Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

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Asian Journal of Plant Sciences 3 (1): 25-30, 2004
ISSN 1682-3974

Cloning and Sequencing of an ACC Synthase cDNA from Asparagus Spears and its Expression During Storage

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Abstract: A 1-aminocyclopropane-1-carboxylate (ACC) synthase cDNA from Asparagus spears was isolated and sequenced as a continuation after cloning, sequencing and expression analysis of ACC oxidase. The partial cDNA clone encoded an mRNA of 968 bp and the derived amino acid sequence was highly homologous to ACC synthase from bamboo banana, rice and wheat. Northern blot analysis showed increase level of pAS-ACS mRNA until 8 h at 20°C, which coincided with ethylene production and the ACC synthase activity, suggesting that the increase might be a response to the wounding associated with harvest.

Key words: ACC synthase, Asparagus spear, gene expression, storage

INTRODUCTION

ACC synthase (EC 4.4.1.14) catalyzes the conversion of S-adenosylmethionine to 1-aminocyclopropane-carboxylate (ACC), one of the rate-limiting steps in ethylene biosynthesis. This enzyme has proven to be quite recalcitrant to biochemical characterization because it is labile and in low abundance in plant tissues. The cloning and expression analysis of ACC synthase genes in many other plant tissues[1-6] has facilitated more biochemical and structural studies of this enzyme. ACC synthase was first cloned from zucchini[7] and since then a number of ACC synthase genes have been cloned from a number of plants. The emerging picture from the study of these genes is that ACC synthase is encoded by a multi-gene family and that these genes are differentially expressed in response to various internal and external inducers[8].

In harvested Asparagus spears increase level of ethylene production and ACC oxidase activity has been reported recently[9] in response to the wounding associated with harvest. As the Asparagus spears are not a large ethylene producing commodity very few research have been conducted on the rates of ethylene production and its deteriorative effects during storage. However, ethylene within the storage environment, whether produced by the stored product or other sources is known to cause a significant stress to many harvested products. The hormone affects the rate of metabolism of many succulent plant products and is generally active at very low concentration. In some recent studies on vegetables like broccoli, cabbage, carrots and lettuce a very small amount of ethylene has been shown to increase the rate of respiration, alter the activity of a number of enzymes and increase membrane permeability. Cellular changes induced by ethylene result in acceleration of senescence and the deteriorative processes that accompany it.

We have previously constructed cDNA libraries from Asparagus spears and isolated cDNA clones for ACC oxidase. Here we report a cDNA sequence encoding ACC synthase along with expression analysis by Northern blotting to elucidate the gene for achieving longer shelf life of Asparagus spears.

MATERIALS AND METHODS

Plant materials: The growth conditions for plant material (*Asparagus officinalis* L. cv. 'Welcome'), spear harvest, enzyme extraction and assay, ethylene production and RNA isolation, were as previously described[9,10]. The term 'top' and 'bottom' refer to the apical and basal 20 of 250 mm spears. After harvest the top and bottom portions were excised and incubated at 20°C for 0, 8, 16, 24 and 48 h under humid and dark conditions.

ACC synthase activity assay: ACC synthase extraction was carried out with 2 g of spear tissues that were homogenized in a mortar and pestle with 4 ml of extraction buffer consisting of 400 mM K-phosphate buffer pH 8.5, 10 μM pyridoxal phosphate, 0.5% mercaptoethanol and 20% glycerol at 2°C. The resulting homogenate was

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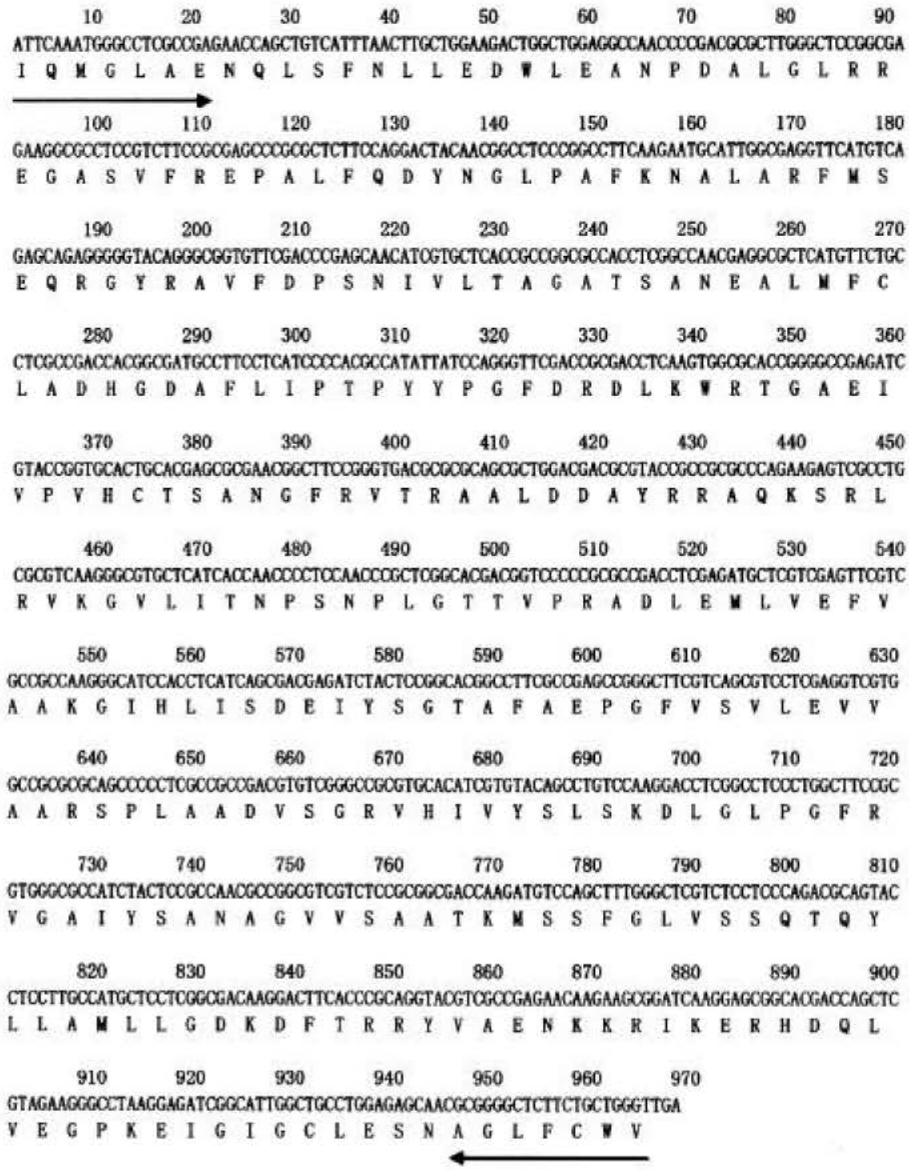

Fig. 1: Nucleotide sequence and deduced amino acid sequence of the cDNA clone corresponding to pAS-ACS. The predicted amino acid sequence is given in single-letter code for each amino acid. The arrows indicate the positions of degenerated primers (sense, antisense) used for RT-PCR. Numbering refers to total nucleotide residues on each line

filtered through 4 layers Kimpwipe and the filtrate was charged on a dry Sephadex G-25 column (1.5 i.d.x7 cm) prepared by centrifuging at 3,000 rpm for 3 min at 4°C just before use. The eluent was recentrifuged at 3,000 rpm for 5 min and was used for the enzyme assay, according to the method described by Lizada and Yang[11].

Amplification of RNA: To amplify poly $(A)^+$ RNA and to isolate ACC synthase internal fragments, degenerate oligonucleotide primers were synthesized from other ACC synthase genes in the database. The primers for PCR were synthesized to two amino acid domains conserved in various ACC synthase genes, IQMGLAE for the sense primer (5′-TYCARATGGGTCTHGCDGAA-3′) and AGLFCWV for the antisense primer (5′-ACCCARCARAASARDCCNGC-3′), respectively. Cloning and sequencing of cDNA, sequence data analysis, probe synthesis and northern blotting were performed as in ACC oxidase and PAL as described in our previous reports[9,10].

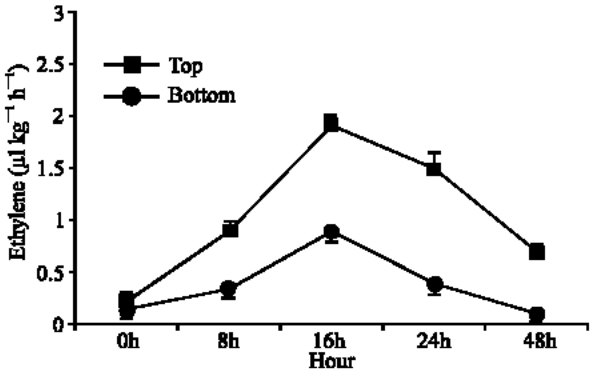

Fig. 2: Changes in ethylene production in the excised top and bottom portion of Asparagus spears. Each point represents the mean of three replicates and bars show SD about mean

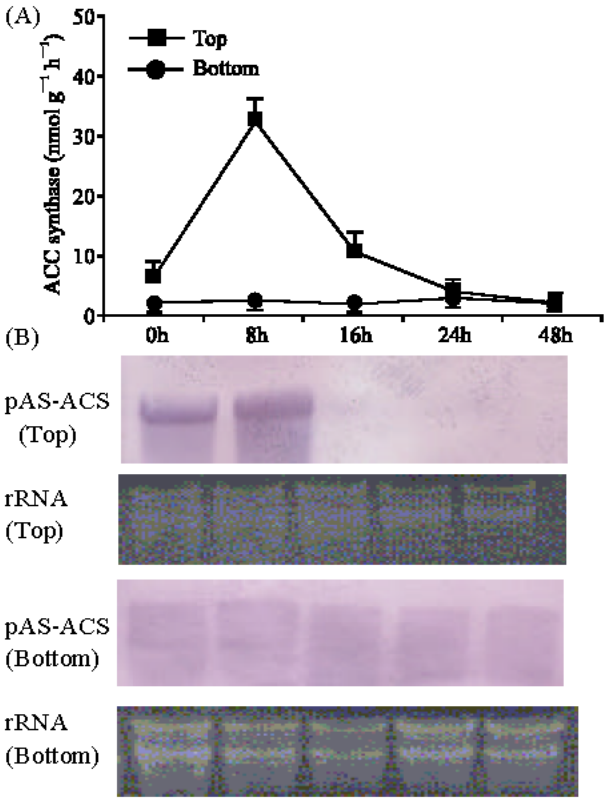

Fig. 3: Changes in ACC synthase activity and expression of pAS-ACS gene in the excised top and bottom portion of Asparagus spears. (A) Changes in ACC synthase activity. Each point represents the mean of three replicates and bars show SD about mean. (B) Northern blot analysis for ACC synthase. Equal loading of RNA was confirmed by staining a gel with ethidium bromide

GenBank accession number: The nucleotide sequence data reported in this paper will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession number AB 111528.

RESULTS

Isolation and identification of cDNA clone: The cDNA pAS-ACS is a partial clone encoding a harvest-induced transcript from Asparagus spears. The encoded mRNA is 968 bp long (Fig. 1) and is highly homologus to the ACC oxidase gene of other plant (Fig. 4). The pAS-ACS sequence is 78.8% identical to ACC oxidase from bamboo (AB 085172), 73.7% identical to ACC oxidase from banana (AF 080258) and 76.8 % identical to the rice ACC oxidase (M 96673), all of which are wound-induced gene (Table 1). Allowing for conservative amino acid substitutions, the similarities are 79.9, 83.2 and 84.9% for the bamboo, banana and rice sequences, respectively.

Wound-induced ACC synthase: ACC synthase activity increased rapidly in the top portion after excision, reaching a peak at 8 h and then declined sharply (Fig. 3A). In the bottom portion, there was no specific inclining or declining pattern of ACC synthase activity throughout the experimental period. In northern blot analysis, the levels of transcripts for ACC synthase increased rapidly in the top portion immediately after excision (Fig. 3B). Expression of ACC synthase gene in top portion remained clearly detectable until 8 h but it was barely detectable in the bottom portion which might be due to the lower activity of ACC synthase in that portion.

A phylogenetic tree was generated from the alignment of the deduced amino acid sequences of pAS-ACS and other ACC synthase gene in the data base. The pAS-ACS (accession no. AB 111528), ACS from bamboo (AB 085172) and rice (M 96673) strongly clustered together in a subgroup, having closest

Table 1: Percentage of nucleotide and deduced amino acid homology between ACC synthase from asparagus and other plants in the databases

Plants	Nucleotide	Amino acid
Bamboo-ACS (AB 085172)	78.8	79.9
Rice-ACS (X85747)	76.8	84.9
Banana-ACS (AF081917)	73.7	83.2
Wheat-ACS1 (U 35779)	73.8	74.9
Pea-ACS (AF 016458)	72.7	73.2
Mung bean-ACS(AB 000679)	66.5	70.6
Apple-ACS (L 31347)	61.5	60.8
Papaya-ACS (U 68216)	66.1	69.2
Tobacco-ACS(X 65982)	64.3	68.8
Wheat-ACS2 (U 35778)	78.8	79.9
Tomato-ACS (M 34289)	67.2	70.8
Papaya-ACS (U 68216)	63.2	64.5
Arabidopsis-ACS (U 23481)	64.9	71.2
Pear-ACS (AF 016458)	67.9	73.9

Asparagus ACS (AB111528) is calculated as 100%

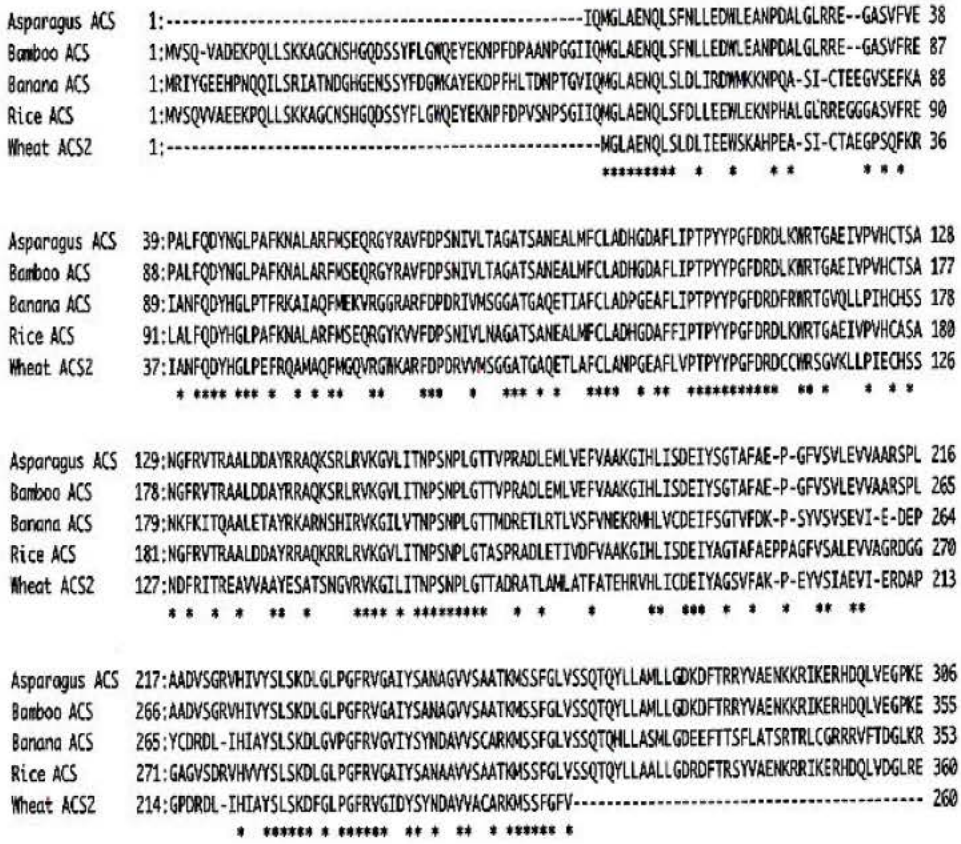

Fig. 4: Comparison of the deduced amino acid sequences from asparagus (AB102677), bamboo (AB 085172), banana (AF 080258), rice(M 96673) and wheat (U 35779) by multi alignment. The amino acid residues are numbered at the beginning and end of the sequences on each line. Asterisks denote the aminoacid residues those are identical. Dashes in the amino acid sequences represent gaps introduced to maximize alignment of the polypeptides

relationship with rice. The same sub group has been identified by another phylogenetic analysis by Matsui *et al.*[6].

DISCUSSION

As a continuation to our understanding of ethylene biosynthesis and its deteriorative effects on Asparagus spears, we have isolated a cDNA for ACC synthase from harvested spear and shown that it is wound induced. The clone, pAS-ACS had high sequence similarity to rice, bamboo and banana cDNA that were also associated with wounding. Northern blot analysis revealed that the expression of pAS-ACS increased in harvested spear tips until 8 h of storage period at 20°C and afterwards it started to decline. Although asparagus is considered as low to medium ethylene producing commodity the excised top and bottom portion of Asparagus spears produced a significant amount of ethylene in response to excision. With a marked rise in ethylene production ACC synthase activity was also rapidly induced in the top portion followed by the significant increase in the expression of ACC synthase gene (Fig. 2, 3A, 3B). In contrast to the top, the activity of ACC synthase in the bottom portion was too low to induce ethylene production. Thus induction of ACC synthase is considered to be primarily important for wound-induced ethylene synthesis. This type of finding was also reported in other vegetables like bamboo shoot[6], tomato[12] and cucumber[13].

Phylogenetic analysis of ACS sequences has revealed the existence of at least two major branches that contain characteristic conserved amino acid sequences, monocotyledon and dicotyledon, These are also subgrouped into wounding, ripening, senescence and auxin induced ACS. The pAS-ACS belonged to the subgroup wound-induced monocotyledon as it was highly homologous to rice ACS and was closely related to bamboo and rice ACS. Our results suggest that induction of ethylene production in harvested asparagus tips is regulated by transcription of pAS-ACS in response to the wounding associated with harvest.

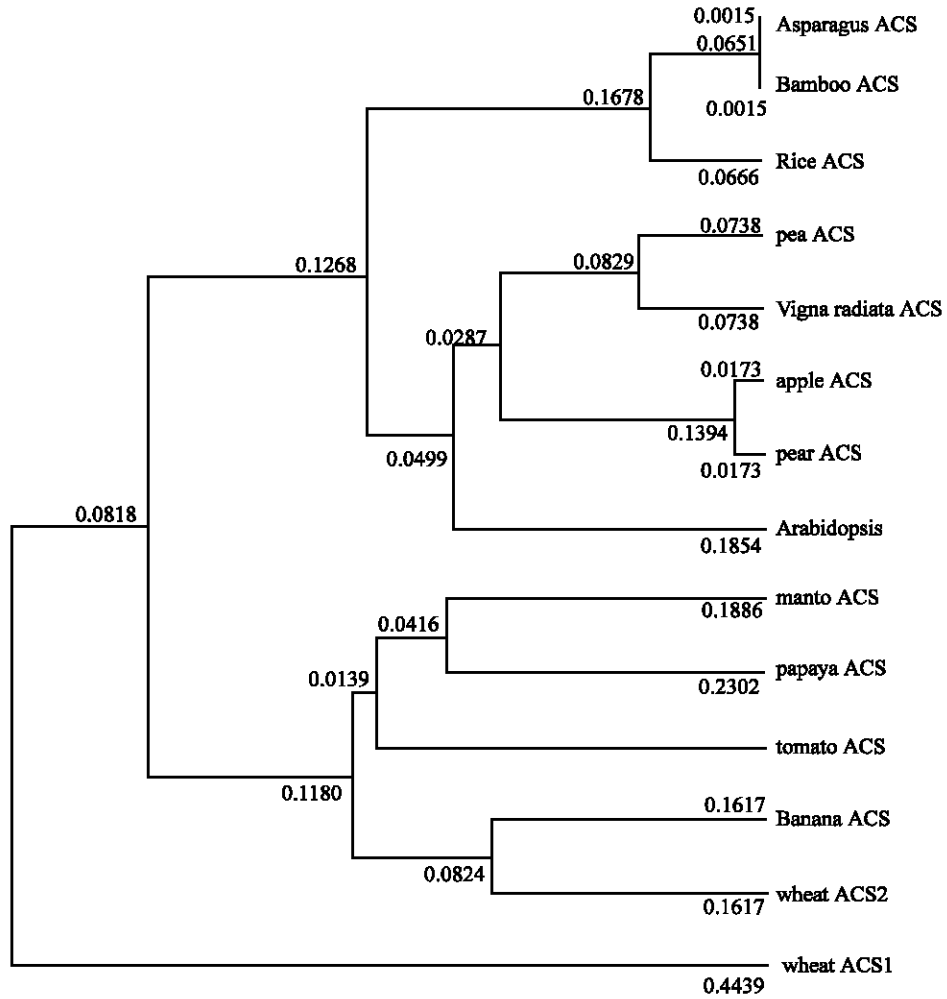

Fig. 5: Phylogenetic tree of the alignment of pAS-ACS deduced amino acid sequence with other ACC synthase in the database. Protein sequences were aligned using UPGMA and phylogenetic tree constructed using GENETYX-MAC software

The work presented here has laid the groundwork for further characterization and regulation of the function of ACC synthase gene in Asparagus spears.

ACKNOWLEDGEMENTS

The financial support of the Ministry of Education, Science, Sports and Culture of Japan under scholarship program for foreign students is gratefully acknowledged.

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