Scientia Horticulturae 119 (2009) 357–361

Contents lists available at ScienceDirect

Scientia Horticulturae

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

Different mechanisms to obtain higher fruit growth rate in two cold-tolerant cucumber (*Cucumis sativus* L.) lines under low night temperature

M. Miao *, Z. Zhang, X. Xu, K. Wang, H. Cheng, B. Cao

Horticulture and Plant Protection College of Yangzhou University, 12 Wenhui East Road, Yangzhou, Jiangsu 225009, PR China

ARTICLE INFO

Article history:
Received 3 June 2007
Received in revised form 23 August 2008
Accepted 27 August 2008

Keywords:
Cucumber
Low night temperature
Fruit growth rate
Assimilate accumulation
Photosynthesis
Sucrose-phosphate synthase

ABSTRACT

One cold-sensitive cultivar (Jinyan 4) and two cold-tolerant inbred lines (NY-1 and XC-1) of cucumber (*Cucumis sativus* L.) were subjected to temperatures of 28 °C/22 °C (day/night, control) or 28 °C/12 °C (day/night, cold treatment) in a 10 h photoperiod (7:00–17:00). Under control conditions, cucumber fruits grew fast during afternoon and early night, and slow during late night and morning. Under 28 °C/12 °C conditions, the two cold-tolerant inbred lines maintained relatively higher fruit growth rates than the cold-sensitive cultivar by different mechanisms. Compared to Jinyan 4, NY-1 fruits had higher growth rates during cold nights while XC-1 fruits grew faster during the next day. Under the 28 °C/12 °C temperature regime, the assimilate accumulation in the fruits of all tested genotypes followed a similar trend with the corresponding fruit growth rates. After a cold night treatment, the net CO_2 assimilation rates of one- and two-fruit plants, which had increased sink demand, were higher than that of plants without fruits in all tested genotypes. This response indicates that feedback inhibition might be an important reason for the reduction of photosynthesis on the next day. In addition, after a cold night treatment, the levels of exportable sugars (sucrose and stachyose) in mature leaves of XC-1 were higher than those measured in Jinyan 4 and NY-1, which might explain why XC-1 fruits had faster assimilate accumulation rates in the next morning. Higher activity of sucrose-phosphate synthase, a key enzyme of sucrose and stachyose biosynthesis, constituted an additional evidence that faster sucrose and stachyose biosynthesis in mature leaves may occur in XC-1 than in Jinyan 4 and NY-1 at that time. In conclusion, our results showed that cucumber genotypes may use different mechanisms to enhance their cold tolerance.

1. Introduction

The Energy-Saving Sunlight Greenhouse, which increases the temperature by sunlight only and maintains the energy during night through insulation without any additional energy supply, is very popular in developing countries and areas where the heating expense is not affordable. In such a greenhouse, at a daytime outdoor temperature of 0 °C, the indoor temperature can rise above 30 °C, and drop to 12 °C or lower at night. In view of the energy crisis all over the world, this type of greenhouse may have great potential application in developed countries in future as well. Thus, the growth and metabolism of crops under this circumstance should be investigated elaborately to improve crop production and breeding for such a greenhouse.

Worldwide, cucumber (*Cucumis sativus* L.) is an important vegetable usually cultivated as a winter season crop in greenhouses. In a diurnal cycle with normal temperature, cucumber fruit grows faster during night than during daytime (Pharr et al., 1985; Verkleij and Hofman-Eijer, 1988), while the assimilate export rate is higher during daytime (Verkleij and Hofman-Eijer, 1988). Several papers have focused on the effect of low night temperature on the metabolism of cucumber seedlings. Kanahamam and Hori (1980) reported that at night temperatures of 5 and 9 °C, the export of ^{14}C assimilated in afternoon was retarded and the assimilate distribution to roots increased. Murakami and Inayama's study (1974) also indicated that low night temperature reduced the assimilate export and increased the starch content in the leaves. It was found that night chilling usually has a negative aftereffect on photosynthesis during the next day (Kurets et al., 1999). Zhou et al. (2004) reported that for cucumber, there are genotypic differences in the factors limiting CO_2 assimilation and in the photo-protection mechanism for night chilling. However, the patterns of fruit growth and carbohydrate translocation of this stachyose-transporting species (Pharr et al., 1985) under normal-day-low-night-

* Corresponding author. Tel.: +86 514 87979394; fax: +86 514 87347537.
E-mail address: mmmiao@yzu.edu.cn (M. Miao).

Abbreviations: SP, sucrose-phosphate synthase; GS, galactinal synthase; SAI, soluble acid invertase.

0304-4238/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.scienta.2008.08.028

temperature thermoperiods (similar to the conditions in Energy-Saving Sunlight Greenhouse) still remain poorly understood. In this study, we used one cold-sensitive cultivar and two cold-tolerant inbred lines of cucumber to investigate how fruit growth and source-sink translocation were influenced by low night temperature under conditions of normal day temperature.

2. Materials and methods

2.1. Plant growth and treatments

Two cold-tolerant inbred lines (NY-1, donated by Known-You Seed Co. Ltd., China, and XC-1, from Tianjin Cucumber Institute, China), and a cold-sensitive cultivar (Jinyan 4, from Tianjin Cucumber Institute, China) of cucumber (*Cucumis sativus* L.) were used in this study. Plants were grown in a climate chamber as described by Miao et al. (2007). To avoid the influence of discontinuous watering or fertilization on the diurnal fruit growth pattern, plants were watered and fertilized continuously with one forth Hoagland nutrient solution by drip irrigators. Lateral branches were removed. Inside the chamber, the temperatures were 28 °C/22 °C (day/night) and the relative humidity was 70%. Light was provided by high-pressure mercury lamps (Philip HPLN 400 W) at about 700 μmol m^{-2} s^{-1} for 10 h per day (7:00–17:00).

Zero, one or two fruits were left on the plants to achieve different sink demands (Miao et al., 2007). In two-fruit plants, anthesis was 3 days apart. To impose the cold treatment, 10 plants without fruits, 40 plants with one fruit and 10 plants with two fruits from each tested genotype were transferred to another chamber in which the night temperature was lowered to 12 °C for 3 days. The same number of plants remained in the control chamber in which the night temperature was further maintained to 22 °C. Cold treatment was started on the day 9 days after anthesis (for two-fruit plants, the second flower was counted). Fruit volume was measured by immersing the fruit into water and measuring the volume change every hour during 3 days treatment.

2.2. ^{14}C labeling and measurement

Each whole plant was placed into a polyethylene chamber with $^{14}CO_2$ at a constant concentration (340 μl l^{-1}) and specific radioactivity (0.5 MBq mg^{-1} C) during the 10 h daytime for labeling (Verkleij and Hofman-Eijer, 1988). The chambers were constructed according to Shishido et al. (1987) and equipped with ventilators to obtain an air speed of 0.5 m s^{-1}. Chambers were put in the growth chamber mentioned above to keep temperature at 28 ± 1 °C and photosynthetic photon flux density at 700 μmol m^{-2} s^{-1} during the labeling. Thirty one-fruit plants of each tested genotype were labeled. Fruits were harvested at 7:00 (start of labeling), 12:00, 17:00 (end of labeling and start of night temperature treatment), 0:00 and 7:00 at the next day, 5 fruits per time. Samples were dried at 80 °C to constant weight, ground into powder, and extracted by shaking for 30 min in 630 g l^{-1} [80% (v/v)] boiling ethanol. The residue was extracted in 14.3 M $HClO_4$:9.7 M H_2O_2 (2:1) for 4 h at 60 °C. The radioactivity of ^{14}C in both the extracted aliquots was counted using a liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA) (Yang et al., 2003).

2.3. Net CO_2 assimilation rate measurement and carbohydrate analysis

After a one-night low temperature treatment, the net CO_2 assimilation rate was measured using a gas exchange system (CID-PS CO_2 Analyzer System, CID, Vancouver, WA) at 9:00, 12:00 and 15:00. The air temperature, air relative humidity, CO_2 concentration and photosynthetic photon flux density were maintained close to the conditions in the climate chamber mentioned above. Sample leaves were collected from the eighth nodes (plants without fruits), fruit-carrying nodes (one-fruit plants) or larger-fruit-carrying nodes (two-fruit plants), respectively.

Starch, sucrose and stachyose were measured at 17:00 (start of night temperature treatment), 0:00, 7:00 (end of night temperature treatment) and 12:00. Sucrose and stachyose were extracted and analyzed with HPLC as described by Miao et al. (2007). Starch was determined according to Pharr et al. (1985). Briefly the leaf residue left after ethanolic extraction for sucrose and stachyose analysis was resuspended in 2.0 ml of 0.2N KOH and boiled for 30 min. After cooling, the pH of the mixture was adjusted with 1N acetic acid to about pH 5.5 and the gelatinized starch was digested to glucose using 20 units of amyloglucosidase (EC 3.2.1.3, dialyzed with 50 mM Na-acetate buffer, pH 4.5). After 3 h of digestion the samples were boiled. The released glucose was detected by HPLC using the sucrose and stachyose assay method mentioned above.

2.4. Enzyme extraction and assay

After a one-night low temperature treatment, mature leaves were collected at 8:00 and 10:00 (1 and 3 h after the start of daytime) from fruit-carrying nodes. Sucrose-phosphate synthase (SPS) (EC.2.4.1.14) and galactinol synthase (GS) (EC 2.4.1.123) were extracted according to the method of Miao et al. (2007). Soluble acid invertase (SAI) (EC 3.2.1.26) was extracted as described by Miron and Schaffer (1991) with modifications. In particular 1 g fresh frozen tissue was homogenized in three volumes of extraction buffer containing 50 mM Mops-NaOH (pH 7.5), 0.5 mM Na–EDTA, 2.5 mM DTT, 0.5% (w/v) BSA and 1% (w/v) insoluble polyvinylpyrrolidone. After centrifugation at 18,000 × g for 30 min, supernatants were dialyzed for 20 h against 25 mM Mops-NaOH (pH 7.5) and 0.25 mM Na_2–EDTA.

SPS activity was assayed according to Robbims and Pharr (1987) with a few modifications. A total reaction volume of 70 μl contained 20 mM UDP-glucose, 8 mM fructose 6-P, 5 mM $MgCl_2$, 50 mM Hepes-NaOH buffer (pH 7.4), and 10 μl leaf extract. Reaction mixture was incubated at 37 °C for 30 min and terminated by addition of 70 μl 1N NaOH followed by boiling for 10 min to destroy unreacted hexoses. Then 0.25 ml of 1% resorcinol in 95% (v/v) ethanol and 0.75 ml 30% (v/v) HCl were added. Tubes were placed in an 80 °C water bath for 8 min, removed and cooled to room temperature. SPS activity was determined by measuring A_{520}. The assay of GS activity was adapted from Karner et al. (2004). The reaction mixture (20 μl) contained 50 mM Hepes-NaOH (pH 7.0), 1 mM dithiothreitol, 5 mM $MnCl_2$, 20 mM myo-inositol, 5 mM UDP-D-galactose, and 10 μl of the extracts. Reaction mixtures were incubated at 30 °C for 15 min and stopped by boiling at 100 °C for 5 min. The amount of formed galactinol was determined by the same HPLC method mentioned above. SAI activity was assayed according to Miron and Schaffer (1991). The reaction mixture contained 0.6 ml of 0.1 M K_2HPO_4, 0.1 M citrate buffer (pH 5), 0.2 ml 0.1 M sucrose, and 0.2 ml of enzyme extract. Incubation was for 30 min at 37 °C. The reaction was stopped and reducing sugars were measured by the HPLC as well.

3. Results

As shown in Fig. 1, of all tested genotypes of cucumber, fruit growth diurnal patterns were similar during the 3 days of cold treatment. Under 28 °C/22 °C, the fruit growth rate of Jinyan 4 was highest at 17:00, declined to a minimum at about 1:00, remained at a low level until the end of night (7:00), increased slowly during