Biochemical and Biophysical Research Communications 393 (2010) 365–370

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Antisense repression of sucrose phosphate synthase in transgenic muskmelon alters plant growth and fruit development

Hongmei Tian, Leyuan Ma, Cong Zhao, Hui Hao, Biao Gong, Xiyan Yu *, Xiufeng Wang *

College of Horticulture Science and Engineering, State Key Laboratory of Crop Biology, Shandong Agricultural University, Tai'an, Shandong 271018, China

ARTICLE INFO

Article history:
Received 19 January 2010
Available online 4 February 2010

Keywords:
Cucumis melo
SPS
Sucrose
Chloroplast ultrastructure

ABSTRACT

To unravel the roles of sucrose phosphate synthase (SPS) in muskmelon (*Cucumis melo* L.), we reduced its activity in transgenic muskmelon plants by an antisense approach. For this purpose, an 830 bp cDNA fragment of muskmelon sucrose phosphate synthase was expressed in antisense orientation behind the 35S promoter of the cauliflower mosaic virus. The phenotype of the antisense plants clearly differed from that of control plants. The transgenic plant leaves were markedly smaller, and the plant height and stem diameter were obviously shorter and thinner. Transmission electron microscope observation revealed that the membrane degradation of chloroplast happened in transgenic leaves and the numbers of grana and grana lamella in the chloroplast were significantly less, suggesting that the slow growth and weaker phenotype of transgenic plants may be due to the damage of the chloroplast ultrastructure, which in turn results in the decrease of the net photosynthetic rate. The sucrose concentration and levels of sucrose phosphate synthase decreased in transgenic mature fruit, and the fruit size was smaller than the control fruit. Together, our results suggest that sucrose phosphate synthase may play an important role in regulating the muskmelon plant growth and fruit development.

Introduction

Sucrose phosphate synthase plays a key role in regulation of carbon assimilation and partitioning of photoassimilate between sucrose and starch in plants [1]. SPS is involved in the synthesis of sucrose by the transfer of glucosyl moiety from UDP-glucose to fructose-6-phosphate, which is dephosphorylated by the action of sucrose-6-phosphate phosphatase (SPP) to finally yield sucrose [2]. Since SPS is the key enzyme in translocation of photoassimilates from sink to source tissues, SPS activity and its regulation have been extensively studied in leaf tissues with the identification of some of its activators and inhibitors [3]. SPS has been proposed to be an important limiting step in source–sink relationships [4], during grain filling in cereals [5,6] and in sugar accumulation in fruits [7–9]. The development and tissue specific distribution of SPS have also been studied and shown to be regulated at the transcriptional level [10].

Most of the previous studies have focused on the regulation of SPS of muskmelon at the enzymatic level under different conditions, and less studies on SPS at biochemistry and molecular level. In the present study, we investigated the contributions of *SPS1* (GenBank Accession No. DQ521271) sucrose phosphate synthase during plant growth and fruit development by expressing a chimeric antisense *SPS1* gene in transgenic muskmelon plants. We analyzed the T_2 generation of transformants throughout plant growth and fruit development, addressing not only the effects on sucrose phosphate synthase activity and sucrose content in fruit, but also the effects on plant growth and fruit development.

Materials and methods

Muskmelon transformation. An 830 bp fragment (nucleotides 2548–3377) from the 5′ end of the *SPS1* cDNA was inserted in the antisense orientation into the binary vector pBI121 (Clontech, Palo Alto, CA) behind the 35S promoter of cauliflower mosaic virus (CaMV). The construct was introduced into *Agrobacterium tumefaciens* LBA4404 [11] via direct DNA transformation [12]. Cotyledons of muskmelon inbred line M01–3 were transformed as described by Fang and Grumet [13]. Two independent transformants were obtained and analyzed. The phenotypes of the T_1 transgenic plants from the T_0 transgenic lines by self-pollinated were very identical. To obtain homozygous plants, T_1 plants were also self-pollinated and T_2 transgenic plants from two T_1 independent lines were analyzed in this study.

Untransformed M01–3 muskmelon plants were used as controls. Transgenic and control muskmelon plants were grown in a greenhouse on an experimental farm at Shandong Agricultural University in Tai'an, China, from February through May 2009, with spacing of 50 cm between plants and 120 cm between rows. Average day/night temperatures were about 30 °C/20 °C. The average daylight was about 12 h. Fertilizer was applied at two stages: a

* Corresponding authors. Fax: +86 0538 8246017 (X. Yu), +86 0538 8242456 (X. Wang).
E-mail addresses: yuxiyan@sdau.edu.cn (X. Yu), xfwang@sdau.edu.cn (X. Wang).

0006-291X/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.01.124

preplant broadcast application of 900 kg ha^{-1} of 14N-6.1P-29.9K, followed by a sidedress application of 150 kg ha^{-1} N at the flowering stage. Irrigation by furrows was applied as needed. Freshly opened female flowers were tagged on the day of hand-pollination to identify fruit of known age. The vines (main shoots) were trained vertically and topped at the 25th node. The other lateral shoots were cut except the lateral shoots setting fruit. Fruit set was recorded 10 d after pollination and the fruit were thinned to leave one per plant.

Plant growth and net photosynthetic rate (P_n) measurement. Plant height, stem diameter and leaf area were determined during the development of transgenic plants and control plants. Twenty plants were sampled for each time point. The net photosynthetic rate was measured with a portable photosynthesis system (LiCor-6400; LiCor Inc., Lincoln, NE, USA). Measurement was made on the uppermost youngest, fully expanded mature leaves of the main stem of transgenic plants and control plants, and was repeated at least six times on each.

Microscope observation. The leaf sub-cellular structure was analyzed by electron microscopy, on sections from the middle portions of fully expanded leaves of the main stem. Three leaves from three plants of the 20 plants used to measure plant growth and net photosynthetic rate (P_n) of transgenic and wild type muskmelons were collected, and two pieces (2×2 mm) of each leaf were taken and used for analysis. The sections were performed in a manner according to that previously described by Yu et al. [14]. The data were analyzed using factorial analysis of variance. Least significant differences (LSDs) at $p \leqslant 0.05$ and $p \leqslant 0.01$ were used to distinguish significantly different means.

Fruit sampling. Female flowers of T_2 generation plants and control plants were hand pollinated and tagged. The control and transgenic fruit were harvested at 5, 10, 15, 20 and 25 DAP and at full-slip (approximately 30 DAP). Five fruit samples of the appropriate age from the 20 plants were pooled to analyze for each data point. The same fruit samples were analyzed for sugars, size, enzymatic activity and real-time quantitative RT-PCR. Sampling continued until the five fruit samples selected were all mature.

Real-time quantitative RT-PCR. Total RNA was isolated using the guanidine isothiocyanate–phenol–chloroform method as described by Sambrook et al. [15]. Specific gene primers were designed from the cDNA sequence of *CmSPS1* to be analyzed using the Primer 5.0 software (PE Applied Biosystems) following the manufacturer's guidelines. For the real-time quantitative RT-PCR analysis, the β-actin gene was used as an internal constitutively expressed control (house-keeping gene). The primers (*CmSPS1* forward-F: 5′-CCATGAAAAGAATGGCAGCTG-3′, reverse-R: 5′-CGGAACTGTCATAGCAAAAGA G-3′; β-actin forward-F: 5′-TGCCCAGAAGTTCTATTCCAG C-3′, reverse-R: 5′-CATAGTTGAACCACTGAGGAC-3′) were synthesized by invitrogen and used at 200 nM final concentration. Each real-time PCR was performed in 25 μl final volume on an FTC2000 thermocycler according to the manufacturer's instructions. Three replicates were run for each sample. The PCR cycles were as follows: 1 cycle of 10 min at 95 °C, followed by 40 cycles each of 5 s at 95 °C, 30 s at 60 °C and 30 s at 72 °C, and the dissociation condition was 95 °C for 15 s, 60 °C for 30 s and 95 °C for 15 s.

Sucrose phosphate synthase extraction and assay. Sucrose phosphate synthase extraction and assay were performed in a manner according to that previously described by Yu et al. [16].

Sugar measurements. Sugars were extracted by grinding flesh tissues (10 g fresh weight) in 80% ethanol, adjusted to pH 7.0 with 0.1 N NaOH, and heated for 5 min at 80 °C. They were then analyzed as described by Lingle and Dunlap [17].

Results

Transgenic muskmelon plants are distinctly weaker than control plants

The antisense *SPS1* transgenic plants and control plants were grown in a greenhouse with the culture conditions described in

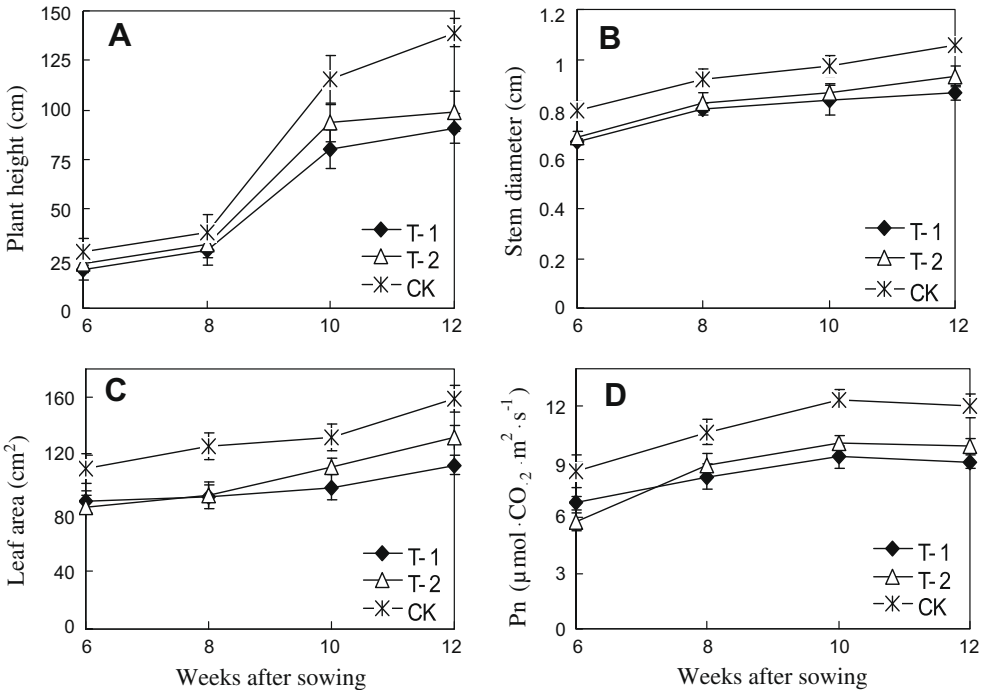

Fig. 1. Plant height (A), stem diameter (B), leaf area (C) and net photosynthetic rate (D) during transgenic plant and control plant development. The antisense *SPS1* transgenic plants and control plants were grown in a greenhouse on an experimental farm of Shandong Agricultural University in Tai'an, China, from February through May 2009. Twenty plants were used to measure plant height, stem diameter and leaf area for each time point. The net photosynthetic rate was measured with a portable photosynthesis system (LiCor-6400; LiCor Inc., Lincoln, NE, USA). Measurement was made on the uppermost youngest, fully expanded mature leaves of the main stem of transgenic plants and control plants, and was repeated at least six times on each. Bars indicate SES.