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Short Communication

S-adenosyl-L-methionine Metabolism Plays Important Roles in Suberization of Potato Tuber

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When plants are wounded, they form a protective layer next to the exposed surface to prevent dehydration and potential penetration by the opportunistic pathogens. This physical barrier termed suberin comprises a specific cell wall modification characterized by both a poly (phenolic) domain and a wax-embedded poly (aliphatic) domain (Bernards and Lewis, 1998; Bernards and Razem, 2001; Razem and Bernards, 2003). Most of the studies on the composition and structure of suberin and its associated materials have been made on the natural or wound-induced periderms of the potatoes (Kolattukudy and Agrawal, 1974; Stark and Garbow, 1992), because quick and uniform suberization is possible in response to the wounding. The polyphenolic domain has been likened to the lignin, which is a three-dimensional polymeric network derived from the monolignols (*p*-coumaryl, conniferyl, and sinapyl alcohols) and a significant amount of hydroxycinnamic acids and their derivatives (Bernards and Razem, 2001; Bernards, 2002). Two of the three types of suberin monomers contain the methyl groups; coniferyl alcohol has one methyl group and synapyl alcohol has two. Therefore, it is quite plausible that the transmethylation reaction is essential to the suberization process. Many biochemical reactions in plants involve the transfer of a methyl group from *S*-adenosyl-L-methionine (SAM). The SAM is not only a co-factor for trans-methylation reactions, but is also a substrate for the syntheses of ethylene, polyamines, biotin, and nicotianamine (Moffatt and Weretilnyk, 2001). In response to the abiotic stresses, the ethylene and polyamine syntheses of plants are stimulated, resulting in the elevated levels of ethylene and polyamine. These can help plants regulate the stress responses, exercise specific tolerance mechanisms, and adapt to their environment (Bouchereau *et al.*, 1999; Bleecker and Kende, 2000). In this paper, we attempted to examine the diverse utilization of SAM for trans-methylation and syntheses of ethylene and polyamines in the potato suberization process via gene expression studies related to the SAM metabolism during suberization.

Solanum tuberosum L. cv. Desiree plants cultured *in vitro* and tubers harvested from the greenhouse were used for suberization tests. Approximately 5 μg of mRNAs isolated from the flesh parts of tubers were subjected to suberization for 6 and 72 h and used for the cDNA synthesis. The synthesized cDNA was ligated with an adaptor of EcoRI and XhoI restriction enzyme sites for directional cloning into Uni-ZAP XP vector using a cDNA library kit (Stratagene). In total, 1,072 ESTs from 6 h cDNA library and 874 ESTs from 72 h cDNA library were isolated and analyzed. Nucleotide and amino acid sequences were aligned with Clustal W and analyzed using the NCBI Blast X search (Altschul *et al.*, 1997). In the course of the gene expression study during suberization of the potato tubers, *in silico* screening of ESTs was performed to isolate cDNAs, whose expressions were induced 6 and 72 h after suberization and related in the SAM metabolism. There is only one pathway known for the synthesis of SAM, a reaction involving the transfer of adenine from ATP to methionine (Met). This reaction is catalyzed by the SAM synthetase (Fig. 1). SAM produced by this reaction is used as a methyl group donor for transmethylation reactions by SAM-dependent methyl-transferases. Each methyltransferase reaction involves the transfer of one methyl group to a specific substrate with the release of one molecule of S-adenosyl-L-homocysteine (SAH), a potent inhibitor of the SAM-dependent methyl-transferases. Thus, the removal of SAH is essential for the continued methyltransferase activity. The only known route for SAH catabolism in eukaryotes is mediated by the SAH hydrolase (Fig. 1). The homocysteine is then remethylated by the Met synthase using 5-methyl-tetrahydrofolate (THF) as a methyl group donor. Three different genes related in the transmethylation reactions, StSAM synthetase, StSAH hydrolase, and StMet synthase, were identified from ESTs in the post-suberization cDNA libraries. Of the total 1946 ESTs from

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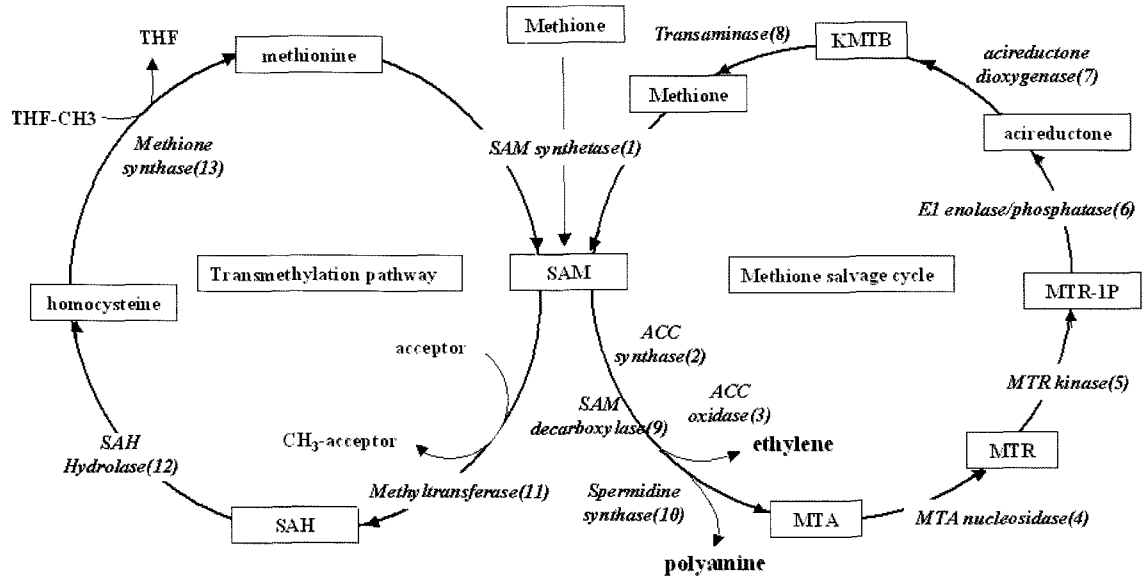

Fig. 1. Transmethylation pathway and methionine salvage pathway for ethylene and polyamine biosyntheses. Methionine (Met) is activated at the expense of ATP into SAM by the SAM synthetase (1). SAM is the substrate of ACC synthase (2). ACC is converted into ethylene by ACC oxidase (3). Resulting MTA enters the Met cycle and is hydrolysed by MTA nucleosidase (4), yielding methylthioribose (MTR) and Ade. MTR is converted back into Met through several successive steps of the Met cycle involving MTR kinase (5), E1 enolase/phosphatase (6), and acireductone dioxygenase (7), which produces KMTB (2-keto-4-methylthiobutyrate) and a transaminase (8). For polyamine synthesis, SAM is decarboxylated by SAM decarboxylase (9) and, with putrescine, spermidine is synthesized from putrescine by spermidine synthase (10). S-Adenosyl-L-homocysteine(SAH) originating from SAM-dependent transmethylation reactions (11) is hydrolysed by SAH hydrolase (12) in a reversible reaction to produce adenosine and homocysteine. Methionine synthase (13) catalyses the transfer of a methyl group from methyltetrahydrofolate (THF-CH_3) to homocysteine, producing tetrahydrofolate (THF) and Met.

"6 and 72 hr post-suberization" cDNA libraries, 19, 10, and 11 ESTs encoded StSAM synthetase, StSAH hydrolase, and StMet synthase, respectively. EST sequences of StSAM synthetase, StSAH hydrolase, and StMet synthetase showed high homologies with those of the potato (98, 97, and 100% identities; GenBank Accession No. DQ222503, DQ252503, and AF082893). The expression profiles were determined during suberization at 0, 6, 12, 24, 36, 72, and 120 h intervals after wounding with StSAM synthetase, StSAH hydrolase, and StMet synthase. The probes were generated by PCR with specific primers (Table 1) designed from the EST sequences. Total RNA was separated and transferred onto the nylon membranes. The membranes were then hybridized with the digoxigenin (DIG)-labeled probes using a PCR DIG probe synthesis kit (Roche Molecular Biochemicals). Target RNAs were detected using a DIG luminescent detection kit. The expression level of StSAM synthetase increased significantly immediately after the wounding and then rapidly decreased until 36 h after the wounding. The expression level of StSAH hydrolase increased immediately after the wounding, upto 72 h, and decreased thereafter. The expression level of StMet synthetase increased immediately after the wounding upto 24 h, and decreased slowly during the suberization process (Fig. 2). These data suggest that a large amount of SAM is required both at the early (6 h) and late (about 72 h) periods of suberization process and transmethylation pathway is important during the whole suberization process.

The polyamines putrescine, spermidine, and spermine are low-molecular-weight polycations that are found in all organisms. They have been implicated in a wide range of biological processes, including plant growth, development, and stress responses (Walden *et al.*, 1997; Bouchereau *et al.*, 1999; Shen *et al.*, 2000). Putrescine is synthesized directly through the decarboxylation of ornithine by ornithine decarboxylase or indirectly from arginine by the arginine decarboxylase. Putrescine is then converted into spermidine and spermine by the addition of propylamino groups from the decarboxylated SAM, which is itself produced from SAM by the action of SAM decarboxylase (SAMDC) (Walden *et al.*, 1997). The enzyme probably posesses the rate-limiting activity that provides the aminopropyl moiety used by spermidine and spermine synthases to convert putrescine into spermidine and spermine, respectively (Pillai and Akiyama, 2004). Potato SAM decarboxylase (StSAMDC) and spermidine synthase

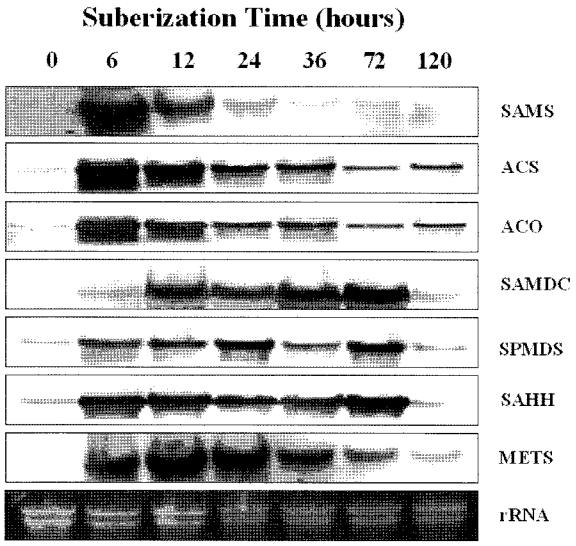

Fig. 2. Expression profiles of genes related to SAM metabolism in potatoes during the suberization process. Total RNA was isolated from potato tubers during suberization at various time intervals after wounding: 0, 6, 12, 24, 36, 72, and 120 h. Northern blotting was performed with probes generated by PCR using cloned potato cDNAs as the templates with specific primers (Table 1) designed from the EST sequences of SAM synthetase (SAMS), SAH hydrolase (SAHH), methione synthase (METS), SAM decarboxylase (SAMDC), and spermidine synthase (SPMDS) genes of the potato. ACS and ACO probes were generated by PCR using potato and tomato cDNAs as the templates with specific primers (Table 1) designed from the sequences of potato *ACS1* and tomato *ACO3* genes (GenBank accession Nos. AB041521 and Z54199).

(StSPMDS) were identified from ESTs of the post-suberization cDNA libraries. Out of 1946 ESTs from the post-suberization cDNA libraries, 4 and 8 ESTs encoded StSAMDC and StSPMDS, respectively. EST sequences of StSAMDC and StSPMDS showed high homologies with those of the potato (99 and 97% identities, GenBank Accession Nos. S74514 and AJ345003). The expression profiles were determined during suberization at various time intervals after wounding with StSAMDC and StSPMDS probes generated by PCR (Table 1). The expressions of StSAMDC and StSPMDS genes showed similar patterns. A moderate increase after wounding was detected, which lasted at similar levels until 72 h, then decreased thereafter (Fig. 2). These data suggest that polyamines are required during the entire suberization process and provide an evidence for the involvement of polyamine in the plant wound responses. In contrast to the animals, plants are unable to mobilize specialized cells devoted to wound healing after the herbivore attact. Although polyamines play an essential role in the wound healing responses in animals, mainly by regulating the

Table 1. Synthetic oligonucleotides used in this study

Primers	Sequences (5' → 3')
SAMS-5'	ATG-GAG-ACT-TTC-TTG
SAMS-3'	TCA-AGC-TTT-AGG-CTT
ACS-5'	CCT-CAT-GGT-GTT-ATT-CAG-ATG
ACS-3'	GTA-CCT-AAT-GGA-TTT-GAT-GGA
ACO-5'	GAA-CTT-CCC-AAT-TAT-CAA-CTT-GGA
ACO-3'	ACT-CAC-TTT-GTC-ATC-TTG-GAA-CA
SAMDC-5'	ATG-GAA-ATG-GAC-TTG
SAMDC-3'	CTA-CTC-CTT-TCC-TTC
SPMDS-5'	ATG-GCA-GAT-GAG-TGT
SPMDS-3'	TCA-TTT-TCC-TTT-GGT
SAHH-5'	ATG-GCT-CTA-TTA-GTC
SAHH-3'	TCA-GTA-CCT-GTA-GTG
METS-5'	ATG-GCA-TCT-CAC-GTT
METS-3'	TCA-CTT-GGC-GCT-GGC

expression of genes encoding the cytoskeletal proteins (Kamińska *et al.*, 1992) and activating the macrophages (Messina *et al.*, 1992), there was no experimental evidence available on the involvement of polyamines in the plant wound responses. However, our data suggest the possible involvement of polyamine in plant wound responses such as suberization pathway. Because polyamines have been implicated in a various plant growth and developmental processes involving cell proliferation and differentiation, morphogenesis, dormancy and germination, embryogenesis, and in the stress response (Walden *et al.*, 1997; Bouchereau *et al.*, 1999), it is plausible that polyamines, produced by increased expressions of StSAMDC and StSPMDS genes after wounding, can induce cell differentiation for the formation of the new wound-healed potato periderm. There are many supporting reports that polyamines play a role in controlling the gene expression, more specifically the cell division. Increased polyamine biosynthesis during the transition from the G1 to the S phase of the cell cycle, preceding the onset of the DNA synthesis in the dividing cells, appears to be a universal phenomenon in animals and plants (Lin *et al.*, 1984 ; Fuller *et al.*, 1997).

Ethylene, a simple gaseous hormone, integrates the external signals with the internal processes, adjusting the plant phenotype to its environment. The two committed steps of ethylene biosynthesis, the conversion of SAM into 1-aminocyclopropane-1-carboxylic acid (ACC) and its subsequent oxidation into ethylene, are regulated by ACC synthase (ACS) and ACC oxidase (ACO), respectively. ACS activity catalyses the rate-limiting step in the biosynthesis of ethylene, where SAM is converted into ACC, and MTA is released as a side product. Many results have been reported for the regulation of wound-elicited ethylene production from leaves. Recent trans-

criptional studies of ACS and ACO gene family members in many plants after the biotic attack and the mechanical wounding revealed high increases the levels of the ACS and ACO genes (Barry *et al.*, 1996; Peck and Kende, 1998; Ge *et al.*, 2000; Ralph *et al.*, 2007; von Dahl *et al.*, 2007). In this study, we also attempted to investigate the involvement of ethylene biosynthesis during suberization process of the potato tuber. However, both ACS and ACO gene clusters involved in the ethylene synthesis could not be found from the gene clusters of ESTs from post-suberization cDNA libraries. The expression profiles of ACS and ACO genes were determined during suberization at various time intervals after wounding. ACS and ACO probes were generated by PCR using potato and tomato cDNAs as the templates with specific primers (Table 1) designed from the sequences of potato ACS1 and tomato ACO3 genes (GenBank accession Nos. AB041521 and Z54199). Expression profiles of ACS and ACO genes showed similar patterns. A rapid increase after wounding was detected, which then decreased after 6 h (Fig. 2). These data suggest that ethylene is also required at the very early stage of suberization process, providing an evidence for the early involvement of ethylene signaling in the potato suberization process.

In conclusion, SAM plays important roles in the potato tuber suberization, not only as a methyl group donor but also as a substrate for the syntheses of ethylene and polyamine during the entire stage of the suberization process. Although SAM is used in numerous reactions, it is not a limiting resource in the plant cells as long as the activated methyl cycle and the Met salvage pathways remain active. The transmethylation cycle mediates the reuse of the thiol and adenyl groups of SAM and ultimately regenerates another molecule of SAM. The Met salvage pathway recoups the 5-methylthioadenosine (MTA) produced during the ethylene and polyamine biosyntheses, again into a molecule of SAM, thus allowing a high rate of ethylene and polyamine biosyntheses even when the pool of free Met is small. Evidently, the potato tuber has effective strategies to reuse and maintain the pool of this high-energy compound, SAM, for an efficient defence against wounding such as tuber suberization process.

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