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# Molecular cloning and expression analysis of spermidine synthase gene during sex reversal induced by Ethrel in cucumber (*Cucumis sativus* L.)

Qiaomei Wang *, Gaofeng Yuan, Haiyan Sun, Puqing Zhao, Yang Liu, Deping Guo

*The State Agriculture Ministry Laboratory of Horticultural Plant Growth, Development and Biotechnology, Department of Horticulture, ZheJiang University, Hangzhou 310029, PR China*

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**Abstract**

A full-length cDNA encoding spermidine synthase *CsSPDS* of 317 amino acids was isolated from cucumber. CsSPDS showed high similarity to SPDSs of other species, with the greatest similarity to SPDS of *Coffea arabica* (identity, 87%). Northern blot analysis revealed that *CsSPDS* was expressed predominantly in rapid growing tissues, such as roots, young leaves and flower buds. The response of *CsSPDS* to the sex reversal of three-leaf stage androecious cucumber seedlings by Ethrel treatment was also examined. Our results showed that the *CsSPDS* was up-regulated in shoot apex during sex reversal process, which correlated with increases of spermidine synthase activity and free spermidine (Spd) content, and a decrease of putrescine (Put) content in shoot apices.
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*Keywords:* Cucumber; Ethylene; Polyamine; Sex differentiation; Spermidine synthase

## 1. Introduction

Polyamines (PAs) are ubiquitous in higher plants, and they play essential roles in plant cell growth and proliferation [1]. Polyamines are also involved in plant morphogenesis, flower bud induction and differentiation, embryogenesis, fruit setting and ripening, and response to environmental stresses [2–6]. Roles of polyamines in sex differentiation are not clear. Early studies have only revealed that different polyamine contents were found in male and female flowers, or during the process of male and female flower formation. It was reported that the stamen contained relatively high level of putrescine (Put) content, while the pistil contained relatively high level of spermidine (Spd) content in tobacco [7]. Other studies suggest that an increase of endogenous Put level is associated with the male flower formation, whereas an increase of Spd content is associated with the formation and development of female flowers in bitter gourd [8], it has also been found that endogenous Put level increases during the differentiation of male flowers in cucumber [9]. These studies collectively suggest the involvement of PAs in sex differentiation. However, the mechanism of polyamine action in sex differentiation remains to be elucidated.

Cucumber is a model plant for studying sex determination in monecious plants [10]. Hormonal regulation of sex determination in cucumber has been widely studied, and the results indicated that ethylene appear to be a critical hormone that promotes the femaleness, because application of 2-chloroethylphosphonic acid (Ethrel), which releases ethylene in plant tissues, promote the formation of female flowers, and a high endogenous ethylene content in shoot apex is associated with female flower development in cucumber [11,12]. It was also reported that some genes involved in ethylene biosynthesis and signal transduction expressed differently among different sex genotypes, and their specific expression patterns were regulated by ethylene [13–15]. But mechanisms underlying physiological correlation of ethylene with sex differentiation are still unclear.

* Corresponding author. Tel.: +86 571 85909333; fax: +86 571 86971121.
*E-mail address:* qiaomeiw@zju.edu.cn (Q. Wang).

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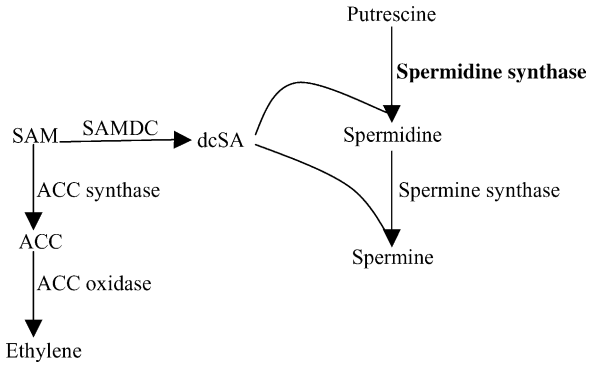


Fig. 1. The polyamine biosynthetic pathway and its connection to ethylene biosynthesis.

Polyamines and ethylene share a metabolic precursor, *S*-adenosyl-methionine (SAM) (Fig. 1). Recently, Chen (2002) reported that application of Ethrel, an ethylene-releasing agent induced an increase of endogenous Spd content and a decrease of Put content in the shoot apex of androecious cucumber, and consequently caused a sex reversal from male to female. Put is metabolized to Spd by spermidine synthase (SPDS) using decarboxylated SAM as an aminopropyl donor. However, no data suggest that changes of spermidine synthase activity or their mRNA levels are associated with sex reversal in cucumber. To understand how PAs biosynthesis is regulated by ethylene, and the possible role of SPDS in cucumber sex differentiation, we want to clone the cucumber *SPDS* gene. We report here the isolation of *SPDS* cDNA and preliminary analysis of SPDS expression in cucumber. Changes in content of free Put and Spd, and spermidine synthase activities in shoot apex were also determined during sex reversal from male to female by Ethrel treatment.

## 2. Materials and methods

### 2.1. Plant materials and sex reversal test

Androecious cucumber line (*Cucumis sativus* L cv. ZH-20) was used for this study. Cucumber seeds were germinated on wet filter paper in a Petri dish at 28 °C in dark for 1–2 days. The resulting seedlings were grown in a greenhouse. Plants were fertilized and irrigated according to normal cultivation practices.

Plants at 3-leaf stage were used to conduct sex reversal test, 1.0 mM Ethrel (2-chlorethyl phosphonic acid) was applied to the shoot apices of androecious cucumber plants with application of distilled water as control. Shoot tips were collected with a razor blade at 2, 4, 8, 12, 24, 48, and 96 h after treatment, and then immediately frozen in liquid nitrogen, and stored at −80 °C until use.

### 2.2. RNA isolation

Total RNA was isolated using the Trizol reagent (Invitrogen Life Technologies, Carlsbad, California, USA) as described in manufacturer's instruction. RNA was detected by spectrophotometer for the ratio of OD260/OD280 and by formaldehyde denatured agarose gel electrophoresis. The RNA samples were stored at −80 °C prior to RACE and RT-PCR analysis.

### 2.3. cDNA cloning

One microgram of total RNA isolated from cucumber shoot was used to synthesize first strand cDNA according to the manual of the Smart™ Race cDNA Amplification Kit (Clontech Laboratories Inc., USA). The first-strand cDNA was synthesized by avian myeloblastosis virus (AMV) reverse transcriptase using oligodT (18) as the anchored primer. To isolate SPDS fragments, two degenerate oligonnucleotide primers corresponding to conserved regions of SPDS were synthesized. 5′-GA[C/T]AT[A/T/C]TG[C/T]GA[A/G]AT[A/T/C]GA[C/T]AA[A/G/CT]ATG-3′corresponding to DICEI-D[N/K]M and 5′-TC[A/G/T]AT[A/G/T]AT[A/G]T[C/G]CA-T[A/G]TG[A/G/C/T]A[A/G]CC-3′corresponding to WLH-M[H/D]IIE. A partial cucumber SPDS cDNA fragment was obtained by PCR using the cDNA as the template and these two primers. The parameters for PCR were 35 cycles of 1 min at 94 °C, 45 s at 52 °C, and 45 s at 72 °C. After the last cycle, the amplification was extended for 10 min at 72 °C. The amplified products were analyzed by gel electrophoresis on 1% and recovered with a Sangon Kit (Sangon, Shanghai, China). The recovered product was cloned into the PUCm-T vector (Sangon, Shanghai, China) by the method described in the instruction manual. Then the 5′ sequence and 3′ sequence of cucumber SPDS was obtained by rapid amplification of cDNA ends PCR (Race-PCR) with the Smart™ Race cDNA Amplification Kit (Clontech Laboratories Inc., USA). The 3′ Race-reaction was carried out with primers of 5′-GGATCCCCGTGTCACTCTTCAT-3′ and 5′-AGCCATT-TTTTGCTTCAGTTGC-3′. The 5′ Race-reaction was carried out with primers of 5′-CTTGTGCAGGCCCGATAGG-3′ and 5′TCAGAACAGCCTTGAGAAATG-3′. The amplified products were analyzed, recovered and sequenced as mentioned above.

### 2.4. Comparison analyses

Nucleotide and deduced amino acid sequences of the SPDS clones were used for a Blast search on the Genebank databases. Sequence alignment was performed with the program of the DNAstar suite (DNAstar, Madison, WI).

### 2.5. RNA blotting analyses

Twenty micrograms RNA samples were separated on a formaldehyde-1.2% agarose gel and transferred onto a Nylon membrane (Hybond-$N^+$, Amersham) in 10 × SSC. After baking for 2 h at 80 °C, the RNA on the membrane was hybridized to the 5′-sequence of *CsSPDS* cDNA, which has been labeled with [$^{32}$P]-dCTP at 65 °C in Church buffer

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