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

**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), relative 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 Willems 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 Arbele 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-
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 (SjS3 and SjS5) were supplied by Professor E. Jacobsen (Wageningen Agricultural University, The Netherlands). Cuttings were grown in a greenhouse 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 partly open 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-isopropylp-naphthalene sodium salt (Kodak)] and an equal volume of phenol, and incubated at 60°C for 10 min. Additional phenol/chloroform/isooamylic alcohol (25:24:1) extractions were performed until no interface remained. RNA was obtained after successive ethanol and 2 M LiCl 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 (SjS3xS2S4) 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 32P-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 cDNA library was differentially screened on nitrocellulose with 32P-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).

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 method (Church and Gilbert 1984). Nucleotide sequence analysis was carried out by the deoxyribonucleotide 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 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 1.3% 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 6x SETS (1x SETS is 0.15 M NaCl, 0.02 M TRIS-HCl pH 7.8, 1 mM EDTA), 5x Denhardt's (1x 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 1x SSC, 0.1% SDS or at 55°C 4x SSC, 0.1% SDS.
Sequences analyses of STS15 cDNA

The sense STS15 cDNA probe showed no hybridization in any tissue examined (Fig. 3A). The hybridization signal was observed in the peripheral and marginal regions of the style (Fig. 3B). No hybridization signal was observed in the style when hybridization signal was detected in the core of the style (Fig. 3D). The hybridization signal from the RNA transcript was observed by the expression of the STS15 gene was determined by Northern and hybridizations of STS15 were performed with the probe of STS15 cDNA.

The expression of STS15 in the different tissues is shown.

Localizatio of STS15 specific expression

el decreased (Fig. 2).

maximum at stage 3. At anthesis, the STS15 RNA was decreased in all tissues analyzed.

To isolate genes expressed in pollinated pistils during

Identification of a gene predominantly expressed in pistils
already present at stage 2, then increased and reached a
was produced with STIS 15 CDNA. The STIS 15 mRNA was
meant, RAP analysis of four stages (see Materials and
To detect STIS 15 transcripts during blastoderm de
issue even after extended exposure.
shown). STIS 15 transcripts were not detected in any other
was the same size as in pills (data not
pas: the signal was the same size as in oocytes and ss-
very weak signal could be observed in ovaries and ss-
not present in complete pills. At lower stringency, a
strongly hybridized to a single mRNA transcript of 0.7

Cold wash was washed in 1x SSC, 0.1% SDS at 55 °C and used for autoradi-
transcripts is induced at the larval pupal transition. The RAP
was produced with the STIS 15 CDNA. The size of the RAP
were produced with the STIS 15 CDNA. The size of the RAP
RNA was isolated from larval stages of Drosophila and equal amounts of
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Fig. 2 Expression of the STIS 15 gene during blastoderm de

Southern hybridization of genomic DNA

Laser scanning micrographs show the developed silver grains were detected in the emulsion by

The probes were hybridized to full-length inserts in a

In this hybridization, we have used according to Receiver et al.

The protocol of American type II SK(-) vector, the sample was immersed in a

Hardesty et al. 1989; White et al. 1986; and Hanson et al.

The probe was a 2.5-kb EcoRI-HindIII fragment labeled with

The probes were hybridized in parenthetical (referred to, Kumphadol, et al. 1989)

In this hybridization, we have used according to Receiver et al.

Cot-1 or Cot-2 DNA (30 pg) was digested with EcoRI or HindIII and

The filters were exposed to X-ray films with an in-

175
The conserved amino acid sequence is rich in basic residues with a potential cleavage site located between

The determination of the STS15 gene in other plant species

Comparison of the STS15 gene and its expression

Amino acid sequences do not display any homology with human forms, except for those in the nucleotide sequence of the STS15 gene. In plants, the STS15 gene is not detectable in the amino acid sequence.
Fig. 4  Nucleotide sequence of the STS 15 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 stslS gene, or a homologue of stslS, is present as one or two gene copies in these plant species. Using the STS 15 probe, two very strongly hybridizing EcoRI fragments were detected and four strong HindIII fragments were visible in digests of S. tuberosum genomic DNA (Fig. 5B). Sequence analysis revealed the presence of an internal HindIII restriction site in the STS 15 cDNA sequence. The number of DNA fragments hybridizing to the STS 15 probe indicate that the stslS gene belongs to a small gene family in dihaploid S. tuberosum.

To determine the conservation of expression of the stslS gene in these plant species, RNA from pistils was isolated and analyzed by northern blot hybridization. STS 15 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, stslS is a gene highly expressed in pistil. Since stslS homology at the genomic level was shown in B. oleracea, P. hybrida, and N. tabacum, stslS is a member of a small gene family which is conserved during evolution. Conservation at the expression level differs for the tested
species; only potato and tobacco showed expression of the \textit{sts15} gene or a gene homologous to \textit{sts15}. Despite the high level of conservation at the genomic level, the expression of \textit{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 \textit{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 styril 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 \textit{fst} gene isolated by Gu et al. (1992) and the \textit{ta20} gene described by Koltunow et al. (1990). The \textit{fst} and \textit{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 calllose-containing cells around the styril channel (Knox 1984). In this zone, Wu et al. (1993) showed the accumulation of CEP (Cys-rich extensin-like protein) mRNAs.

The localization of STS15 expression primarily in the styril cortex combined with its maximum expression just before anthesis suggests that the gene products of \textit{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 \textit{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.

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