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MINIREVIEW ARTICLE

Profiling the aminopropyltransferases in plants: their structure, expression and manipulation

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Abstract Polyamines are organic polycations that are involved in a wide range of cellular activities related to growth, development, and stress response in plants. Higher polyamines spermidine and spermine are synthesized in plants and animals by a class of enzymes called aminopropyltransferases that transfer aminopropyl moieties (derived from decarboxylated S-adenosylmethionine) to putrescine and spermidine to produce spermidine and spermine, respectively. The higher polyamines show a much tighter homeostatic regulation of their metabolism than the diamine putrescine in most plants; therefore, the aminopropyltransferases are of high significance. We present here a comprehensive summary of the current literature on plant aminopropyltransferases including their distribution, biochemical properties, genomic organization, pattern of expression during development, and their responses to abiotic stresses, and manipulation of their cellular activity through chemical inhibitors, mutations, and genetic engineering. This minireview complements several recent reviews on the overall biosynthetic pathway of polyamines and their physiological roles in plants and animals. It is concluded that (1) plants often have two copies of the common *aminopropyltransferase* genes which exhibit redundancy of function, (2) their genomic organization is highly conserved, (3) direct enzyme activity data on biochemical properties of these enzymes are scant, (4) often there is a poor correlation among transcripts, enzyme activity and cellular contents of the respective polyamine, and (5) transgenic work mostly confirms the tight regulation of cellular contents of spermidine and spermine. An understanding of expression and regulation of aminopropyltransferases at the metabolic level will help us in effective use of genetic engineering approaches for the improvement in nutritional value and stress responses of plants.

Keywords Polyamines · Spermidine synthase · Spermine synthase · Transgenic manipulation · Genetic manipulation · Putrescine

Abbreviations

Agm	Agmatine
ADC	Arginine decarboxylase
APT	Aminopropyltransferase
Cad	Cadaverine
CHA	Cyclohexylamine
dcSAM	Decarboxylated S-adenosylmethionine
DCHA	Dicyclohexylamine
MTA	5′-methylthioadenosine
ODC	Ornithine decarboxylase
ORF	Open reading frame
PA	Polyamine
Put	Putrescine
SAM	S-adenosylmethionine
SAMDC	S-adenosylmethionine decarboxylase
Spd	Spermidine
SPDS	Spermidine synthase
Spm	Spermine
SPMS	Spermine synthase
tSpm	Thermospermine
tSPMS	Thermospermine synthase
UTR	Untranslated region
WT	Wild type

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Introduction

Polyamines are ubiquitously distributed cationic compounds in living organisms. They play important roles in numerous cellular functions in diverse physiological processes (Handa and Mattoo 2010; Igarashi and Kashiwagi 2010). Most common PAs found in higher organisms are diamine Put, triamine Spd, and tetraamine Spm. Recently, a structural isomer of Spm called tSpm, initially reported in prokaryotes, has also been identified in plants (Knott et al. 2007). In addition to the common PAs, several unusual PAs are prevalent in thermophilic archaea and bacteria, whose major function is to stabilize the DNA and RNA of these organisms at high temperatures (Oshima 2010). These unusual PAs can be broadly classified into two groups; long-chain PAs (e.g. homocaldohexamine, caldohexamine, homocaldopentamine) and branched PAs (e.g. tetrakis(3-aminopropyl)ammonium, mitsubishine). Unusual PAs in thermophiles vary among species and with changes in temperature at which they grow. For example, certain thermophilic eubacteria and archaea contain branched PAs as major species while others have only long-chain PAs (Hamana et al. 1999). Moderate thermophiles, e.g., *Bacillus* and *Thermoplasma* often do not possess long-chain or branched PAs (Hamana et al. 1999). Another unusual PA, sym-homospermidine has recently been reported in *Thermus thermophilus* (Oshima 2010).

Besides involvement of the unusual PAs in thermal stabilization, several long-chain PAs are implicated in cell wall formation and wall architecture determination of siliceous diatoms (Kröger et al. 2000). Long-chain PAs found in *Thalassiosira pseudonana* were N-methylated polypropyleneimine derivatives (up to 20 repeat units) that are bound to Orn, Put, Spd, and Spm. They can exist either free or in conjugation with polycationic peptides called silaffins (Kröger et al. 2000). Long-chain PAs in coordination with silaffins have been shown to polymerize monomeric silicic acid to polymeric silica, the major cell wall component of silicaceous diatoms.

The diamine Put is synthesized either from Orn or from Arg; the former is brought about directly by ODC and the latter by ADC and several additional enzymes, which vary among species (e.g. Agm ureohydrolase or agmatinase in *E. coli*, Agm iminohydrolase and N-carbamoylputrescine amidohydrolase in plants). Production of triamines and tetraamines, on the other hand, is catalyzed by a class of enzymes called APTs, which transfer an aminopropyl residue to an amine acceptor on another PA, generating MTA as a byproduct. The aminopropyl moiety in this reaction is derived from dcSAM, which is produced from decarboxylation of SAM by SAMDC.

polyamine substrate + dcSAM
$\rightarrow 5' -$ methylthioadenosine + higher polyamine.

The APT Spd synthase (SPDS, EC 2.5.1.16) is specific to its amine acceptor Put and produces Spd, whereas Spm synthase (SPMS, EC 2.5.1.22) and tSPM synthase (tSPMS, EC 2.5.1.79) show substrate specificity to Spd and synthesize Spm and tSpm, respectively. In addition to those widely distributed APTs, a different PA biosynthetic pathway found in *T. thermophilus* reveals a novel APT which uses Agm as substrate to produce an intermediate aminopropyl-Agm, which is then hydrolyzed to Spd (Oshima 2010). Recently, a new kind of APT, called Agm/Cad APT (ACAPT), has been reported from hyperthermophile archaeon *Pyrococcus furiosus*, whose substrate specificity ranges from diamines like Put, Cad, Agm, and 1,3-diaminopropane to unusual substrates like sym-norSpd (Cacciapuoti et al. 2007). The other APTs involved in the biosynthesis of long-chain and branched PAs in thermophilic bacteria have not been fully characterized (Oshima 2010 and references therein).

The physiological functions of APTs have been studied in a variety of organisms using different techniques, including chemical inhibitors, mutations, knockouts, transgenic expression, etc. A number of recent reviews on PAs have covered topics such as their metabolism and regulation, roles in growth and diseases, importance in plant development and stress response, and cellular functions (Handa and Mattoo 2010; Igarashi and Kashiwagi 2010); however, most of them have been broad-based and not focused on the enzymes involved. Specific to APTs, a few reviews have described their structure, functions, and genetics mostly with emphasis on animal enzymes (Ikeguchi et al. 2006; Pegg and Michael 2010). This review summarizes recent progress and future applications of studies with plant APTs, including relevant comparisons with animal and microbial enzymes. Also, the discussion is mostly limited to three common APTs found in plants, namely, SPDS, SPMS, and tSPMS.

Biochemical properties of aminopropyltransferases

Enzyme activity of SPDS and SPMS is measured either by following the incorporation of radio labeled aminopropyl moiety from ^{14}C-dcSAM into the product (Hibasami and Pegg 1978) or the conversion of ^{14}C-Put/Spd into the respective higher PA (Porta et al. 1981). In either case, the labeled product is separated after dansylation by thin-layer chromatography (TLC) or HPLC (High Performance Liquid Chromatography) and quantified by counting radioactivity. An alternate approach is to measure the byproduct MTA from dcSAM, which is separated by TLC, paper electrophoresis or phosphocellulose ion exchange chromatography (Enomoto et al. 2006 and references therein). However, these procedures are cumbersome,

time-consuming, and expensive; thus, information available about APTs is rather scant as compared with the Put biosynthetic enzymes like ADC and ODC. Lee and Cho (1997) have described yet another protocol based on radioimmunoassay of the byproduct MTA, which is more sensitive and time saving. It also eliminates the interference caused by PA oxidases when radioactive PAs are measured. Enomoto et al. (2006) reported a high-throughput approach also based on the immunoassay of MTA but with fluorescent labeling instead of radioisotope labeling. Activity of tSPMS is measured by a similar isotopic assay as for SPMS (Knott et al. 2007).

Details on enzyme kinetics are available for human and a few other APTs. The human SPDS showed a K_{cat} value of 1.9 s^{-1} at pH 7.5, 37°C; K_m values for dcSAM and Put were 20 and 0.9 μM, respectively (Wu et al. 2007). The human SPMS K_{cat} value was 32 s^{-1} and a K_m of 800 μM for Spd and 0.45 μM for dcSAM (Wu et al. 2008). *Thalassiosira pseudonana* tSPMS showed the highest enzyme activity at 55°C with optimal pH range from 9.4–9.6. The K_m values for substrates were 91 μM for dcSAM and 104 μM Spd (Romer et al. 2008). The optimal pH and temperature for soybean (*Glycine max*) SPDS activity were 8.5 and 37°C, respectively; K_m value was 0.426 μM for dcSAM and 32.45 μM for Put (Yoon et al. 2000). Whereas SPMSs in monocots have a PEST–rich region at the C-terminus, potentially indicating a faster turnover, only limited experimental data are available on the turnover of APTs in most organisms (Rodríguez-Kessler et al. 2010).

At the molecular level, most known APTs are homodimeric,although a few, such as those found in thermophiles and diatoms, are presumed to be tetramers (Ikeguchi et al. 2006; Knott et al. 2007; Pegg and Michael 2010). Wu et al. (2007) showed that the N-terminal and C-terminal domains of human hSPDSs are involved in the dimerization process, with the central part having the catalytic domain. *E. coli* SPDS, which also consists of a similar N-terminal domain and C-terminal catalytic domain, has a much larger substrate-binding pocket than the other enzymes, which might be responsible for its broader substrate specificity (Zhou et al. 2010).

Monomers of human SPMS also have N-terminal domain associated with dimerization, a central β-strand domain, and a C-terminal catalytic domain. Compared with hSPDS, the hSPMS has Ala^{127} instead of a Trp^{28} (in hSPDS) at the corresponding position in the active site; the latter residue serves as a spatial block to binding of larger substrate in hSPDS, which accounts for its high specificity to Put (Wu et al. 2008).

Crystal structures of SPDSs and SPMSs from several other organisms are also available (Pegg and Michael 2010 and references therein). Although tSPMS has been recently classified apart from SPMS, sequence comparisons show highly conserved substrate-binding sites in both, with a replacement of Asp by Glu at the dcSAM-binding site in *T. pseudonana* tSPMS (Romer et al. 2008). Interestingly, an earlier study on *Arabidopsis thaliana* APTs demonstrated that *At*SPDS2 monomers may interact with *At*SPDS1 and also with *At*SPMS, forming heterodimers in vitro; however, this is inconsistent with the crystal structure of animal APTs, which are homodimeric (Panicot et al. 2002). Since no follow-up work on Arabidopsis has been published, the underlying mechanism of this interaction is still unknown (Ikeguchi et al. 2006). The purified *Pyrococcus furiosus* ACAPT enzyme is also a homodimer, which is highly thermo-stable up to 108°C, with Cad further increasing its stability to 112°C (Cacciapuoti et al. 2007).

Inhibitors of aminopropyltransferases

For obvious reasons of the involvement of PAs in a multitude of growth and developmental processes, and their metabolism being targeted for cancer and antiparasitic chemotherapy, a plethora of inhibitors of animal APTs has been synthesized and tested to manipulate cellular contents of Spd and Spm. The body of literature on this topic is voluminous, particularly involving animals and animal cell cultures (reviewed by Ikeguchi et al. 2006). Inhibitors have also been useful to study the role of APTs in cellular functions, morphogenesis, growth, and differentiation in plants.

Pollen development in kiwi fruit (*Actinidia deliciosa*) was inhibited by SPDS inhibitor CHA and methylglyoxal-bis-(guanylhydrazone) (MGBG, an inhibitor of SAMDC); there also was a significant reduction in the Spd content (Falasca et al. 2010). In white spruce (*Picea glauca*) inhibition of SPDS and SPMS by DCHA reduced somatic embryogenesis (Meskaoui and Trembaly 2009). In tobacco thin layer cultures, CHA caused hypertrophy in the subepidermal and parenchymal cell layers (Altamura et al. 1993). Nuclear division was delayed in cells treated with CHA or CHA + Spd, and higher frequency of amitotic cells was observed in CHA-treated cells as compared with the control; the latter was recovered to some extent by exogenous Spd.

Tanimoto and Matsubara (1995) found that both CHA and aminopropyl-CHA (APCHA, an inhibitor of SPMS) significantly decreased bulblet formation from bulb scale explants of lily (*Lilium* sp.); Spd and Spm reversed the effects in both cases. Recently, in a detailed study of the role of Spd in development of spermatids in male gametophyte of the water fern *Marsilea vestita*, Deeb et al. (2010) found that CHA or *SPDS* gene silencing by SiRNA inhibited spermatid differentiation by interfering with

nuclear elongation and microtubule organization; this effect was not fully reversed by Spd.

In two fungal pathogens of plants (*Gaeumannomyces graminis* and *Pyrenophora avenae*), SPDS inhibition by CHA reduced mycelial growth (West and Walters 1989; Mackintosh and Walters 1997) and caused a reduction in powdery mildew infection in barley (West and Walters 1988); it also decreased virulence of *Sclerotinia sclerotiorum* in tobacco (Gárriz et al. 2003).

Genomic structure and evolution

Genes encoding APTs have been identified and cloned from a diverse group of organisms, representing all major kingdoms. Most prokaryotes only contain genes for SPDS; however, Spm and tSpm have also been found in many bacteria, indicating that the presence of *SPMS* and *tSPMS*-like genes in bacteria cannot be ruled out (Minguet et al. 2008; Rodríguez-Kessler et al. 2010). Fungi and animals have genes for both SPDS and SPMS, while plants apparently may have all three common APT genes. Unlike other genes of the PA biosynthetic pathway enzymes in plants (e.g. *ADC*, *ODC* and *SAMDC*), whose main ORFs do not contain introns; eukaryotic *APT* genes do have introns within their ORFs (Figs. 1, 2, 3, 4, 5). The exon–intron organization of plant APT genes seems to be highly conserved (Minguet et al. 2008; Rodríguez-Kessler et al. 2010), with major variability occurring near the 5′- and the 3′-UTRs. Most plant *SPDS* genes have nine exons and *SPMS* genes have ten or eleven exons (Figs. 1, 2). Furthermore, the arrangement and sizes of exons are also conserved between the two gene families, signifying that the two enzymes participate in similar biochemical

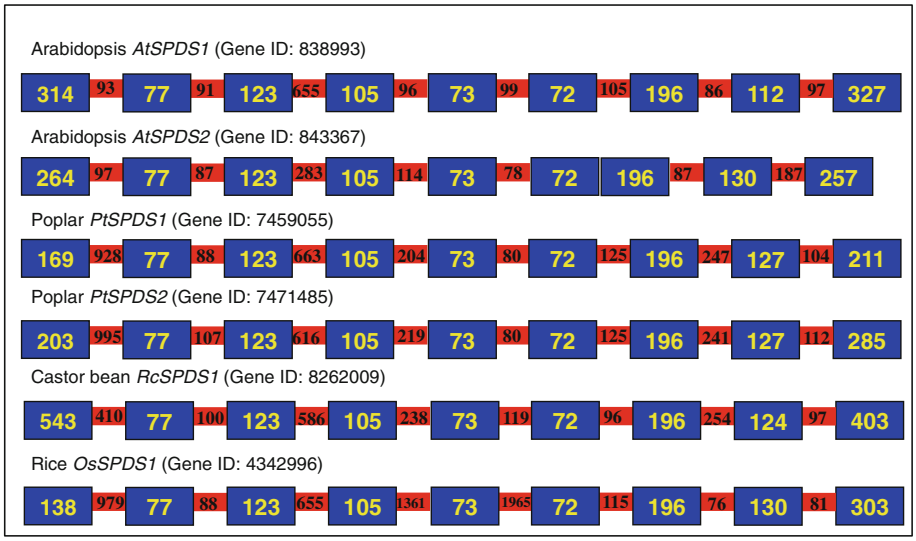

Fig. 1 Exon-intron organization of plant *SPDS* genes. *Blue boxes* indicate exons and *red lines* indicate introns. The sixth exon of *AtSPDS2* is derived from the fusion of exon 6 and 7 of other plant *SPDSs* (Modified from Rodríguez-Kessler et al. 2010)

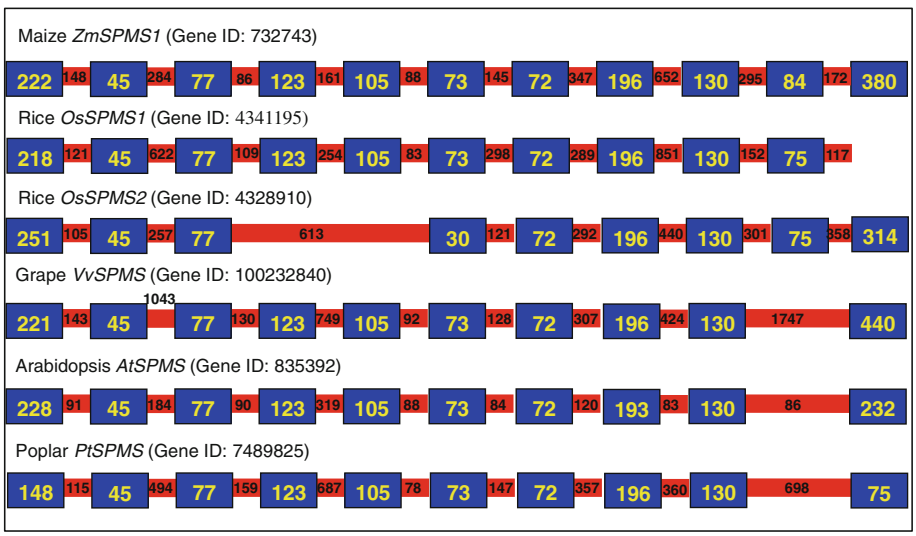

Fig. 2 Exon-intron organization of plant *SPMS* genes. *Blue boxes* indicate exons and *red lines* indicate introns. *OsSPMS2* lacks Exon 4, 5 and partial exon 6 of other plant *SPMSs*. Dicot (Arabidopsis, poplar and grapes) *SPMSs* do not have the tenth exon of monocot (maize and rice) *SPMSs* (Modified from Rodríguez-Kessler et al. 2010)

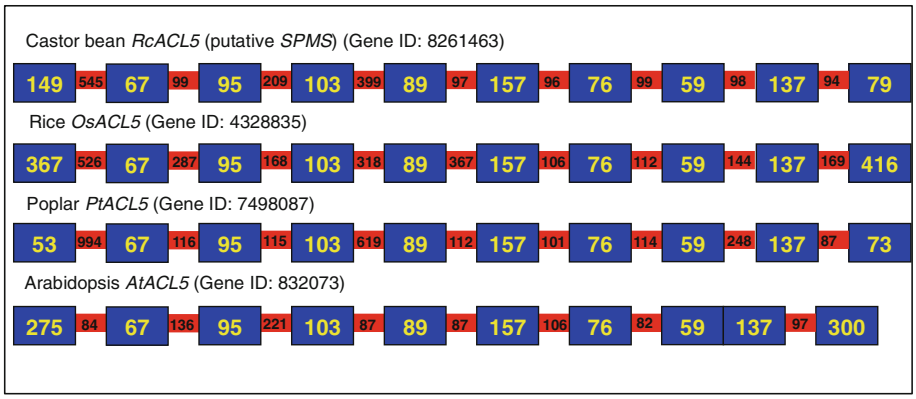

Fig. 3 Exon-intron organization of plant *tSPMS* genes (*ACL5*). *Blue boxes* indicate exons and *red lines* indicate introns. The eighth exon of *AtACL5* is derived from the fusion of exon 8 and 9 of other plant *ACL5s* (Modified from Rodríguez-Kessler et al. 2010)

reactions and may have evolved from a common ancestral gene.

As compared with *SPDS* genes, monocot *SPMS* genes contain an additional exon (the 10th exon) at the 3′-end of the ORF, which is absent in the dicot *SPMS*s (Fig. 2). This exon encodes a PEST-rich region that might contribute to the regulation of SPMS protein degradation in monocots (Rodríguez-Kessler et al. 2010). The genomic organization of plant *tSPMS* (*ACL5*) genes, which contain ten exons in the ORF, is also conserved, but it is completely different from that of *SPDSs* and *SPMS* (Fig. 3), thus leading to the suggestion of its independent origin.

Figure 4 shows that the genomic organization of animal *SPDSs* and *SPMS*s are quite different from their plant counterparts, but overall they are highly conserved among themselves. Almost all mammalian *SPDS*s shown in Fig. 4a have eight exons, except the mouse gene, whose 5th exon (230 bp) apparently is a fusion product of the 5th (84 bp) and the 6th (146 bp) exons of other mammalian *SPDS*s. Main exon variations are seen at the 5′ and the 3′ends. Zebrafish *SPDS* shows the same exon pattern as that in mammals; on the other hand, exon 2 is absent in chicken *SPDS*. Some exons in tick (*Ixodes scapularis*) *SPDS* are perhaps missing due to its incomplete sequence; nevertheless, its 2nd, 3rd and 4th exons are identical in size with the corresponding exons in higher animals. Greater variation in exon organization exists in primitive animals, e.g., nematode (*Loa loa*).

Animal *SPMS* genes, which contain 11 exons in most cases, have a different organization pattern from the corresponding *SPDS*s (Fig. 4b); however, the 2nd (121 bp) exon is identical in all cases. The animal *SPMS* genes are also highly conserved in their genomic organization amongst species. Among the exons found in all vertebrates, only the first and last, which contain 3′- and 5′-UTRs, vary in size.

Some fungal *SPDS* genes do not have introns, and those that do have them show a conserved five-exon pattern with the 2nd through 4th exons being consistent in size (Fig. 5). On the other hand, *SPMS* genes with introns are found only in a few fungi; these genes typically do not show much conservation among species (Fig. 5).

In terms of the evolution of *APT*s, an ancestral *SPDS* (probably prokaryotic) is believed to have given rise to others by functional diversification. Independent origins are suggested for fungal, plant, and animal *SPMS* genes (Minguet et al. 2008). Fungal *SPMS* genes presumably originated from an ancestor common with fungal *SPDS* (Minguet et al. 2008). With respect to plant *SPMS* genes, it has been proposed that they may have been derived from a plant *SPDS* by more recent gene duplication and function diversification (Minguet et al. 2008; Pegg and Michael 2010; Rodríguez-Kessler et al. 2010). Animal *SPMS*s, on the other hand, are believed to have a more ancient ancestor than plant or fungal genes from which animal *SPDS*s also originated (Minguet et al. 2008; Pegg and Michael 2010). Since the presence of *tSPMS* is only reported in prokaryotes and some plants, it can be argued that the acquisition of *tSPMS* in plants may have occurred via horizontal gene transfer from Archaea or bacteria. More details on various arguments about the evolution of *APT*s can be found in Minguet et al. (2008); Pegg and Michael (2010); and Rodríguez-Kessler et al. (2010).

Mutants of aminopropyltransferases in microbes and animals

In order to investigate physiological and developmental functions of APTs, mutations of corresponding genes provide a time-tested approach. Mutants for all major APT genes have been selected, induced or recognized in all groups of organisms, including humans.

A full spectrum of *APT* mutants of *E. coli* and several other bacteria has been analyzed for their biochemical and growth-related phenotypes (Cohen 1998; Wortham et al. 2007). As an example, *SPDS* mutant strains of *E. coli* (*speE*) show major reductions in Spd and Spm contents and

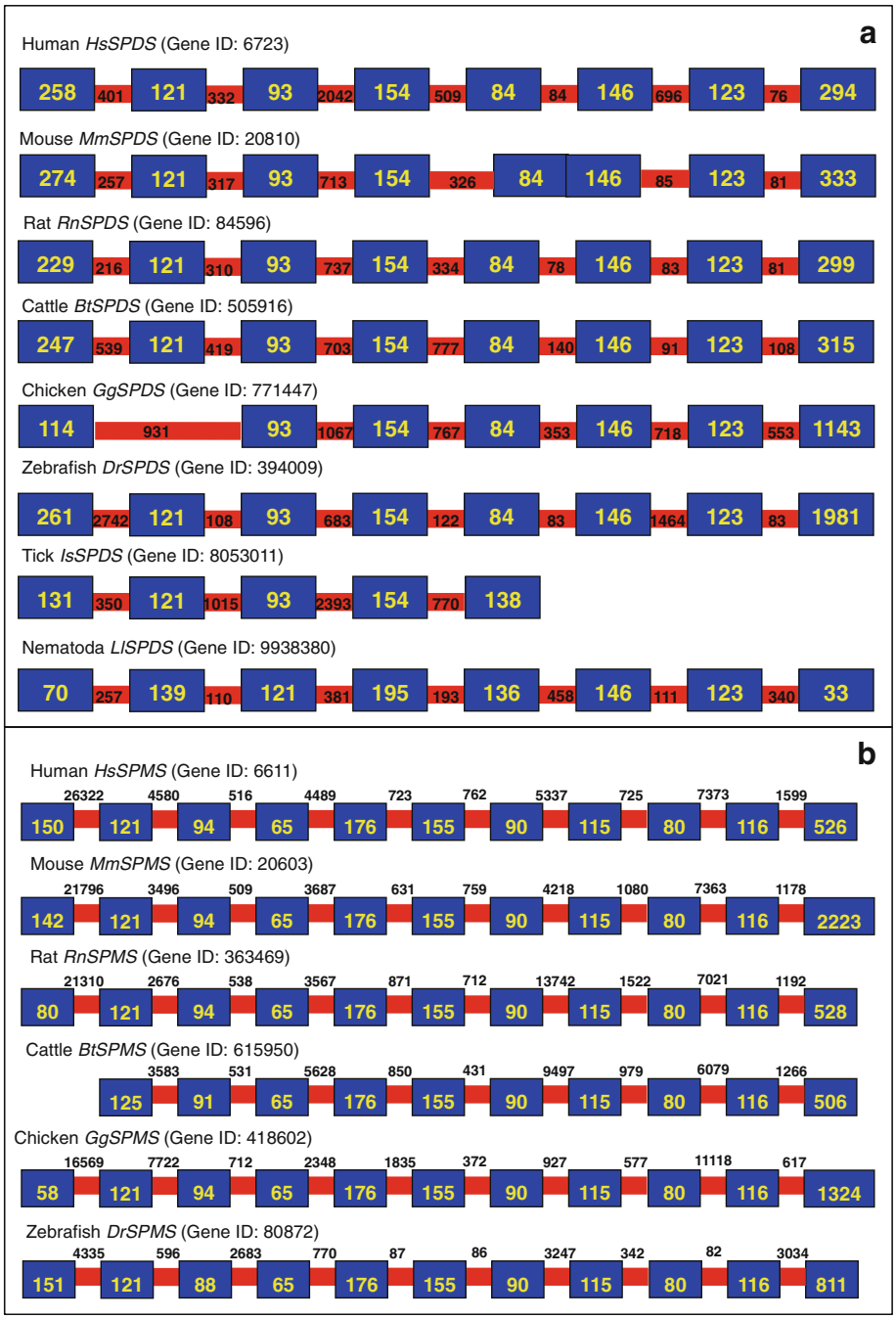

Fig. 4 Exon-intron organization of animal *SPDS* (**a**) and *SPMS* (**b**) genes. *Blue boxes* indicate exons and *red lines* indicate introns. The fifth exon of *MmSPDS* is the fusion of exon 5 and 6 from other animal *SPDSs*. *GgSPDS* lacks exon 2 of other animal *SPDSs*. Several exons are likely to be missing for *IsSPDS* because of incomplete sequence

increased accumulation of Put; this is often accompanied by poor growth (but not lethality) as compared with WT strains. The ability of accumulating exogenously applied PAs decreased (sixfold with Put and 30-fold with Cad) in one of the mutants in comparison with the WT; growth retardation was improved by exogenous addition of PAs, e.g., Spd, Spm, homo-Spd, Cad, and Put. Transformation of the *speE* mutant with *Rhodopseudomonas* homo-*SPDS* showed functional complementation. Chattopadhyay et al. (2009b) have recently constructed a mostly PA-free strain of *E. coli* by deletion of all genes involved in PA biosynthesis (Δ*speA*, Δ*speB*, Δ*speC*, Δ*speD*, Δ*speE*, Δ*speF*, Δ*cadA* and Δ*ldc*), and still found it to be viable under normal growth conditions; however, at high O_2 (95%) or under anaerobic conditions, this strain did not grow well.

A *speE* mutation in *Streptococcus pneumoniae* showed reduced Spd content and lesser colonizing efficiency (i.e. virulence) in mouse either alone or in competition with the WT strain (Shah et al. 2011). Mice infected with the mutant strain lived longer than those infected with the WT strain while no significant difference in growth kinetics was

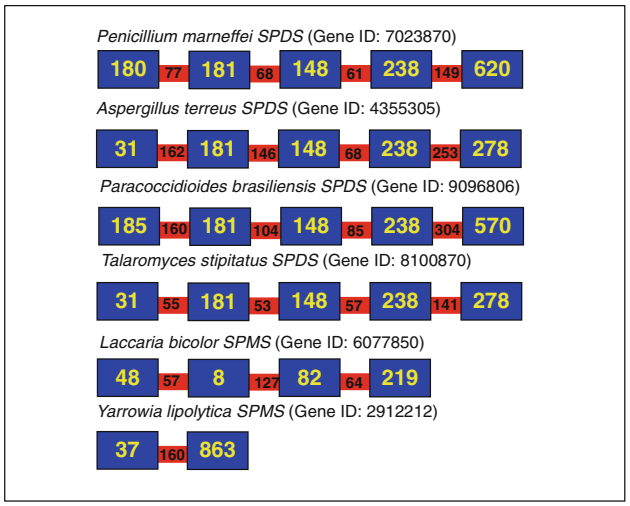

Fig. 5 Exon-intron organization of fungal *APT* genes. Fungal (*Laccaria bicolor* and *Yarrowia lipolytica*) *SPMSs* do not have conserved exon–intron pattern. Other fungal *SPMS* sequences available in NCBI do not contain introns

observed in the mutant versus the WT. Proteome analysis of the mutant strain showed reduction in expression of several key virulence factors (e.g. capsular polysaccharide, zinc metalloprotease, amino acid transporters) of this pathogen.

A yeast *SPDS* mutant (*spe3*Δ), which lacked Spd and Spm when grown in PA-deficient medium (Hamasaki-Katagiri et al. 1997), had its growth restored by addition of Spd or by transformation with a plasmid-containing *SPE3* gene. Although exogenous Spd had no effect on its Spm content, growth was fully restored, indicating that Spm may not be essential for growth. On the other hand, addition of Spm did restore 50% of the growth of the mutant indicating back-conversion of Spm to Spd in this organism. The dispensability of Spm in yeast (as in some plants) is further supported by the observation that a *SPMS* mutant of yeast (*spe4*Δ) showed the absence of Spm without adverse effects on growth (Hamasaki-Katagiri et al. 1998). In line with conclusions from bacteria and yeast, inhibition of SPDS activity negatively impacted growth and virulence in the parasitic protozoan *Trypanosoma brucei*, which causes African sleeping sickness. RNAi-mediated knockdown of *SPDS* resulted in cell growth arrest apparently due to decreased intracellular Spd, which was recovered by exogenous supply of Spd, but not Put (Xiao et al. 2009). In another parasitic protozoan *Leishmania donovani*, *SPDS*-null mutant (Δ*spdsyn*) showed arrested growth after few divisions and eventual lethality (Roberts et al. 2001); exogenous supply of Spd reversed the lethality in the mutant.

Spermidine synthase also plays a vital role in controlling virulence mechanism of *Ustilago maydis*, the causal agent of common smut in maize (Valdés-Santiago et al. 2009). In this fungus, a chimeric bifunctional gene *SPE-SDH* encodes SPDS and saccharopine dehydrogenase; the latter is required for Lys biosynthesis. The Δ*spe-sdh* mutant showed attenuated virulence in maize accompanied by inhibition of transition from yeast-stage to mycelium-stage in its life cycle. Complementation of the mutant by WT gene reversed virulence of the fungus. A similar *SPDS-SDH* gene (*SPE3-LYS9*) has been reported from the human pathogen *Cryptococcus neoformans* (Kingsbury et al. 2004).

Microarray analysis of *SPDS* and *Spm oxidase* double mutant (Δ*spe3*.Δ*fms1*) in yeast showed differential responses to exogenously applied Spd or Spm vs. the WT (Chattopadhyay et al. 2009a); Spd affected a higher number of genes versus Spm. A twofold up-regulation of 247 genes and tenfold up-regulation of 11 genes was observed in response to exogenous Spd. Genes involved in sulfur metabolism, from sulfate uptake to homocysteine and Met syntheses were highly up-regulated; several others that encode for transporters of Met, methyl-Met, and SAM were also induced. While genes associated with Arg, Orn and citrulline metabolism, and for biotin synthesis showed induction, those belonging to categories that are involved in nucleic acid metabolism and stress responses were down-regulated. Only a few genes (about 18) showed significant induction in response to exogenous Spm and belonged to the same group of genes that were induced by Spd.

The importance of SPMS in normal growth and development in mammals is well known. A mutation of human *SPMS* gene caused X-linked recessive condition called Snyder-Robinson Syndrome (SRS), which is associated with mental retardation and defective skeletal development (Ikeguchi et al. 2006; Pegg and Michael 2010 and references therein). A male mouse strain (called Gy) with X-chromosomal deletion (that is missing the *SPMS* gene) showed a lack of SPMS activity as well as cellular Spm and exhibited a wide range of abnormalities including deafness, size reduction, sterility, and shorter life span (Ikeguchi et al. 2006; Pegg and Michael 2010 and references therein).

Mutants of aminopropyltransferase genes in plants

In addition to randomly observed/selected mutants in many plant species, site-directed or experimentally induced mutations have been extremely useful in plants like *Arabidopsis thaliana*, whose genome has been fully sequenced and well characterized. In this species, genome-wide mutagenesis has been created using T-DNA and *Ds* transposon insertions, thus allowing identification of mutants for all *APT* genes (Table 1). As mentioned earlier, Arabidopsis has two *SPDS* genes, one *SPMS* gene, and one *tSPMS* called *ACL5*.

Table 1 Characterization of Arabidopsis mutants of aminopropyltransferase genes

Mutant	Mutagen	Mutation loci/type	Phenotype/biochemical alteration	References
spds1-1	T-DNA insertion	Insertion in exon 7	No phenotypic change observed, no alteration in PAs	Imai et al. (2004b)
spds2-1	T-DNA insertion	Insertion in intron 3	Same as *spds1-1*	Imai et al. (2004b)
spds2-2	T-DNA insertion	Insertion in exon 7	Same as *spds1-1*	Imai et al. (2004b)
spds1-1/ spds2-1	T-DNA insertion	See *spds1-1* and *spds2-1*	Embryo lethal; largely increased Put, decreased Spd and Spm	Imai et al. (2004b)
spms-1	T-DNA insertion	Insertion in the intron 1	No phenotypic change; increased Spd, decreased Spm	Imai et al. (2004a)
acl5-1	Methane-sulfonate	1 bp substitution in exon 4	Dwarf inflorescence stems, incomplete xylem development; lack of tSpm, increased Put, Spd and Spm	Hanzawa et al. (2000); Imai et al. (2004a); Kakehi et al. (2008); Rambla et al. (2010)
acl5-3	Ds insertion	Insertion in intron 6	Dwarfism phenotype same as *acl5-1*	Hanzawa et al. (2000)
acl5-4	Ds insertion	Large deletion from the excision of *acl5-3* Ds transposon	Dwarfism phenotype same as *acl5-1*	Hanzawa et al. (2000); Muñiz et al. (2008)
spms-1/ acl5-1	T-DNA insertion and Methane-sulfonate	See *spms-1* and *acl5-1*	Dwarfism phenotype same as *acl5-1*, hypersensitive to salinity and drought; lack of tSpm, decreased Spm, increased Spd	Imai et al. (2004a); Yamaguchi et al. (2006, 2007); Rambla et al. (2010)
Thickvein	Diepoxybutane	1 bp deletion in exon 7 of *ACL5*	Dwarfism, thicker veins, disruption of auxin polar transport	Clay and Nelson (2005)

Single-gene T-DNA insertion mutants of either *SPDS1* or *SPDS2* in Arabidopsis exhibited no phenotypic abnormality under normal growth conditions and no significant alteration in cellular PA contents, suggesting functional complementation of the two homologs (Imai et al. 2004b). Seeds of *spds1/spds2* double mutant were not viable with embryos arrested at the heart-torpedo transition stage. A considerable reduction in Spd concomitant with a small decrease in Spm was seen in the double-mutant seeds. On the other hand, Put content was elevated remarkably, presumably the result of blockage in its conversion to Spd. The embryo-lethality in the double mutant was reversible by transgenic expression of At*SPDS1* under a constitutive promoter, suggesting that SPDS is indispensable during embryo development. Due to the seed/embryo lethality, fully grown double mutants are not available; thus, phenotypes in other stages of development are not known. Development of conditional mutations in one or the other gene should help resolve this situation.

An Arabidopsis mutant with T-DNA insertion in the *SPMS* gene showed lower Spm and higher Spd but no notable phenotype under normal growth conditions, leading to the suggestion that Spm may not be required for growth of this plant (Imai et al. 2004a; Rambla et al. 2010). On the other hand, an *acl5* mutant contained no detectable tSpm but accumulated Spd and Spm (Kakehi et al. 2008; Rambla et al. 2010). This mutant was earlier reported to have a severe defect in internode elongation, smaller rosette leaves, reduced number of flowers, and abnormal surface of the siliques (Hanzawa et al. 2000; Imai et al. 2004a). Antisense expression of *ACL5* in Arabidopsis also resulted in a similar dwarf phenotype (Hanzawa et al. 2000), and heat shock-inducible transgenic expression of *ACL5* cDNA in the *acl5* mutant produced detectable endogenous tSpm and restored the internode dwarfism (Hanzawa et al. 2000; Kakehi et al. 2008). Interestingly, an increase in defective transcripts of *acl5* in the mutant, combined with its reduction after exogenous tSpm treatment, suggests a negative feedback regulation of *ACL5* gene expression (Hanzawa et al. 2000; Kakehi et al. 2008). The expression of *SAMDC4* was also up regulated in the *acl5* mutant, which was reversed by tSpm. This indicates a possible metabolic co-regulation of *ACL5* and *SAMDC4*; the latter would supply dcSAM required for the production of tSpm (Kakehi et al. 2010). Similar co-ordination in the expression patterns of several PA biosynthetic genes and also those involved in the biosynthesis of their substrates (Orn and Arg from Glu) has been reported by Page et al. (2007, 2010) in control as well as a high Put- producing transgenic cells of poplar (*Populus nigra x maximowiczii*).

Another Arabidopsis mutant called '*thickvein* (*tkv*)', whose phenotype resembled the *acl5* mutant, was identified to have a single base deletion in exon 7 of *ACL5* gene, which resulted in a frame shift in the ORF. Anatomical study of the mutant revealed increased number of vascular cells, which resulted in thicker veins in leaves and inflorescence of the mutant plants. Furthermore, abnormality of vein structure was accompanied by disruption of polar

auxin transport in the inflorescence stalk, which implicates an interaction of PAs with plant growth hormones for the observed phenotype, and shows that tSpm may be involved in this interaction (Clay and Nelson 2005). However, in a more recent study by Vera-Sirera et al. (2010), an increase in cellular contents of the natural auxin IAA and up-regulation of IAA marker *DR5::GUS* expression were seen in *acl5* hypocotyls, which showed that IAA transport from apical meristem in the mutant seedlings may be adequate. Therefore, the authors provided an alternative explanation that the defect in xylem and the retarded growth in the mutant may actually be responsible for defective auxin transport in the inflorescence stalk. To complicate the matters further, Muñiz et al. (2008) had attributed incompletely developed xylem of *acl5* to premature cell death, therefore, suggesting that xylem specification may be determined by the lifetime of xylem elements, which is under the control of *ACL5*.

Rambla et al. (2010) recently reported that a double mutant (*spms/acl5*) in Arabidopsis that almost completely lacked tSpm, and had significantly lower Spm but elevated Spd, was phenotypically identical to the *acl5* mutant of Imai et al. (2004a). This again indicates that Spm probably does not play a major role in the development of Arabidopsis. However, Yamaguchi et al. (2006) had earlier shown that the double mutant was hypersensitive to NaCl and KCl (but not to $MgCl_2$ or mannitol) as compared with the WT plants and exogenous application of Spm reversed the NaCl hypersensitivity. Furthermore, the salt hypersensitivity was alleviated by a Ca^{2+}-channel inhibitor, which along with growth arrest of the mutant on Ca^{2+}-depleted medium indicated a Ca^{2+} deficiency in the *spms/acl5* double mutant. Later it was shown by Yamaguchi et al. (2007) that in addition to salinity, *spms/acl5* double mutant displayed hypersensitivity to drought as well, which was also reversed by exogenous Spm. Greater water loss in the mutant was attributed to defective stomata closure which might also involve regulation of Spm-modulated Ca^{2+} channel and K^+ flux. Thus, it can be argued that Spm is perhaps essential or at least plays a role in stress responses, even though its role in development may be doubtful. Furthermore, this role may be mediated by regulating Ca^{2+}-permeable channel, which controls cellular Ca^{2+}homeostasis (Yamaguchi et al. 2006).

Expression patterns of aminopropyltransferase genes in plants

Organ- and tissue-specific expression

As described earlier, most plants contain two genes for *SPDS* and one or two genes for *SPMS* and/or *tSPMS*. While their coding sequences show a high degree of homology within and between the gene families, little is known about the homology between their promoter sequences or about the regulation of their expression in various tissues and cells in a given plant. Five different approaches, namely northern hybridization, RT-PCR, QRT-PCR, in situ *hybridization*, and promoter::reporter fusion have been employed to study organ- tissue-specific gene expression of the four APT genes, each showing different levels of specificity for expression at tissue, organ, and cell levels. In addition, microarray data on the expression of some of these genes in Arabidopsis are available. Still, the information is quite sporadic and inconsistent, thus leading to inconclusive assessment of their role during development or in response to stress in plants.

Semi-quantitative northern blot analysis was used by Hanzawa et al. (2002) to study the presence of *AtSPDS1* and *AtSPDS2* mRNAs in 7-day-old seedlings, mature leaves, stem internodes, inflorescences, and siliques of *A. thaliana*. It was observed that mRNAs of both genes were present in all organs, with higher levels being detected in the seedling roots. Transcripts of *AtSPMS* and *AtACL5* were also present ubiquitously, but more in the stem internodes, flower buds, and roots (Hanzawa et al. 2000, 2002). However, the probes used in this study were *AtSPDS1* and *AtSPDS2* 3′-UTRs (with >51% identity between them), and *AtSPMS* and *AtACL5* cDNAs (also with ~51% identity between them), which might not have been able to distinguish those homologues completely.

Later, Urano et al. (2003) using semi-quantitative RT-PCR reported constitutive presence of *AtSPDS1* transcripts in all organs (flowers, buds, immature and mature siliques, upper and lower stems, and cauline and rosette leaves); *AtSPDS2* mRNA was also found in all organs but at relatively lower levels in the mature siliques and upper stems. In the same study, it was seen that while *AtSPMS* mRNA was present in all organs examined, transcripts of *AtACL5* were more abundant in immature siliques, cauline leaves, and roots than other organs. Subtle discrepancies in results of the two studies might simply be due to the techniques used. Detailed cell- and tissue-level expression analysis of *AtACL5* using a technique with higher specificity (promoter::reporter fusion) by Clay and Nelson (2005) revealed its procambium-confined expression in bent cotyledon embryos, primary roots, young leaves as well as during inflorescence development.

Charles Rice (unpublished) in our lab studied the expression profile of *AtSPMS* and *AtACL5* using the promoter::*GUS* fusion approach in all tissues and organs of Arabidopsis during its entire life. Overall, the expression of *AtSPMS* was found to be high in young developing tissues with continued but weaker expression in the vascular tissue of mature plants. A similar expression profile was observed

for *AtACL5*; however, more expression was observed in the meristematic and elongating regions of young organs. More recently, detailed studies of *AtSPDS1* and *AtSPDS2* genes in our lab using the same technique (Lin Shao unpublished) have revealed more subtle differences of expression patterns of these genes in different cell/tissue types as well as in response to various forms of abiotic stress.

Microarray data (Genevestigator—www.genevestigator.com) also reveal constitutive presence of *AtSPDS1* and *AtSPDS2* transcripts in all organs and tissues in Arabidopsis. Between the two homologs, *AtSPDS1* shows relatively higher expression especially in flowers and siliques; at the same time, transcripts of *AtSPMS* are also ubiquitous in these organs. On the other hand, *AtACL5* expression is much less in most organs than the other *APT*s except in hypocotyls and stems.

In addition to Arabidopsis, *APT* genes have been identified in several other plants and their organ- tissue-specific expression patterns have been characterized in some. In 1999, Alabadi and Carbonell isolated two *SPDS* genes from pea (*Pisum sativum* L. cv. Alaska), which were expressed differentially in different tissues. Northern blots showed a ubiquitous presence of *PsSPDS1* and *PsSPDS2* transcripts; however, the former was more abundant in actively growing tissues (shoot tips and young leaves) whereas the latter was predominant in the stem. The expression of *PsSPDS1* was up-regulated in ovaries in the very early stages of fruit development, and *PsSPDS2* displayed high expression in later stages. Having 85.7% sequence identity in the coding region, cDNAs of the two homologs, which were used as probes, again might raise the question of probe specificity. Enzyme activity of SPDS during fruit development paralleled the expression of *PsSPDS1*.

Two *SPDS* genes have been reported in apple (*Malus sylvestris* var. *domestica*); one of them, i.e. *MdSPDS2*, apparently generates two mRNA variants (*MdSPDS2a* and *MdSPDS2b*) by alternative splicing at the first exon (Zhang et al. 2003). Due to the low specificity of northern blot probes to distinguish between *MdSPDS1* and *MdSPDS2a*, transcripts of one or both were detected in all tissues with greater abundance in young leaves and fruits (Zhang et al. 2003). On the other hand, *MdSPDS2b* was not detected by northern blot but was shown to be present by RT-PCR in mature leaves and shoots, which suggested its specific role in later stages of vegetative development in contrast to the other two transcripts during early development and reproductive tissue formation (Zhang et al. 2003).

Low-level expression of *MdACL5* was found in young leaves and flower buds but not in mature leaves (again by northern blots), which along with its absence in cell suspensions indicated its expression mostly in highly differentiated cells (Kitashiba et al. 2005). However, the greatest abundance of *MdACL5* transcripts in fruits at 19 days after full bloom (DAF), followed by a gradual decline during the later part of fruit development, suggests a possible role of *MdACL5* in cell division (Kitashiba et al. 2005). He et al. (2008b) later suggested that the mechanism of how *MdACL5* functions in cell division could perhaps be through the facilitation of protein synthesis by ACL5 protein forming a complex with a translation elongation factor eEF-1A based on the protein interaction detected by yeast two-hybrid analysis.

Expression pattern for *MdSPMS* was similar to that of *ACL5* in leaves and flower buds (Kitashiba et al. 2005). In developing fruits, two peaks of *MdSPMS* mRNA accumulation at cell enlargement (61 DAF) and ripening (174 DAF) stages implicated this enzyme in these two processes. In contrast to *MdACL5*, *MdSPMS* was expressed in cell suspensions with a decline from initial stationary phase to the exponential growth phase (0–14 d), and an increase towards second stationary phase (16–20 d of culture). The higher expression of *MdSPMS* in later stages of fruit development and later part of the cell suspension cultures strongly suggests its relevance in either mature cell formation or stationary phase of growth (Kitashiba et al. 2005). It should be pointed out that the probes used to detect apple *SPMS* and *ACL5* also have more than 50% sequence identity.

Olive (*Olea europaea* L.) *SPDS* (*OeSPDS*) expression has been investigated by QRT-PCR and Fluorescence in situ Hybridization (FISH) in flowers and fruits of two cultivars (Arbequina and Picual), which differ in fruit size and shape (Gomez-Jimenez et al. 2010). Only one copy of *SPDS* was identified in this species and its transcript levels were similar in closed and open flowers. In early stages of developing fruits, in comparison with flowers, an increase in expression at 14 days post-anthesis was observed in both cultivars. Equally high expression was detected in leaves but stem had the lowest transcript abundance. Tissue- cell-specific analysis by FISH revealed localization of *OeSPDS* mRNA in the ovules of young flowers but with different patterns in the two cultivars. The expression was confined to integument cell layers and inner epidermis in Arbequina ovules, whereas, in Picual ovules, widespread expression was seen. In developing fruits of both, *OeSPDS* transcripts were localized in the fruit mesocarp and exocarp where cells undergo active division. While Spd level did not correlate with *SPDS* transcripts, a correlation with *SAMDC* expression and enzyme activity was observed. Thus, it was suggested that Spd synthesis was regulated mainly by SAMDC which influences fruit size by regulating cell division (Gomez-Jimenez et al. 2010).

Ubiquitous expression of *Citrus clementina SPDS* (*CcSPDS*) and *SPMS* (*CcSPMS*) detected by northern blots

using full-length cDNA probes was seen in internodes, shoot apices, flowers, ovaries, and developing fruits (Trénor et al. 2010); however, *CcACL5* transcripts were confined to shoot apices, flowers, and ovaries. Furthermore, by using QRT-PCR, a decline in *CcSPDS* transcripts and an increase of *CcSPMS* transcripts were observed in gibberellic acid (GA)-induced developing fruits; expression of *CcACL5* remained unchanged. Notably, this study showed a positive correlation between changes in PA titers and *APT* expression during early fruit development. On the other hand, *CcSAMDC* transcripts did not exhibit a strong correlation with PA contents, showing that *SAMDC* expression might not be important in regulating PA titers during early fruit development.

In *Lotus japonicus,* a legume, *LjSPDS* and *LjSPMS* transcripts (QRT-PCR) were higher in developing nodules than the roots, with the highest level being at early nodulation stages (10 d post-inoculation—DPI) with *Rhizobium*, followed by a decline during the later stages (14, 21 and 28 DPI—Efrose et al. 2008). Spatial expression analysis by in situ hybridization showed presence of both transcripts in inner cortical cells, vascular bundles, and central core of nodules. A discordance of Spd and Spm levels with transcripts of their biosynthetic enzymes was suggested to be due to post-translational regulation, changes in catabolism, and alternative PA source from bacteria. There also was an up-regulation of *LjSPDS* and *LjSPMS* expression in non-symbiotic tissues (stems) but down-regulation in leaves; again without a positive correlation with the PA titers (Efrose et al. 2008).

In the male gametophyte of fern *Marsilea vestita*, transcripts of *MvSPDS* were first seen only in the jacket cells at up to 4 h of development, then in the spermatogenous cells at 6 h, eventually becoming more abundant in the spermatids at later stages of development (8 h). The temporal and spatial variation of Spd during gametophyte development coincided with transcript levels (Deeb et al. 2010). The authors pointed out that the dramatic increase in *SPDS* transcripts in spermatids at later developmental stages was due to unmasking of stored mRNA by newly produced Spd, leading to further rise in Spd within a short time.

In poplar cell suspension cultures, *pSPDS1* transcripts were more abundant than *pSPDS2*, as seen by QRT-PCR; both transcripts increased substantially upon sub-culture into the fresh medium (Page et al. 2007, 2010). Expression of *pSPDS1* continuously rose until day 3 and then decreased by day 7, whereas *pSPDS2* gradually declined after the first day of transfer. In a high Put producing transgenic cell line over-expressing a mouse *ODC*, transcripts of both genes displayed similar expression pattern except that the up-regulation of *pSPDS2* was also seen initially (up to day 3). This was in accordance with an increase in Spd in the cells.

Expression of aminopropyltransferase genes in response to abiotic stresses and phytohormones

One of the most discussed topics about the physiological roles of PAs in plants is their potential involvement in abiotic stress responses, many of which also involve other phytohormones, particularly abscisic acid, i.e., ABA (Alcázar et al. 2006). The arguments in favor of a positive role of PAs in increasing or imparting stress tolerance are based upon three types of studies: (i) increases in cellular PAs in response to applied stress, (ii) protection from abiotic stresses by exogenous application of PAs, and (iii) enhanced stress tolerance of transgenic plants producing/accumulating higher amounts of cellular PAs. In this regard, foliar PAs have also been proposed as reliable indicators of abiotic stress in forest trees in which the symptoms of stress and its impact on tree growth and forest productivity may not have yet appeared (Minocha et al. 2000). On the other hand, suggestions have also been made that increased metabolism of PAs may actually cause damage to plants/plant cells, perhaps through increased production of H_2O_2 from their catabolism (Mohapatra et al. 2009). While in most cases, it is Put which is strongly and positively correlated with stress tolerance, in a few cases at least, Spd and/or Spm also increase. Likewise, their increased production through genetic engineering has also been shown to impart stress tolerance (discussed below). A few studies have directly analyzed the expression of *APT* genes in plants in response to a variety of abiotic stress treatments; results of representative studies are discussed here.

Increasing salt concentration was reported to delay Arabidopsis seed germination (Bagni et al. 2006). In response to long-term salt treatment (21, 31 and 38 days) of mature plants, *AtSPDS1* mRNA levels increased slightly (as seen by RT-PCR) while *AtSPMS* and *AtACL5* increased to a greater extent, concomitant with an increase in Spm (Bagni et al. 2006; Tassoni et al. 2008). This is consistent with the results of Urano et al. (2003) based on northern blots, except that in their study *AtACL5* transcripts decreased after short-term NaCl treatment (24 h). A recent study using QRT-PCR (Naka et al. 2010) confirmed the results of Urano et al. (2003) about the decrease in *AtACL5* transcripts using seedlings subjected to short-term salt treatment (1–3 days). This discrepancy shows that *AtACL5* responds differently to the concentration of salt and/or to the duration of treatment. In terms of PA contents, Put increased initially (at 1 day) and then declined at 2–3 days. On the other hand, Spd and tSpm decreased while Spm increased; thus, changes of PAs paralleled the expression of *APT* genes.

In maize (*Zea mays*), both *ZmSPDS1* and *ZmSPMS1* (initially designated as *ZmSPDS2*) were responsive to

NaCl; whereas *ZmSPDS1* was up-regulated by increasing NaCl concentration in long-term treatment of 7 days, *ZmSPMS1* was induced only by short-term treatment of 1 day (Rodríguez-Kessler et al. 2006; Jiménez-Bremont et al. 2007). An increase in Put, Spd, and Spm upon long-term treatment with 25 mM NaCl was reported. Proline and total chlorophyll content were also affected by salt treatment. Proline accumulated 10 days following treatment with higher salt concentration (150 and 400 mM NaCl), whereas chlorophyll content rose after short-term treatment and went down notably after 14 days. Up-regulation of *Panax ginseng PgSPDS* in roots was seen in response to salinity accompanied by increased Put and Spd contents (Parvin et al. 2010).

When 3- to 4-week-old Arabidopsis plants were removed from soil and subjected to dehydration (Urano et al. 2003; Alcázar et al. 2006), the expression of *AtSPDS1* in detached leaves and *AtSPMS* in both 4-week-old plants and detached leaves increased dramatically; this was not accompanied by parallel changes in Spd and Spm contents. On the other hand, Put was elevated in both studies concomitant with increased *AtADC2* expression, indicating a tighter regulation of cellular Spd and Spm homeostasis than Put as suggested earlier by Bhatnagar et al. (2001) for poplar cells. A recent report (Alcázar et al. 2011) suggests that back-conversion of Spm to Put contributes to this tight homeostatic regulation of Spd.

Cold treatment (4°C) for 2 to 5 h caused a decrease in Spm in 4-week-old Arabidopsis plants but *AtACL5* transcripts increased (Urano et al. 2003). In *P. ginseng* roots, *PgSPDS* expression was induced gradually by chilling until 8 h post-treatment and showed a decline from 12 to 24 h; then maximum accumulation was observed at 48 h. This was in parallel to changes in Spd content. Parvin et al. (2010) found that jasmonate, mannitol, and $CuSO_4$ treatments also induced *PgSPDS* expression in roots; changes in PA contents in this study were not reported.

In studies from our lab (Rice, unpublished), both *AtSPMS* and *AtACL5* were induced in response to drought and (100 mM) salt stress in Arabidopsis plants 20 days or older; younger plants could not be used because of extremely high GUS activity (dense blue color) in the untreated (control) promoter::*GUS* fusion transformants. No change in expression was observed in any other organ aside from rosette and cauline leaves in 35 day old plants. Changes in GUS expression in roots were also not discernible due to the high level of GUS activity in the control (untreated) plants. There was a slight increase in expression in response to chilling stress, but expression soon decreased over a 24-h period. *AtSPMS* was also induced in response to wounding.

A substantial increase in Arabidopsis *AtSPDS2* expression (detected by promoter::*GUS* fusion) at nematode-feeding site on roots coincided with increased Spd level suggesting its possible role in nematode parasitism (Hewezi et al. 2010).

The *APT* genes also respond positively to various phytohormones. For example, treatment with ABA, a hormone most closely associated with abiotic stress, increased Put level by almost threefold in Arabidopsis seedlings within hours; this was accompanied by a dramatic (~sevenfold) increase in *AtADC2* transcripts (Rambla et al. 2010). ABA treatment induced *AtSPMS* expression to the same extent (i.e. ~sevenfold); however, except Put, the other PAs remained unaltered. Earlier studies of Hanzawa et al. (2002) and Urano et al. (2003) had shown that *AtSPMS* expression in the seedlings as well as 4-week-old plants increased rapidly (2 h) after treatment with 100 μM ABA. The discordance between variation of *AtSPMS* transcripts and Spm titer suggested post-transcriptional and/or post-translational regulation of this enzyme. In addition to Arabidopsis, pronounced stimulations of maize *ZmSPDS1* and *ZmSPMS1* transcripts were detected in detached leaves in response to ABA within 6 h (Jiménez-Bremont et al. 2007) as was the case with accumulation of *PgSPDS* mRNA in *P. ginseng* roots at 8–12 h after treatment with ABA (Parvin et al. 2010).

Rambla et al. (2010) found that in response to auxin (50 μM IAA), both Put and tSpm contents increased by 1.5 and 2.5 fold, respectively, in Arabidopsis seedlings at 8 h post-treatment, which paralleled an up-regulation of *AtADC2* and *AtACL5* transcripts. Similar increases in *AtACL5* and *AtSPDS2* expression were observed in earlier studies by Hanzawa et al. (2000, 2002) in response to IAA and kinetin, respectively; no major phenotypic change was reported in either case.

Microarray data on expression of *APT*s in response to abiotic stresses and hormones in Arabidopsis seem to be consistent with studies discussed above, e.g., up-regulation of *AtSPMS* by salinity, drought, osmotic stresses and ABA treatment, and *AtACL5* up-regulation in response to drought, cold, and auxin treatment (www.genevestigator.com).

Transgenic manipulation of polyamines via aminopropyltransferases

While inhibitors of biosynthetic and catabolic enzymes provide an effective means of regulating a biochemical step, this approach has severe limitations, e.g., problems associated with uptake, transport, and metabolism of the inhibitor, as well as the lack of specificity of the inhibitor in most cases. Genetic manipulation through transgene expression (*a.k.a.* genetic engineering) enables us to overcome some of these problems; of course, it introduces some other equally important issues. Nevertheless, this

approach has been used extensively for experimentally altering PA metabolism in plants, more so than in animals. Genetic manipulation is suitable for both up- and down-regulation of a metabolic step. Almost every gene in the core PA biosynthetic pathway has been the target of overexpression and/or inhibition through antisense or a similar approach. Plants with up-regulated expression of *SPDS* have been produced to study the effects of increased Spd production during development and to test for stress responses (Table 2), whereas relatively few studies on transgenic manipulation of plant *SPMS* or t*SPMS* have been reported.

Franceschetti et al. (2004) were the first to report transgenic constitutive expression of a *Datura stramonium SPDS* cDNA in tobacco; transgenic plants displayed increased SPDS as well as SAMDC enzyme activities. However, increases in enzyme activities were not proportionate to the increases in transcripts, indicating post-transcriptional and/or post-translational regulation. The Spd to Put ratio increased in transgenic plants either due to increase in Spd and/or decrease in Put, but Spm level was not affected. Interestingly, almost no alteration in the total PA content was seen, suggesting a tight regulation of the total cellular PA levels in transgenic plants. Morphologically, the transgenic plants were shorter and had fewer internodes; flowering was also delayed.

Over-expression of a *Cucurbita ficifolia CfSPDS* cDNA under a CaMV 35S promoter in Arabidopsis resulted in five- to sixfold increase in SPDS enzyme activity (Kasukabe et al. 2004), with an accompanying increase of up to 2 and 1.8 fold in Spd and Spm, respectively. No morphological change was observed in the transgenic plants. Tolerance of these plants to various stresses including low temperature, salinity, hyper-osmosis, drought, and oxidative stress was

Table 2 Genetic manipulations of APT aminopropyltransferase genes in plants

Plant	Transgene	Approach	Phenotype/biochemical alteration	Reference
Tobacco	*Datura stramonium SPDS*	Over-expression under *CaMV* 35S promoter	Put/Spd increased, Spm and total PA content unaltered in leaf tissues. SPDS and SAMDC activity increased in leaves	Franceschetti et al. (2004)
Arabidopsis thaliana	*Cucurbita ficifolia SPDS*	Over-expression under *CaMV* 35S promoter	Increased SPDS activity, Spd and Spm in leaves; enhanced tolerance to low temperature, salinity, hyper-osmosis, drought and oxidative stresses	Kasukabe et al. (2004)
Sweet potato (*Ipomoea batatas)*	*Cucurbita ficifolia SPDS*	Over-expression under *CaMV* 35S promoter	Increased Spd content in leaves and storage roots; less effect of salinity, drought and week light on storage root growth, less damage on photosynthesis by chilling and heat stresses	Kasukabe et al. (2006)
Pear (*Pyrus communis* L. 'Ballad')	Apple (*Malus sylvestris* var. *domestica*) *SPDS*	Over-expression under *CaMV* 35S promoter	Reduced shoot heights of seedlings, elevated Put, Spd and Spm; enhanced tolerance to salt, osmosis and heavy metal stresses along with increased antioxidant activity	He et al. (2008a); Wen et al. (2008, 2009, 2010)
Arabidopsis thaliana acl5 mutant	*Arabidopsis thaliana ACL5*	Expression under a heat shock-inducible promoter	Restore the dwarfism phenotype of *acl5* mutant, produced detectable tSpm absent in mutant	Hanzawa et al. (2000); Kakehi et al. (2008)
Arabidopsis thaliana	*Arabidopsis thaliana SPDS2*	Over-expression under *CaMV* 35S promoter	Increased susceptibility to cyst nematode *H. schachtii* infection	Hewezi et al. (2010)
Tomato (*Solanum lycopersicum*)	Apple (*Malus sylvestris* var. *domestica*) *SPDS*	Over-expression under *CaMV* 35S promoter	Put, Spd and Spm all increased in fruits; higher carotenoid contents accumulated especially lycopene	Neily et al. (2010)
Tomato (*Solanum lycopersicum*)	Yeast *SPDS*	Over-expression under *CaMV* 35S promoter	Increased Spd, decreased Put and Spm in leaves and developing fruits; fruits showed increased lycopene content, delayed ripening, longer shelf life and reduced shriveling	Nambeesan et al. (2010)
Tomato (*Solanum lycopersicum*)	Yeast *SPDS*	Over-expression under a fruit-ripening specific promoter E8	Increased Spd, Spm in developing fruits; fruits showed increased lycopene content, delayed ripening, longer shelf life and reduced shriveling	Nambeesan et al. (2010)
Fern (*Marsilea vestita*)	Fern (*Marsilea vestita*) *SPDS*	RNAi by double stranded RNA in male gametophyte	Reduction of Spd, development arrest at cell division stage, defects in microtubules, base bodies and spermatid chromatin condensation	Deeb et al. (2010)
Arabidopsis thaliana	Antisense *Arabidopsis thaliana SPDS1*	Antisense *AtSPDS1* under *CaMV* 35S promoter	No PA or phenotypic change in WT background. Resembled *spds1-1/spds2-1* double mutant phenotype (seen in Table 2) when expressed in *spds2* mutant	Imai et al. (2004b)

enhanced. Data from cDNA microarray of transgenic plants revealed up-regulation of several stress-responsive transcription factor genes in them under chilling treatment. The authors concluded that increased Spd possibly plays a role in stress-signaling pathways. The same group (Kasukabe et al. 2006) later used the same *CfSPDS* to produce transgenic sweet potato (*Ipomoea batatas*) plants, again using a constitutive promoter. The transgenic plants tolerated salinity and drought stress more than the WT plants in terms of growth and starch content of storage roots. Furthermore, suppression of storage root formation by low light was abated, and damage by chilling and heat stress on photosynthesis was reduced. The authors further inferred that increased tolerance to various environmental stresses might at least partially be ascribed to higher antioxidant activity in these plants.

When apple *MdSPDS1* gene was constitutively expressed in European pear (*Pyrus communis* L.—Wen et al. 2008), contents of all three PAs in transgenic seedlings were elevated; the plants exhibited reduced shoot height and increased tolerance to salt, heavy metal, and osmotic stresses. He et al. (2008a) later showed that in response to NaCl and mannitol treatments, the transgenic plants showed higher antioxidant capacity. Follow-up studies showed that the transgenic seedlings were more tolerant to Al also (Wen et al. 2009). A small increase in Spd ($\sim$1.2 fold) was seen in both WT and transgenic lines upon Al treatment although values of the former were lower than the latter. Interestingly, an increase in Put ($\sim$1.3 fold) was found in transgenic lines while in WT seedlings it declined by $\sim$30%. This was accompanied by higher antioxidant activity, and greater accumulations of Ca and other ions in the transgenic plants, implying that increased Spd improved Al and heavy metal tolerance through affecting oxidative status of the cells and their inorganic ion balance (Wen et al. 2009, 2010). The above results collectively lead to the hypothesis that antioxidant activity and metal chelator properties of Spd may be involved in the enhanced tolerance to heavy metal stresses in transgenic plants. The results in this regard are similar to the report of Mohapatra et al. (2010) on increased accumulation of Ca in a high-Put transgenic cell line of poplar.

Several papers from A.K. Mattoo's group have described changes including delayed ripening, longer shelf life, reduced shriveling, increased lycopene content, and major alterations in the metabolic profile of transgenic tomato fruits transformed with yeast *SAMDC* and *SPDS* genes regulated either constitutively or by a fruit-ripening specific promoter (Nambeesan et al. 2010; Mattoo et al. 2010 and references therein). The extended shelf-life of transgenic fruits was ascribed to delay in post-harvest senescence, perhaps due to the accumulation of Spd. In another study by Neily et al. (2010), all three PAs increased in transgenic tomato fruits expressing *MdSPDS1* under a constitutive promoter, among which Spm showed the least increase. Primary metabolism in transgenic tomato fruits was also altered during ripening, with metabolites such as malate, galactose, Glu, Gln, Phe, and GABA being significantly higher in the transgenic fruits. Furthermore, transgenic fruit had higher lycopene content due to apparent increases in its biosynthetic genes and down-regulation of degradation genes. The results show that increased PA contents have pleiotropic effects on cellular metabolism as well as the transcriptome and the metabolome, a phenomenon also observed in transgenic poplar cells with enhanced Put production (Mohapatra et al. 2009, 2010; Page et al. 2007, 2010).

As described earlier, *SPDS*, but not *SPMS* or *ACL5* is one of the favored targets for transgenic manipulation of PA content in plants. It is noteworthy that overexpression of *SPDS* in different plants increased Spd and/or Spm levels, but only to a small extent (maximum about two-threefold increase) in comparison with similar studies with the manipulation of Put via *ODC* or *ADC*, where >tenfold increases have been reported (Bassie et al. 2000; Bhatnagar et al. 2001). A thorough review of the literature on changes in PA contents of plants in response to genetic manipulation or stress treatments shows overall wider fluctuations in Put than Spd/Spm, leading to the conclusion that Spd/Spm metabolism in plants is more tightly regulated than that of Put (Bhatnagar et al. 2001, 2002). In poplar cells overproducing Put, its catabolism also increased concomitantly (Bhatnagar et al. 2002). No parallel studies on Spd catabolism during its increased biosynthesis are reported. It was also reported by Bhatnagar et al. (2002) that the half-life of Put in poplar cells was about 6–7 h in contrast to that of Spd and Spm, which was estimated to be >35 h.

Another important point to note in most transgenic studies is that the rise in Spd content in transgenic plants is often not proportionate to the increase of SPDS enzyme activity or *SPDS* transcripts, which strongly indicates that SPDS is not the primary rate-limiting enzyme for Spd production. Also, it appears that manipulation of SAMDC is a more efficient way to elevate Spd/Spm than SPDS, which indicates that the substrate dcSAM is limited in cells and acts as a major rate-limiting factor in aminopropyl transfer reactions. Besides, the decline of Put accompanying increase in Spd in some of the studies discussed above implies that limitation of the precursor Put may also restrain the capability to increase Spd/Spm. Accumulation of Spd in some cases where Put was increased (either via *ODC* or *ADC* over-expression), is also supportive of this idea.

In addition to transgenic over-expression, antisense and RNAi approaches have also been used to down-regulate expression of *APT* genes in plants. Antisense expression of

AtSPDS1 in Arabidopsis did not affect the phenotype, as was the case with the *spds1* mutant discussed above (Imai et al. 2004b). Likewise, lines homozygous for antisense *SPDS1* and mutated *spds2* resembled *spds1/spds2* double mutants. Silencing of *MvSPDS* in Marsilea male gametophytes achieved by RNAi arrested their development during cell division and resulted in reduced Spd. It turned out to be that cell division deficiency caused by lower Spd was due to a defect in the formation of basal bodies and microtubules and the failure of chromatin condensation in spermatids (Deeb et al. 2010).

Conclusions and future perspectives

A review of the literature on aminopropyltransferases in plants reveals a few salient points:

1. Plants often have two copies of the common *SPDS* genes, which exhibit redundancy of function, with the exception of a few cases where the two genes are independently regulated.
2. The genomic organization of all *aminopropyltransferase* genes is highly conserved.
3. Direct enzyme activity data as well as biochemical properties of enzymes for all APTs are scant and have not been done with purified enzymes; moreover, differences in the biochemical properties of the two or more gene products are not known in any case.
4. Often there is a poor correlation among transcripts, enzyme activity, and cellular contents of the respective PA. This is true both for the native genes/enzymes as well as for the transgenes. While this discrepancy is often explained in terms of post-transcriptional and post-translational regulation of the enzyme production, the availability of the substrates (PAs and dcSAM) and rates of catabolism of the products, etc., there is rarely direct experimental evidence provided for such hypotheses.
5. Transgenic work mostly involves the use of constitutive promoters, which often lead to homeostatic adjustment of the plants/cells to steady-state levels of PAs and do not permit the study of changes in plant metabolism in response to transient changes in APT activity or PA concentrations, which is most often the case in nature, e.g., in response to short-term stress or during development. Overall, the results from transgenic work confirm a tighter regulation of the cellular contents of Spd and Spm versus that of Put.

With our increasing ability to analyze nucleic acids down to the level of a single cell, the gap between our knowledge about gene transcription and the homeostatic metabolic regulation of the cellular contents of specific metabolites is widening rapidly. Therefore, further biochemical work is warranted to establish direct correlations between mRNA levels and enzymatic activities on the one hand and between biosynthetic enzyme activities and the accumulation of Spd and Spm in plants/cells on the other. Modern techniques of metabolic flux analyses using stable or radioactive isotopes, combined with our ability to experimentally regulate transcription of transgenes in genetically engineered plants/cells using inducible promoters, should provide suitable means of analyzing these correlations, especially in the well-studied model plant species. This information will also provide reliable means of genetic manipulation (via gene transfer as well as mutagenesis) of the cellular contents of Spd and Spm to realistically assess their role in plant development and stress responses, and to design plants with desired phenotypes, including those with enhanced nutritional properties.

Finally, the lethality of knockout mutants of some *APT* and other PA biosynthetic genes hampers our ability to analyze the functions of these genes in different developmental stages and under different growth conditions. Conditional mutations will serve as a more efficient approach to study the loss of function of these important genes. Alternatively, by using inducible and tissue-specific promoters, gene expression can be turned on or off at a specific stage and in specific cell/tissue types, which will reveal unambiguous insights into the functions and regulation of the genes of interest. A clear understanding of the expression and regulation of APTs at the metabolic level will help us in effective use of genetic engineering approaches for the improvement of nutritional value and stress responses of plants.

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