Plant Molecular Biology **41:** 115–124, 1999.

Fertilization and wounding of the style induce the expression of a highly conserved plant gene homologous to a *Plasmodium falciparum* surface antigen in the wild potato *Solanum chacoense* Bitt.

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Received 1 April 1999; accepted in revised form 26 July 1999

Key words: fertilization-induced gene, *Plasmodium falciparum* surface antigen, *Solanum chacoense*, wild potato, wound-induced gene

Abstract

Pistil tissues are actively involved in pollen tube growth and respond to the presence of the growing pollen tubes by modulating the expression of specific genes. Once fertilization has occurred, complex developmental programs lead to embryogenesis, ovary maturation, and seed set. In order to understand the early events that follow pollination and fertilization we have used a subtractive hybridization approach to characterize genes which are related to pollination and fertilization events. One cDNA clone isolated and named SPP30 (*Solanum* pollinated pistil) was found to share significant sequence identities with a *Plasmodium falciparum* (malaria parasite) surface antigen and a yeast gene of unknown function. Searches in recent EST databases also revealed that SPP30 homologues are found in both monocot and dicot species. The presence of this conserved gene in evolutionarily distant organisms such as yeast, *Plasmodium*, and plants suggests that it codes for an essential cellular function. This is also strengthened by its extremely high sequence conservation in both monocots and dicots where virtually all substitutions tolerated are conservative.

Abbreviations: DPA, days post-anthesis; DPP, days post-pollination; DPW, days post-wounding.

Introduction

Sexual reproduction in flowering plants involves multiple and successive interactions between the male gametophyte, the pollen grain, and the female reproductive structure, the pistil. After landing on the stigma surface where the pollen adheres and hydrates, the pollen grain grows a protruding tube which is most probably guided by both the physical architecture and chemotrophic cues originating from the style and the ovary (Sanders and Lord, 1989; Ray *et al.*, 1997; Wolters-Arts *et al.*, 1998). The two sperm nuclei carried at the tip of each growing pollen tube then enter the ovule through the micropyle and fuse with the egg and the central cell nuclei of the embryo sac, thus forming the zygote and the endosperm, respectively (Russell, 1992).

From pollen grain hydration on the stigmatic surface to the delivery of the sperm cells in the embryo sac, numerous cell-cell interaction events take place between the growing pollen tube and the extracellular matrix of the style-transmitting tissue. During pollen tube growth the pistil plays active roles including pollen tube guidance and nourishment (Cheung, 1996; Wilhelmi and Preuss, 1997). Furthermore, in self-incompatible species, like *Solanum chacoense*, the pistil acts as an efficient sieve, enabling only

The nucleotide sequence data reported will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number AF136010 (SPP30 cDNA).

pollen tubes carrying an *S* allele different from the ones expressed in the style to reach the ovary (Matton *et al.*, 1994, 1997). Pollination itself induces multiple responses, the most dramatic being ovule development in orchid flowers (Zhang and O'Neill, 1993). The best characterized responses to pollination are the increase in ACC synthase and ACC oxidase gene expression leading to ethylene production and to perianth senescence (O'Neill *et al.*, 1993). Other pollination-induced events include cell deterioration and death of transmitting-tissue cells throughout the path of pollen tube growth, as well as the deglycosylation of stylar transmitting tissue-specific proteins and the poly(A) tail shortening from some transmitting tissue-specific mRNAs (Wang *et al.*, 1993, 1997). Differential screening strategies have been recently used in potato (van Eldik *et al.*, 1997b) and apple (Dong *et al.*, 1998b) to isolate pollination-inducible genes. Genes involved in basic metabolic functions including ribosomal protein, histone H2B, translation elongation factor 1α, and vacuolar ATPase were found to be up-regulated following pollination in apple (Dong *et al.*, 1998a). Genes involved in flavonoid biosynthesis were also found to be up-regulated in both apple (Dong *et al.*, 1998a) and potato (van Eldik *et al.*, 1997a,b) after pollination. Other pistil genes also characterized as being up-regulated after pollination include an *Arabidopsis* E2-related ubiquitin-conjugating enzyme (Watts *et al.*, 1994), two barley calreticulins (Chen *et al.*, 1994), a peroxidase (Chandra-Sekhar and Heij, 1995), a tobacco receptor-like protein kinase (Li and Gray, 1997) and an apple homologue of the DAD1 (defender against cell death 1) gene, an inhibitor of programmed cell death in *Caenorhabditis elegans* (Dong *et al.*, 1998b). Although successful in isolating genes of moderate to high abundance, differential screening is not sensitive enough to isolate weakly expressed genes after pollination and fertilization. *In vitro* fertilization with isolated gametes would substantially enrich the up-regulated fraction of target genes, but production of a large number of isolated gametes is limiting, difficult to master and limited to a few amenable species (Kranz and Dresselhaus, 1996).

In order to characterize the early responses following pollination and fertilization we have begun the isolation of pollination- and fertilization-induced genes through differential display, subtractive hybridization and virtual subtraction approaches in *Solanum chacoense*, a self-incompatible relative of the potato. In this study, we report the isolation of a ubiquitous and highly conserved plant gene which shows strong sequence identity with a *Plasmodium falciparum* (malaria parasite) surface antigen.

Materials and methods

Plant material

The diploid ($2n = 2x = 24$) *Solanum chacoense* Bitt. self-incompatible genotypes used include line PI 458314 (which carries the S_{11} and S_{12} self-incompatibility alleles) and line PI 230582 (which carries the S_{13} and S_{14} alleles) which were originally obtained from the Potato Introduction Station (Sturgeon Bay, WI). Plants were grown in greenhouses with 14 h of light per day.

Library construction and screening

The pollinated pistil cDNA library was made from 5 μg of poly(A)$^+$ mRNA isolated from compatibly pollinated pistils harvested 48 h after pollination in the ZAP express pBK vector following the manufacturer's instructions (Stratagene, La Jolla, CA). The subtracted cDNA pool was made by suppression subtractive hybridization (Diatchenko *et al.*, 1996) with poly(A)$^+$ mRNA from pistils 48 h after pollination as tester mRNAs and poly(A)$^+$ mRNA from unpollinated pistils as driver mRNAs following the manufacturer's instructions (PCR-select; Clontech, Palo Alto, CA). The subtracted cDNA pool was PCR-amplified, radiolabeled and used to screen the ZAP-pBK 48 h post-pollination library. Hybridizing phage plaques were scored and, after *in vivo* excision, tested individually by RNA slot blot analysis (Sambrook *et al.*, 1989) to confirm their induction by pollination and fertilization.

Isolation and gel blot analysis of RNA and DNA

Total RNA was isolated as described previously (Jones *et al.*, 1985). Polyadenylated RNA was prepared by oligo(dT) spin column chromatography as described in the mRNA separator kit protocol (Clontech). RNA concentration was determined by measuring its absorbance at 260 nm and verified (adjusted if necessary) by agarose gel electrophoresis and ethidium bromide staining. To confirm equal loading of total RNA on RNA gel blots, a 1 kb fragment of the *S. chacoense* 18S RNA was PCR-amplified and used as a probe. The oligonucleotides

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GACATTACAGTGCTAAGCGATCTAGAGAGAGAGTTGAAGGAAGAAGATGATAAATGTAGG 60
                                              M  I  N  V  G

GAAGATCAAACAGTACTCAAATGTTCTTGAGAGACCCCTCAGCAAAGGCAAACAAGAGGT 120
  K  I  K  Q  Y  S  N  V  L  E  R  P  L  S  K  G  K  Q  E  V

CAGTTTGAGTGCTTTTGCTTTCTTGTTTTCGGAGCTTGTTCAGTACAATCAAACTCAAGT 180
  S  L  S  A  F  A  F  L  F  S  E  L  V  Q  Y  N  Q  T  Q  V

GGACAACATTACTGAATTAGAGCGAAGGTTAGAGGATGCTGGCTATGCGGTTGGAGCCCG 240
  D  N  I  T  E  L  E  R  R  L  E  D  A  G  Y  A  V  G  A  R

AATTTTAGAACTTCTCTGCCATAGGGAAAAGGGAAACAGAAGGGAGACGCGACTACTGGG 300
  I  L  E  L  L  C  H  R  E  K  G  N  R  R  E  T  R  L  L  G

TATATTATCATTTGTGCATAGCACAGTATGGAAGGTTTTGTTTGGGAAGGTTGCTGACTC 360
  I  L  S  F  V  H  S  T  V  W  K  V  L  F  G  K  V  A  D  S

TCTTGAGAAAGGCACTGAACATGAAGATGAATATATGATTAGTGAGAAGGAGCTCCTTGT 420
  L  E  K  G  T  E  H  E  D  E  Y  M  I  S  E  K  E  L  L  V

CAACAGATTCATTTCAATTCCAAAAGATATGGGTGCTTTCAACTGCGGCAGTTTCGTTGC 480
  N  R  F  I  S  I  P  K  D  M  G  A  F  N  C  G  S  F  V  A

GGGTATTGTAAGGGGAGTTCTTGAAAATGCAGGTTTCCCAGCAGTAGTGACAGCTCATTT 540
  G  I  V  R  G  V  L  E  N  A  G  F  P  A  V  V  T  A  H  F

TGTTCCTGTGGAGGGGCAGCACCGACCCCGGACAACAATTTTGATAAAATTTGCTGAAGA 600
  V  P  V  E  G  Q  H  R  P  R  T  T  I  L  I  K  F  A  E  E

GGTACTAAGAAGGGAAGCAACACTAGGTTGATGTTTATTTGCCATTATATTTGGTGCAAA 660
  V  L  R  R  E  A  T  L  G  *

ATGCTTGAACAACATTATGATGAGATGACAAATGAAAGATCCTTTGATTGTTCAACACAT 720
CTTAGGTTCATGTCAATCCTAAGATCATACAATTGCCAGTTGTGACATTATTTTCTATAG 780
TACTATAGTTGACTTGTTCAATGCATCTTAGGTTTACCGCGATAAATTGTATCAAAACAT 840
CAAGCATTTATCTTCTTTGGTGTAAAATATTTGCACTTTGGAAGGTGATTTCTGGAAATT 900
TTATGTTGTTGATGAGCTATGAGTAGAAAGTTATTTGAGACAAGTTAAAAAAAAAAAAAA 960
AAAAAAA                                                       967
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Figure 1. Nucleotide and deduced amino acid sequence of the *S. chacoense* SPP30 cDNA. The sequence of the coding strand of the SPP30 cDNA insert is shown together with the deduced amino acid sequence. The start of translation was chosen as the first methionine in the sequence, which is also in good agreement with the plant and animal (ACAATGGC) initiation context (Fütterer and Hohn, 1996).

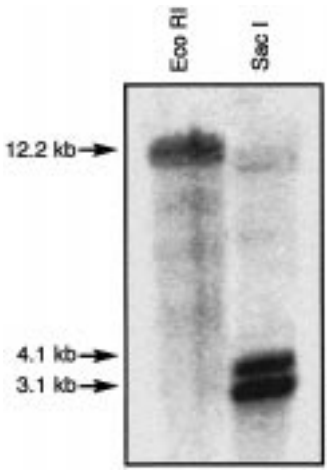

Figure 2. DNA gel blot analysis of the SPP30 gene. Genomic DNA (10 μg) isolated from *S. chacoense* leaves was digested with *Eco*RI or *Sac*I restriction enzymes and probed with the complete *Eco*RI/*Xho*I SPP30 cDNA insert. Molecular weight markers appear on the left.

used (5′-TCGATGGTAGGATAGTGGC-3′ and 5′-GCATAGCTAGTTAGCAGG-3′) were derived from highly conserved regions determined from a ClustalW alignment of 18S RNA sequences from *S. avicular*, *S. lycopersicon*, *S. melongena*, *S. petophylum* and *S. tuberosum*. Genomic DNA isolation was performed via a modified CTAB extraction method (Murray and Thompson, 1980; Reiter *et al.*, 1992). DNA gel blot analysis, including restriction, electrophoresis and capillary transfer onto a positively charged nylon membrane (Hybond N+, Amersham Pharmacia Biotech, Baie D'Urfé, Canada) were performed as described in Sambrook *et al.* (1989). Hybridization of the membrane was performed under high-stringency conditions at 65 °C as described by Church and Gilbert (1984) for 16 to 24 h and after hybridization the membrane was washed at room temperature, once with 4× SSC/0.1% SDS for 1 h, twice with 1× SSC/0.1% SDS at 50 °C for 30 min and twice with 0.1× SSC/0.1% SDS at 55 ° for 30 min (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0). RNA gel blot analyses were performed as described in Sambrook *et al.* (1989), following the formaldehyde denaturing protocol. RNAs were capillary-transferred onto Hybond N+ nylon membranes and cross-linked (120 mJ/cm^2) with a Hoefer UVC 500 UV Crosslinker. Hybridization of the membranes was performed under high-stringency conditions at 45 °C in 50% deionized formamide, 5× Denhardt's solution, 0.5% SDS, 200 μg/ml denatured salmon sperm DNA and 6× SSC for 16 to 24 h. After hybridization, the membranes were washed at room temperature once with 2× SSC/0.1% SDS for 1 h, twice with 1× SSC/0.1% SDS at 50 °C for 30 min and twice with 0.1× SSC/0.1% SDS at 55 °C for 30 min. Probes for both DNA and RNA gel blot analysis were synthesized from random-labeled isolated DNA inserts (Roche Diagnostic, Laval, Canada) with α-^{32}P-dCTP (ICN Biochemicals, Irvine, CA). The membranes were autoradiographed at −85 °C with one intensifying screen on Kodak Biomax MR film (Interscience, Markham, Canada).

Phytohormone and wounding treatments

Flowers were locally sprayed with aqueous solutions of either indoleacetic acid (IAA), indolebutyric acid (IBA), naphthaleneacetic acid (NAA), abscisic acid (ABA), gibberellin (GA_3), etephon, or kinetin once per day on two consecutive days, and pistils were collected for total RNA isolation 48 h after the

first treatment. All phytohormones were purchased from Sigma/Adrich (Oakville, Canada) and sprayed as 10 μM solutions. For wounding, a small forceps was used to slighly crush leaves and styles, and tissues were harvested 48 h after the wounding. Tubers were sliced and incubated in a moist Petri dish, also for 48 h.

Results

Isolation of the SPP30 cDNA

In order to characterize genes involved in pollen-pistil interactions and fertilization events we used a subtractive hybridization approach (Diatchenko *et al.*, 1996). A subtracted cDNA pool was made from compatibly pollinated pistils 48 h after pollination from which were subtracted common mRNAs from mature unpollinated pistils. The pool of subtracted cDNAs was then PCR-amplified and used to screen a 48 h post-pollination *Solanum chacoense* pistil cDNA library made in the λZap-pBK vector. Positives plaques were purified and plasmids were rescued by *in vivo* excision. cDNA clones were tested individually by RNA slot-blot hybridization to determine if they were up-regulated following pollination and/or fertilization events (data not shown). The nucleic acid sequence as well as the deduced amino acid sequence of one of the cDNA clones isolated are shown in Figure 1. The SPP30 clone (*Solanum* pollinated pistil) codes for a cDNA of 967 bp, including a short 5′-untranslated leader of 46 nucleotides (nt) and a 315 nt 3′ UTR. The size of the SPP30 cDNA corresponds to the size of the mRNA, as determined by RNA gel blot analyses (1.0 kb), suggesting that the SPP30 cDNA is full-length or near-full-length. The SPP30 cDNA codes for a 21.8 kDa protein with a predicted pI of 7.35. PCR amplification of *S. chacoense* genomic DNA with oligonucleotides complementary to the 5′ and 3′ ends of the SPP30 cDNA produced fragments of identical size, suggesting that the SPP30 gene is most probably intronless (data not shown).

Gene copy number of SPP30

A Southern blot of genomic DNA isolated from *S. chacoense* leaves was probed with the *Eco*RI-*Xho*I complete SPP30 cDNA insert. Only one 12 kb hybridizing fragment can be detected from the *Eco*RI-digested DNA and two hybridizing bands of 3.35 and 2.8 kb, respectively, are detected from the *Sac*I digestion (Figure 2). Since there is one *Sac*I restriction site in the cDNA sequence (position 414), this strongly suggest that the SPP30 is a single-copy gene in *S. chacoense*. A garden blot (data not shown) also indicates that weaker hybridizing fragments can be detected in other solanaceous plants (eggplant, tomato, ground cherry, petunia) as well as in Brassicaceae (*Arabidopsis thaliana*, *Brassica oleracea*).

Sequence analysis

Homology searches in DNA and protein databases revealed striking similarities with a *Plasmodium falciparum* (malaria parasite) blood stage antigen (Knapp *et al.*, 1989) and an unknown protein from yeast chromosome IV obtained from the Yeast Genome Sequencing Project (GenBank accession number U33050). An animo acid sequence comparison is shown in Figure 3. All three proteins share significant sequence identities throughout their entire sequence, except for two insertions found in the yeast gene, one located at the N-terminus of the protein, and a larger one from amino acid position 109 to 169 (numbering according to the YDR472w protein from *Saccharomyces cerevisiae*). In order to compare equivalent regions, the insertions from the yeast protein were removed to calculate amino acid identities. The SPP30 gene from *S. chacoense* shares 41% amino acid identity (60% similarity) with the 41-2 antigen from *P. falciparum* and 39% amino acid identity (57% similarity) with the YDR472w protein from *S. cerevisiae*. The 41-2 antigen and the YDR472w protein sequences share 33% amino acid identity (50% similarity). All three deduced proteins have similar isoelectric points, ranging from 6.34 to 8.11 and similar amino acid compositions. Hydrophilicity plots revealed the presence of a hydrophobic region of 11 amino acids flanked by negatively charged residues (D or E) in the N-terminal region for all three proteins (underlined in Figure 3). This region was previously suggested to be a signal peptide targeting sequence for the *P. falciparum* 41-2 surface antigen (Knapp *et al.*, 1989).

A search in EST databases yielded four recently described plant gene sequences highly homologous to SPP30 from *Arabidopsis thaliana*, rice, maize and hybrid aspen. Table 1 shows the nucleotide and amino acid sequence identities between these plant ESTs and SPP30. For amino acid sequence identities, the EST sequences were translated and amino acid sequence identities were determined with the open reading frame that could be assembled with these partial sequences. Nucleotide sequence identities be-

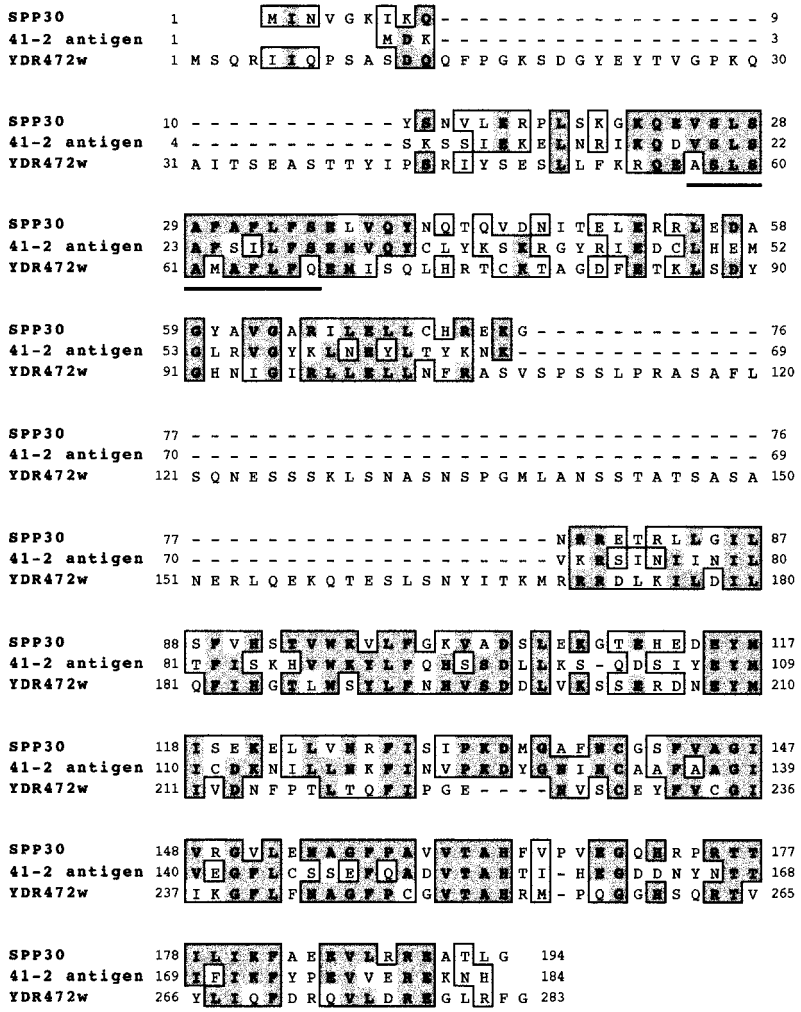

Figure 3. Alignment of the SPP30 deduced protein sequence with the *Plasmodium falciparum* and yeast homologues (GenBank accession number J04656 and U33050, respectively). The underlined region corresponds to the hydrophobic domain of 11 amino acids flanked by negatively charged residues (D or E) in the N-terminal region that was previously suggested to be a signal peptide targeting sequence for the *P. falciparum* 41-2 surface antigen (Knapp *et al.*, 1989). Identical and similar amino acid residues are boxed in dark gray and light gray shading respectively.

tween these ESTs and the SPP30 cDNA clone ranged from 74% to 80% while amino acid sequence identities ranged from 87% to 96%. Almost all amino acid substitutions between these sequences were found to be highly conservative.

Abundance of SPP30 mRNA in mature tissues and time-course accumulation after pollination

Tissue-specific expression of SPP30 was determined in mature tissues of *S. chacoense*. An RNA gel blot analysis (Figure 4) indicates that SPP30 is expressed in all tissues at low levels, except pollen and pollen tubes, where no hybridization signal could be detected even after prolonged exposure. Highest expression is seen in floral tissues, mainly in pollinated pistils 48 h after pollination, as expected from our subtractive hybridization screen.

In order to determine if the increase in mRNA abundance seen 48 h after pollination (Figure 4) is pollination- and/or fertilization-dependent, we performed an RNA time-course analysis after pollination (Figure 5). SPP30 mRNA levels increased very slightly from 0 to 36 h after pollination, reached a peak 48 h after pollination and declined to unpollinated levels by 96 h after pollination. Since most pollen tubes reach the ovary 36 h after pollination,

Table 1. Percentage of nucleotide and amino acid sequence identities between the *S. chacoense* SPP30 cDNA and plant ESTs.

	S. chacoense	*Arabidopsis*	Aspen	Rice	Maize
S. chacoense	100	77 (87)[1]	80 (90)[2]	74 (87)[3]	78 (96)[4]
Arabidopsis		100	76	73	72
Aspen			100	73	76
Rice				100	84
Maize					100

The maize EST came from an inflorescence (immature ear) cDNA library (GenBank accession number AI065654). The rice EST came from a root cDNA library (GenBank D24245). The *Arabidopsis thaliana* EST came from a mixed tissue cDNA library (GenBank T88475). The hybrid aspen (*Populus tremula* × *Populus tremuloides*) EST came from a meristematic cambial zone cDNA library (GenBank AI165198 and AI162891).
[1]Partial sequence of 168 amino acids (5′ and 3′ regions of the EST combined).
[2]Partial sequence of 80 amino acids.
[3]Partial sequence of 97 amino acids.
[4]Partial sequence of 124 amino acids.

and fertilization occurs in *Solanum* spp. from 36 h onward (Clarke, 1940; Williams, 1955; and our unpublished observations), the maximum SPP30 mRNA accumulation reached at 48 h after pollination suggests that it is most likely a fertilization-dependent event. Alternatively, the transient increase observed in pollinated tissue may be developmentally regulated. To test this hypothesis, SPP30 mRNA abundance was also determined in unpollinated pistils from the day before anthesis until five days after anthesis (DPA). SPP30 mRNA levels were found to be unaffected by the age of the flower (data not shown). To rule out a possible slow response to pollination, we took advantage of the gametophytic self-incompatibility system of *S. chacoense* and performed an incompatible (self) pollination. In incompatible pollinations, pollen tubes are generally arrested in the top half of the style. No significant SPP30 mRNA increase could be detected in either styles or ovaries after a 48 h incompatible pollination (data not shown), suggesting that the increase in SPP30 mRNA abundance observed is a fertilization-dependent event.

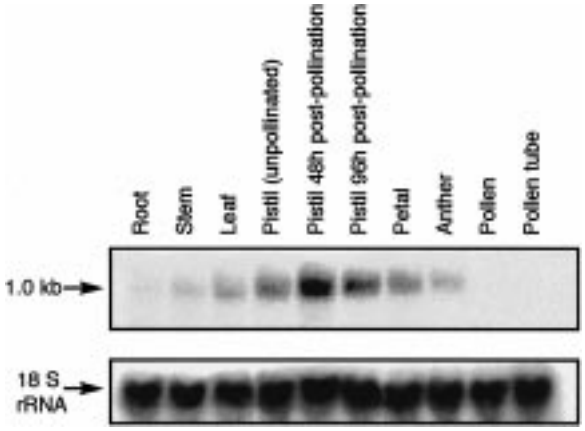

Figure 4. RNA gel blot analysis of SPP30 transcript levels in mature tissues. Upper panel: 10 μg of total RNA from various tissues was probed with the 1.0 kb complete *Eco*RI/*Xho*I SPP30 cDNA insert. Lower panel: same as upper panel except that the membrane was stripped and reprobed with an 18S ribosomal cDNA probe from *S. chacoense*.

Wounding of the style triggers SPP30 mRNA expression at a distance in the ovary

In many species, pollination is known to induce cellular deterioration of specific cells or tissues including the secretory cells in the stigmatic region and the transmitting tissue of the style (Cheung, 1996). To determine if cell death caused by wounding could trigger SPP30 mRNA accumulation in the style or at a distance in the ovary, the styles of young flowers were slightly crushed with tweezers, and the styles and ovaries were collected separately 48 h later. Figure 6 shows that wounding of the style induced SPP30 mRNA accumulation in the style itself and at a distance in the ovary, to levels comparable to a 48 h compatible pollination. Pollination had almost no effect on SPP30 mRNA levels in the style. Therefore, localized cellular deterioration and death in the transmitting tissue of the style, caused by pollination (either compatible or incompatible), is not sufficient to trigger SPP30 mRNA accumulation, while more extensive damage (wounding), in both the style cortex and transmitting tissue, induced SPP30 mRNA accumulation in

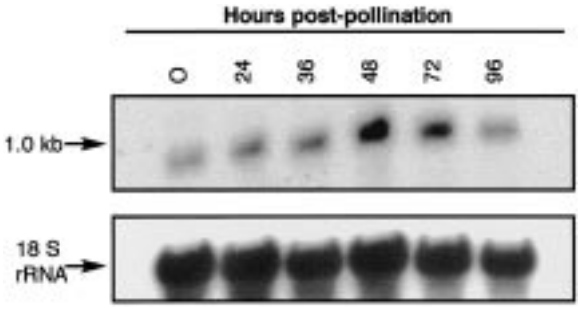

Figure 5. Temporal expression pattern of SPP30 mRNA levels in pistil tissues during a compatible pollination. SPP30 transcript levels were determined by RNA gel blot analysis following a compatible pollination (pollen from a $S_{11}S_{12}$ genotype, female recipient from a $S_{13}S_{14}$ genotype). Upper panel: 10 μg of total RNA from whole pistil (stigma, style and ovary) was collected at various times after pollination and probed with the 1.0 kb SPP30 cDNA insert. Lower panel: same as upper panel except that the membrane was stripped and reprobed with an 18S ribosomal cDNA probe from *S. chacoense*.

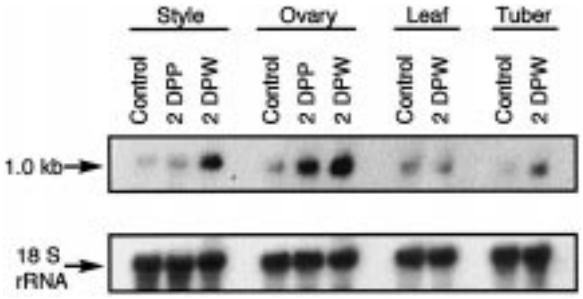

Figure 6. Effect of pollination and wounding on SPP30 mRNA levels. SPP30 transcript levels were determined by RNA gel blot analysis of wounded tissues 2 days after wounding (DPW) or 2 days after pollination (DPP). Total RNA from various tissues (10 μg) was probed with the 1.0 kb SPP30 cDNA insert. Styles were either pollinated or slightly crushed with tweezers and collected two days later (DPP and DPW respectively). Unpollinated and unwounded styles served as controls. Ovaries from the same pistils were also collected two days after either pollination (2 DPP) or wounding (2 DPW). Lower panel: same as upper panel except that the membrane was stripped and reprobed with an 18S ribosomal cDNA probe from *S. chacoense*.

the style and at a distance in the ovary. Since wounding of the style induced SPP30 mRNA accumulation in styles and ovaries, we tested if wounding could also induce SPP30 mRNA accumulation in leaves and tubers. Figure 6 also shows that SPP30 mRNA levels increase in wounded (sliced) tubers, but not in wounded leaves.

Hormonal control of mRNA expression

The nature of the wound signal that triggers SPP30 mRNA accumulation in the ovary remains to be determined, but plant hormones have been implicated

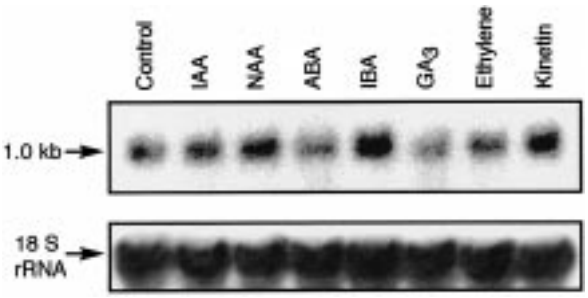

Figure 7. Effect of hormonal treatments on *S. chacoense* flowers. Upper panel: SPP30 transcript levels were determined by RNA gel blot analysis of unpollinated pistil tissues collected 48 h after various hormonal spray treatments (10 μM solutions). Total RNA (10 μg) from whole pistil (stigma, style and ovary) was probed with the 1.0 kb SPP30 cDNA insert. Lower panel: same as upper panel except that the membrane was stripped and reprobed with an 18S ribosomal cDNA probe from *S. chacoense.*

in many physiological processes during flowering and fertilization. To test if SPP30 mRNA expression was under hormonal control, we sprayed *S. chacoense* flowers, bearing flowers at different developmental stages, once per day for two consecutive days, with various phytohormones. Pistils (stigma, style and ovary) were then collected from open mature flowers. An RNA gel blot analysis of the effect of hormonal treatments on SPP30 mRNA expression in pistils of treated flowers is shown in Figure 7. Of the auxins used, IBA had the strongest effect and induced a significant increase in SPP30 mRNA expression. Kinetin also induced SPP30 mRNA accumulation while ABA, GA_3, and ethylene had little or no effect. To determine if stress hormones known to induce wound or defense responses could be mediating the SPP30 mRNA increase in style and ovary, flowers were individually sprayed with jasmonic acid (JA) or salicylic acid (SA), or treated in an airtight chamber with the volatile compound methyl jasmonate (MeJA). ABA, JA and MeJA are well known mediators of wound-responsive genes (Peña-Cortés *et al.*, 1989; Hildmann *et al.*, 1992; Wasternack and Parthier, 1997), and jasmonates and SA are also involved in mediating defense gene expression (Reymond and Farmer, 1998). None of the stress hormones used induced SPP30 mRNA accumulation in styles or ovaries (not shown).

Discussion

While searching for genes involved in pollen-pistil interactions and fertilization events, we have characterized a gene with strong sequence similarities to

a blood surface antigen from the malaria parasite *P. falciparum* and to a yeast gene of unknown function. Significant, albeit weaker sequence identities, were also found through pattern-hit initiated BLAST (Zhang *et al.*, 1998) with genes of unknown function in man and in the nematode *C. elegans* (GenBank accession numbers AC005757 and Z83113, respectively). Although no function has been attributed to this gene in any of these organisms, the presence of such a highly conserved gene in plants, yeast, animals and *P. falciparum* (an Apicomplexa, a group of unicellular endoparasites) suggests an ancient and essential cellular function. Furthermore, SPP30 homologues in *Arabidopsis*, maize, rice, and hybrid aspen are extremely similar (around 90% amino acid identity) and almost all amino acid substitutions are highly conservative, again suggesting an essential function.

The SPP30 plant gene as well as the ESTs from *Arabidopsis*, maize, rice, and aspen are more closely related to the *P. falciparum* 41-2 antigen than to the yeast gene, which has two large insertions (one immediately N-terminal and a second one in the middle of the protein). Interestingly, Apicomplexan parasites, closely related to dinoflagellate algae, possess a vestigial plastid that has been hypothesized to be a non-photosynthetic derivative of the dinoflagellate plastid (McFadden *et al.*, 1996, 1997). The presence of this vestigial plastid in *P. falciparum* prompted us to verify if the 41-2 antigen was encoded within the *P. falciparum* 35 kb circular plastid DNA. No match could be found; thus, the 41-2 antigen in *P. falciparum* is nuclear-encoded.

Hydrophilicity plots revealed the presence of a hydrophobic region of 11 amino acids flanked by negatively charged residues in the N-terminal region for all SPP30 homologues (underlined in Figure 3). This region was suggested to be a signal peptide targeting sequence for the *P. falciparum* 41-2 surface antigen, which was localized, by immunoelectron microscopy, on the schizont membrane, on the internal surface of the parasitized erythrocyte, and on membranous structures in the erythrocyte cytoplasm (Knapp *et al.*, 1989). Western blot analysis showed that the 41-2 antigen migrates at a molecular mass of 29 kDa which suggested that the protein could be post-translationaly modified, though no experimental evidence (e.g. deglycosylation) was provided (Knapp *et al.*, 1989). Although putative N-glycosylation sites are present on both SPP30 and the 41-2 antigen, they are not conserved in their positions. Furthermore, only one cysteine residue is conserved between the SPP30 homologues, suggesting that no disulfide bridges would be involved in maintaining an identical tertiary structure. Since the putative signal peptide previously proposed is wrongly positioned (not immediately N-terminal) and that signal peptide predictive programs (SignalP, PSORT) did not detect any N-terminal sequences which could serve as an ER targeting sequence, the SPP30 protein and its homologues are most probably cytoplasmic proteins. Although labeling of the *P. falciparum* 41-2 antigen was detected in membrane structures by immunoelectron microscopy, the 41-2 antigen was also detected in the cytoplasm and found by western blot analysis in both the soluble and membrane fractions of the schizont, but not in the schizont culture supernatant (Knapp *et al.*, 1989). This, combined with the presence of two other conserved hydrophobic regions (amino acid region 73–85 and 130-147, 41-2 antigen numbering) could suggests that the SPP30 homologues, most probably localized in the cytoplasm, might loosely interact with membranes via these three hydrophobic regions, although none of these regions are long enough to be a membrane anchoring or spanning domain.

Expression patterns of the SPP30 cDNA clone also indicate that it most probably encodes a very basic cellular function as it is expressed to detectable levels in all tissues with the exception of pollen and pollen tubes. Highest expression is observed transiently in ovaries after fertilization as determined by RNA gel blot analyses (Figures 4 and 5). Since the increase observed in SPP30 mRNA accumulation only occurs after fertilization and both incompatible pollinations or compatible pollinations where pollen tubes have not yet reached the ovary do not induce a similar SPP30 mRNA increase, this suggests that pollination per se is insufficient to trigger SPP30 gene expression, and that the transient SPP30 mRNA increase is a fertilization-dependent event.

Phytohormones are known to have profound effects on floral development, fertilization and fruit development. Of the hormonal treatments, IBA had the strongest effect on SPP30 mRNA accumulation in pistils. Auxins, in general, are known to be capable of inducing parthenocarpic fruit development in many solanaceous species (Gustafson, 1936). In our hands, IBA treatment consisting of imbibing the stigma surface with a 1% IBA solution, consistently gave fruit, albeit of smaller than normal size, and absolutely seedless (data not shown). Of the phytohormone treatments, IBA had the strongest effect

on SPP30 mRNA levels in pistils (Figure 7). Treatments with IAA were ineffective in promoting fruit development under the same experimental conditions, and only slightly increased SPP30 mRNA levels in sprayed pistils (Figure 7). These results suggest that cell growth and differentiation induced either by fertilization, parthenocarpic fruit development or wounding might be triggering the accumulation of SPP30 mRNAs, although involvement of the SPP30 gene product in the wound response is less likely since no wound hormone treatments could induce SPP30 mRNA accumulation.

Although of unknown function, the very high level of sequence conservation among SPP30 plant homologues in both monocots and dicots, combined with the presence of a similar gene in both yeast and *Plasmodium falciparum*, suggest an ancient and essential cellular function for the SPP30 gene. To understand the role of this gene in plant development, transgenic plants overexpressing the SPP30 cDNA in either sense or anti-sense orientation are currently being produced.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada and by Le Fonds pour la Formation des Chercheurs et l'Aide à la Recherce (FCAR, Québec). We thank Sylvain Lebeurrier and Gabriel Téodorescu for plant care and maintenance, and Dr Dwight U. Beebe for critically reviewing the manuscript.

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