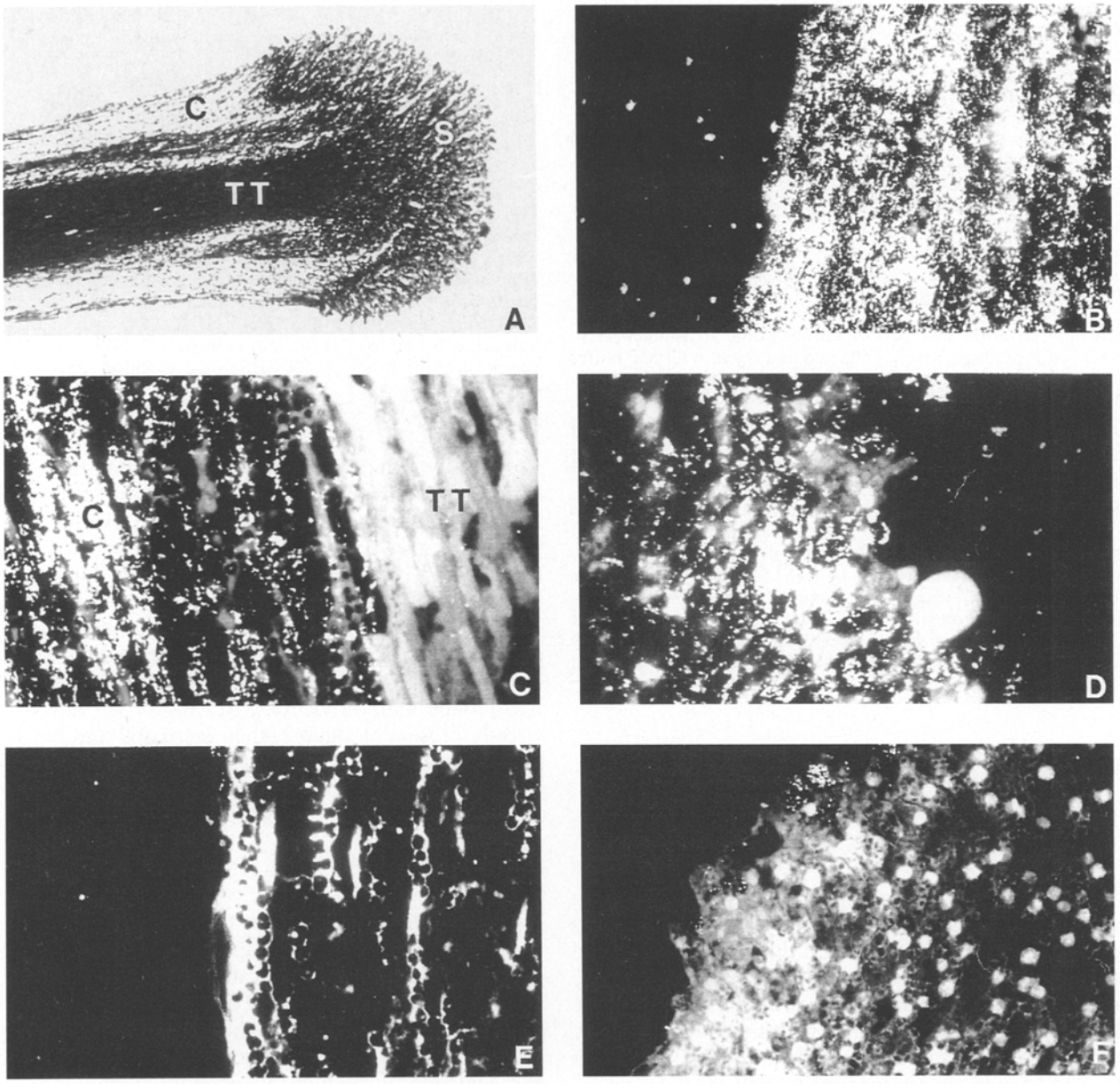

Fig. 3A–F Spatial expression pattern of the *sts15* gene in potato pistils. **A** Bright-field illumination of a longitudinal section of a potato pistil stained with toluidine blue, showing the stylar cortex *C*, the transmitting tissue *TT* and the stigma *S*. ×20. **B–F** In situ hybridization of STS15 in longitudinal sections stained with ethidium bromide. **B** Antisense STS15 probe hybridizing with the stylar cortex. ×520. **C** Antisense STS15 probe only hybridizing in the cortex, no hybridization signal in the transmitting tissue. ×520. **D** Antisense STS15 probe hybridizing with the stigma of the pistil. ×520. **E** Sense STS15 probe. No hybridization signal in the stylar cortex or epidermis. ×360. **F** Sense STS15 probe. No hybridization signal in the stigma. Ethidium bromide staining of the nuclei. ×360. Transmitting tissue *TT* and stylar cortex *C*

kDa. The deduced amino acid sequence is rich in leucine and serine and does not contain any tryptophan. A hydrophobic putative signal peptide of 23 amino acid residues is predicted from the STS15 cDNA sequence, together with a potential cleavage site located between amino acid 23 and 24 (Von Heijne 1986). Computer analysis also revealed a transmembrane helix located in the signal peptide from amino acids 2 to 20. The protein has a predicted high isoelectric point of 9.24. Four potential N-glycosylation sites are located on amino acids 15, 37, 75 and 96. The STS15 cDNA nucleotide and amino acid sequences do not display any homology with sequences in the computer databases.

Conservation of the *sts15* gene and its expression in other species

The conservation of the *sts15* gene in other plant species was determined on genomic and expression levels (Fig. 5). Genomic DNA was isolated from young leaves of *Brassica oleracea, Nicotiana tabacum* cv. Petit Havana, *Petunia hybrida* and *Solanum tuberosum*, and analyzed

```
  1  aaaaggtcatgtcaattgtcataccaaaaacatgaagcatctttcctctttcttcctatt   60
  1                                    M  K  H  L  S  S  F  F  L  F    10

 61  tctctctttatttaacctaacattctatggctcaacagcccaaaatttaatccaaacaac  120
 11   L  S  L  F  N  L  T  F  Y  G  S  T  A  Q  N  L  I  Q  T  T    30

121  ttgcaaatcatgttccaaaaatgaatcaagtattacgtatggcttttgcacttcatctct  180
 31   C  K  S  C  S  K  N  E  S  S  I  T  Y  G  F  C  T  S  S  L    50

181  acaagctgctccggcgagtcaatgtgctactctccgtggccttggaatgatttctattag  240
 51   Q  A  A  P  A  S  Q  C  A  T  L  R  G  L  G  M  I  S  I  R    70

241  attaattcgatacaatgttactgatacgaggtgtcacgttaaaatgttgttgaaggacaa  300
 71   L  I  R  Y  N  V  T  D  T  R  C  H  V  K  M  L  L  K  D  K    90

301  gaatttggatctttataataggagtcgcttgaaagtttgtttagatctttattctgacgc  360
 91   N  L  D  L  Y  N  R  S  R  L  K  V  C  L  D  L  Y  S  D  A   110

361  gatccctactatcaagcttgctatgaagagttacaatacgaagaaatattatgatgcgaa  420
111   I  P  T  I  K  L  A  M  K  S  Y  N  T  K  K  Y  Y  D  A  N   130

421  tatacaaataagtgcaattatagctaccgctacgacatgtgaagatggatttaaggagaa  480
131   I  Q  I  S  A  I  I  A  T  A  T  T  C  E  D  G  F  K  E  K   150

481  agaaggtgctgtgtcaccgttaactatgagaaatgataatactttcaattatctgcaat  540
151   E  G  A  V  S  P  L  T  M  R  N  D  N  T  F  Q  L  S  A  I   170

541  tgcactttctgttatgaatcttgttatgaataataatggataaattggtcgatattgatc  600
171   A  L  S  V  M  N  L  V  M  N  N  N  G                       183

601  aagtacttgttcgtgcattaaaaaaaaaaaaaaaaaa                         638
```

Fig. 4 Nucleotide sequence of the STS15 cDNA and its deduced amino acid sequence. The stop codon is shown in *boldface letters* and putative N-glycosylation sites are *underlined*

by southern hybridization. The presence of two faint hybridizing fragments in *B. oleracea*, one strong band in *P. hybrida* and two bands in *N. tabacum* (Fig. 5A) indicated that the *sts15* gene, or a homologue of *sts15*, is present as one or two gene copies in these plant species. Using the STS15 probe, two very strongly hybridizing *Eco*RI fragments were detected and four strong *Hin*dIII fragments were visible in digists of *S. tuberosum* genomic DNA (Fig. 5B). Sequence analysis revealed the presence of an internal *Hin*dIII restriction site in the STS15 cDNA sequence. The number of DNA fragments hybridizing to the STS15 probe indicate that the *sts15* gene belongs to a small gene family in dihaploid *S. tuberosum*.

To determine the conservation of expression of the *sts15* gene in these plant species, RNA from pistils was isolated and analyzed by northern blot hybridization. STS15 transcripts were detectable in *S. tuberosum* and at a low level in pistils of *N. tabacum* (Fig. 5C). In contrast to potato, STS15 cDNA hybridized with two transcripts of 0.7 kb and 0.75 kb in tobacco. Shorter exposure of the blot revealed that the very strong hybridization signal observed in *S. tuberosum* pistils consists of transcripts of one length.

Discussion

For a better understanding of the process of pollination, it is important to identify genes specifically expressed in all different tissues of the pistil and establish their functional role during pollination. All the pistil-specific genes previously isolated are expressed in the stigmatic tissue and/or in the transmitting tissue of the pistil (Sims 1993; Gasser and Robinson-Beers 1993; Nasrallah and Nasrallah 1993).

As can be concluded from the northern blot analysis, *sts15* is a gene highly expressed in pistil. Since *sts15* homology at the genomic level was shown in *B. oleracea*, *P. hybrida*, and *N. tabacum*, *sts15* is a member of a small gene family which is conserved during evolution. Conservation at the expression level differs for the tested

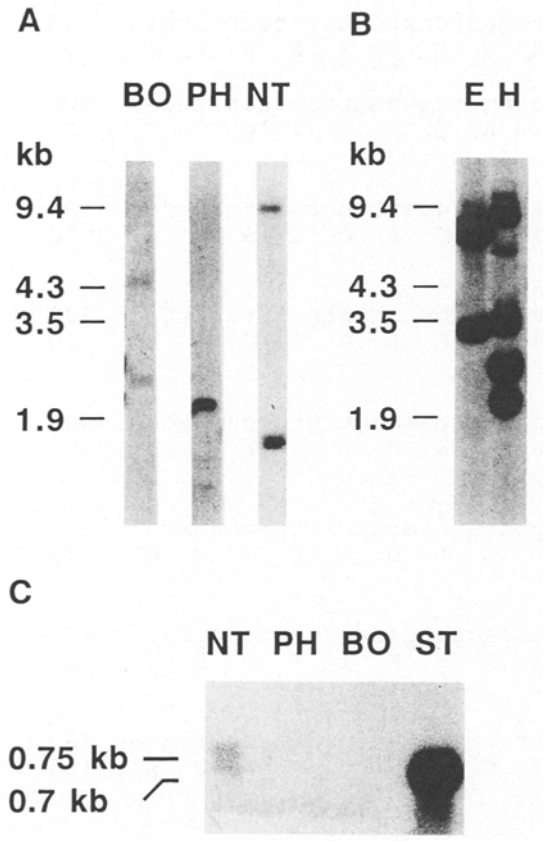

Fig. 5A–C Conservation of the *sts15* gene in other plant species. **A** Southern blot analysis of genomic DNA of the following species: BO *Brassica oleracea*, PH *Petunia hybrida*, NT *Nicotiana tabacum* cv. Petit Havana. Genomic DNA was digested with *Eco*RI and probed with STS15 cDNA. Molecular size of digested lambda DNA fragments is indicated at the left. Following hybridization the blot was washed in 1× SSC, 0.1% SDS at 60° C and used for autoradiography. **B** Southern blot analysis of genomic DNA of *Solanum tuberosum*. Genomic DNA was digested with *Eco*RI (*E*) or *Hin*dIII (*H*) and probed with STS15 cDNA. Molecular size of digested lambda DNA fragments is indicated at the left. Following hybridization the blot was washed in 1× SSC, 0.1% SDS at 60° C and used for autoradiography. **C** Northern blot analysis of the conservation of the *sts15* expression. Total RNA was isolated from pistils of: NT *Nicotiana tabacum*, PH *Petunia hybrida*, BO *Brassica oleracea*, ST *Solanum tuberosum*. The size of the hybridizing transcripts is indicated at the left. Following hybridization the blot was washed in 1× SSC, 0.1% SDS at 55° C and used for autoradiography

species; only potato and tobacco showed expression of the *sts15* gene or a gene homologous to *sts15*. Despite the high level of conservation at the genomic level, the expression of *sts15* is not conserved in the Solanaceae. In contrast to the presence of a transcript of 0.7 kb in potato, an additional transcript of 0.75 kb is present in tobacco pistils (Fig. 5C). This may have been caused by the use of two different polyadenylation sites in the tobacco gene.

The *sts15* gene is the first pistil-specific gene described that is expressed in the cortex of the pistil. The localization of STS15 expression in the stylar cortex (Fig. 3) is, so far, unique among pistil-specific genes. However, localization of expression of STS15 in the pistil epidermis as well cannot be excluded. The other described genes with expression in the cortex are the flower-specific *fst* gene isolated by Gu et al. (1992) and the *ta20* gene described by Koltunow et al. (1990). The *fst* and *ta20* genes are expressed in the cortex of the style, but also in petals, stamens, and other parts of the pistil. Furthermore, in some species the cortex is demarcated from the transmitting tissue by a zone of callose-containing cells around the stylar channel (Knox 1984). In this zone, Wu et al. (1993) showed the accumulation of CELP (Cys-rich extensin-like protein) mRNAs.

The localization of STS15 expression primarily in the stylar cortex combined with its maximum expression just before anthesis suggests that the gene products of *sts15* do not play a direct role in processes during or after pollination even though the temporal expression pattern is similar to that of the potato *S* genes (Kaufmann et al. 1991). The deduced protein sequence of the STS15 cDNA has no homology with other sequences in the databases and could not, therefore, elucidate a possible function for *sts15*. The specific expression of STS15 in the parenchymatous tissue of the cortex suggests that these celltypes are highly differentiated and may play a different role than in other organs.

Most of the pistil-specific genes isolated so far are expressed in the tissues that come into close contact with the pollen or pollen tube (stigma and transmitting tissue). However, the mature form and proper functioning of the stigma and style depend on a developmental program that includes all tissues of the pistil. The spatial and temporal patterns of expression of STS15 strongly suggest that the gene is developmentally regulated and may have a specific function in the cortex. To further understand the process of pollination, knowledge both of the tissues in direct contact with the pollen tubes and of the other parts of the pistil is necessary. To this end, greater effort should be made to isolate and characterize genes involved in the development of the pistil cortex and epidermis.

Acknowledgements We thank Wim van den Brink and Gerard van der Weerden for excellent greenhouse work and Theo Laeijendecker for photography. Critical manuscript editing was provided by Anton Croes and Celestina Mariani. This work was supported by the EC Bridge program BIOT 900172.

References

Angerer LM, Cox KH, Angerer RC (1987) Demonstration of tissue-specific gene expression by in situ hybridization In: Berger S, Kimmel A (eds) Guide to molecular cloning techniques. (Methods in enzymology, vol 152) Academic Press, London, pp 649–661

Atkinson AH, Heath RL, Simpson RJ, Clarke AE, Anderson MA (1993) Proteinase inhibitors in *Nicotiana alata* stigmas are derived from a precursor protein which is processed into 5 homologous inhibitors. Plant Cell 5:203–213

Baldwin TC, Coen ES, Dickinson HG (1992) The ptl1 gene expressed in the transmitting tissue of Antirrhinum encodes an extensin-like protein. Plant J 2:733–739

Budelier KA, Smith AG, Gasser CS (1990) Regulation of a stylar transmitting tissue-specific gene in wild-type and transgenic tomato and tobacco. Mol Gen Genet 224:183–192

Chen CG, Cornish EC, Clarke AE (1992) Specific expression of an extensin-like gene in the style of *Nicotiana alata*. Plant Cell 4:1053–1062

Chen CG, Mau SL, Clarke AE (1993) Nucleotide sequence and style-specific expression of a novel proline-rich protein gene from *Nicotiana alata*. Plant Mol Biol 21:391–395

Cheung AY, May B, Kawata EE, Gu Q, Wu HM (1993) Characterization of cDNAs for stylar transmitting tissue-specific proline-rich proteins in tobacco. Plant J 3:151–160

Church GM, Gilbert W (1984) Genomic sequencing. Proc Natl Acad Sci USA 81:1991–1995

Cox KH, De Leon DV, Angerer M, Angerer RC (1984) Detection of mRNAs in sea urchin embryos by in situ hybridization using asymmetric RNA probes. Dev Biol 101:485–502

Devereux J, Haeberli P, Smithies O (1984) A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res 12:387–395

Esau K (ed) (1977) Anatomy of seed plants, 2nd edn. Wiley, New York

Frankis R, Mascarenhas JP (1980) Messenger RNA in the ungerminated pollen grain: a direct demonstration of its presence. Ann Bot 45:595–599

Gasser CS, Budelier KA, Smith AG, Shah DM, Fraley R (1989) Isolation of tissue-specific cDNAs from tomato pistils. Plant Cell 1:15–24

Gasser CS, Robinson-Beers K (1993) Pistil development. Plant Cell 5:1231–1239

Goldberg RB, Hoshek G, Tam SH, Ditta GS, Breidenbach RW (1981) Abundance, diversity, and regulation of mRNA sequence sets in soybean embryogenesis. Dev Biol 83:201–217

Goldman MHD, Pezzotti M, Seurinck J, Mariani C (1992) Developmental expression of tobacco pistil-specific genes encoding novel extensin-like proteins. Plant Cell 4:1041–1051

Goldman MHS, Goldberg RB, Mariani C (1994) Cell ablation of the stigmatic secretory zone causes female sterility in tobacco. EMBO J 13(13):2976–2984

Gray JE, McClure BA, Bönig I, Anderson MA, Clarke AE (1991) Action of the style product of the self-incompatibility gene of *Nicotiana alata* (S-RNase) on in vitro grown pollen tubes. Plant Cell 3:271–283

Gu Q, Kawata EE, Morse MJ, Wu HM, Cheung AY (1992) A flower-specific cDNA encoding a novel thionin in tobacco. Mol Gen Genet 234:89–96

Hanson DD, Hamilton DA, Travis JL, Bashe DM, Mascarenhas JP (1989) Characterization of a pollen-specific cDNA clone from *Zea mays* and its expression. Plant Cell 1:173–179

Herrero M, Arbelo A (1989) Influence of the pistil on pollen tube kinetics in peach (*Prunus persica*). Am J Bot 76(10):1441–1447

Herrero M, Dickinson HG (1979) Pollen-pistil incompatibility in *Petunia hybrida*: changes in the pistil following compatible and incompatible intraspecific crosses. J Cell Sci 36:1–18

Ichimura K, Yamamoto Y (1992) Changes in the amount and composition of stylar canal excudate after self-pollination or cross-pollination in self-incompatible *Lilium longiflorum* Thumb. J Japan Soc Hort Sci 61(3):609–617

Kaufmann H, Salamini F, Thompson RD (1991) Sequence variability and gene structure at the self-incompatibility locus of *Solanum tuberosum*. Mol Gen Genet 226(3):457–466

Knox RB (1984) Pollen-pistil interactions In: Linskens HF, Heslop-Harrison J (eds) Encyclopedia of plant physiology, vol 17. Springer, Berlin Heidelberg New York, pp 508–608

Koltunow AM, Truettner J, Cox KH, Wallroth M, Goldberg RB (1990) Different temporal and spatial gene expression patterns occur during anther development. Plant Cell 2:1201–1224

Konar RN, Linskens HF (1966) Physiology and biochemistry of the stigmatic fluid of *Petunia hybrida*. Planta 71:372–387

Kroh M, Miki-Hirosige H, Rosen W, Loewus F (1970) Incorporation of label into pollen tube walls from myoinositol-labeled *Lilium longiflorum* pistils. Plant Physiol 45:92–94

Kroh M, Labarca C, Loewus F (1971) Use of pistil exudate for pollen tube wall biosynthesis in *Lilium longiflorum* In: Heslop-Harrison J (ed) Pollen: development and physiology. Butterworth, Toronto, pp 273–278

Labarca C, Loewus F (1973) The nutritional role of pistil exudate in pollen tube wall formation in *Lilium longiflorum*. Plant Physiol 52:87–92

Lee HS, Huang S, Kao TH (1994) S proteins control rejection of incompatible pollen in *Petunia inflata*. Nature 367:560–563

Lord EM, Sanders LC (1992) Roles for the extracellular matrix in plant development and pollination: a special case of cell movement in plants. Dev Biol 153:16–28

Mascarenhas JP (1990) Gene activity during pollen development. Annu Rev Plant Physiol Plant Mol Biol 41:317–338

Murfett J, Atherson TL, Mou B, Gasser CS, McClure BA (1994) S-RNase expressed in transgenic *Nicotiana* causes S-allele-specific pollen rejection. Nature 367:563–566

Nasrallah JB, Nasrallah ME (1993) Pollen-stigma signalling in the sporophytic self-incompatibility response. Plant Cell 5:1325–1335

Newbigin E, Anderson MA, Clarke AE (1993) Gametophytic self-incompatibility systems. Plant Cell 5:1315–1324

Ori N, Sessa G, Lotan T, Himmelhoch S, Fluhr R (1990) A major stylar matrix polypeptide (sp41) is a member of the pathogenesis-related proteins superclass. EMBO J 9:3429–3436

Reijnen WH, Van Herpen MMA, De Groot PFM, Olmedilla A, Schrauwen JAM, Weterings KAP, Wullems GJ (1991) Cellular localization of a pollen-specific messenger RNA in situ hybridization and confocal laser scanning microscopy. Sex Plant Reprod 4:254–257

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning – a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory press, Cold Spring Harbor, NY

Sanger F, Nicklen S, Coulsen AR (1977) DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Sedgley M, Blessing MA, Bonig I, Anderson MA, Clarke AE (1985) Arabinogalactan-proteins are localized extracellularly in the transmitting tissue of *Nicotiana alata* Link and Otto, an ornamental tobacco. Micron Microsc Acta 16:247–254

Sims TL 81993) Genetic regulation of self-incompatibility. Cri Rev Plant Sci 12:129–167

Van Herpen MMA (1984) Extracts from styles, developed at different temperatures, and their effect on compatibility of *Petunia hybrida* in excised-style culture. Acta Bot Neerl 33:195–203

Van Went JL, Willemse MTM (1984) Fertilization In: Johri BM (ed) Embryology of angiosperms. Springer, Berlin Heidelberg New York, pp 273–309

Von Heijne G (1986) A new method for predicting signal sequence cleavage sites. Nucleic Acids Res 14:4683–4690

Wang H, Wu HM, Cheung AY (1993) Development and pollination regulated accumulation and glycosylation of a stylar transmitting tissue-specific proline-rich protein. Plant Cell 5:1639–1650

Wu HM, Zou JT, May B, Gu Q, Cheung AY (1993) A tobacco gene family for flower cell wall proteins with a proline-rich domain and a cysteine-rich domain. Proc Natl Acad Sci USA 90:6829–6833