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Effects of the Temperature Lowered in the Daytime and Night-time on Sugar Accumulation in Sugarcane

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Abstract : Sugarcane (*Saccharum* spp.) is a major crop grown for sucrose production. In Japan, its sucrose concentration is highest in winter. We examined the effects of the temperature lowered in the daytime and night-time (LDT and LNT, respectively) on sugar assimilation. Since photosynthetic and respiration rates change with temperature, we assumed that plants under LNT (LNT plants) would have low respiration rates and thus high sugar yields, whereas those under LDT (LDT plants) would have low rates of photosynthesis and thus low sugar yields. However, because of their acclimatisation to the reduced temperatures, LNT and LDT plants had sugar yields that were similar, or superior, to those of control plants. Sugar yield depends on biomass and sugar concentration; the stems of LNT and LDT plants did not grow as tall as those of the controls, but the sucrose concentrations in their stems were higher than in the controls. ^{13}C analysis revealed no difference in the partitioning of photosynthates to the soluble sugar fraction between control plants and those treated with low temperature. Control plants had higher glucose concentrations in the stem than treated plants, in which new photosynthates appeared to be partitioned preferentially into sucrose. Low temperature enhanced the sucrose concentration in the sugarcane stem not by improving the carbon budget, but by promoting the partitioning of carbon to stored sucrose.

Key words : ^{13}C, Internode, Photosynthesis, Respiration, Sucrose, Translocation.

Sugarcane is an important source of biomass for ethanol production. It is also a major crop grown for sucrose production, providing up to 60% of the world's sugar supplies (Grivet and Arruda, 2002). In Japan, the sugar concentration and sugar yield of sugarcane increase in late autumn and winter (Terauchi et al., 1999b, 2000).

Sugar yield depends on two factors: plant biomass and stem sucrose concentration (Ebrahim et al., 1998). Since more carbohydrate is needed to grow a bigger biomass and to store sucrose at higher concentrations, a good whole-plant carbon budget would seem to induce a higher sugar yield. Carbohydrate is produced in the leaves and translocated to the stem (Moore, 1995). Photosynthesis and respiration gain and consume carbon, respectively, and both are affected by temperature (Yamori et al., 2005). So if the daytime temperature remains optimal, a lower temperature in the night-time may promote net carbon gain; conversely, a lower temperature in the daytime may decrease carbon gains.

Several researchers reported the effects of the difference between daily maximum and minimum temperature on crop yield (Sato and Ikeda, 1979; Nicholls, 1997; Dhakhwa and Campbell, 1998; Gent and Ma, 1998; Wilkens and Singh, 2001; Peng et al., 2004; Lobell, 2007; Lobell and Ortiz-Monasterio, 2007). A large range of diurnal change in temperature reduces crop yield owing to water and heat stresses and harm induced by hot days (Dhakhwa and Campbell, 1998; Lobell, 2007). On the other hand, the positive effect of a large diurnal temperature range is earlier fruit set, earlier ripening, and an increase in fruit size in tomato (Gent and Ma, 1998), and an increase in yield in soybean (Sato and Ikeda, 1979). However, few reports have considered the carbon budget changes that may result from a lowered temperature.

Even if the whole plant has a good carbon budget, growth and storage compete for substrates (Hatch and Glasziou, 1963; Bonnett et al., 2006; Inman-Bamber et al., 2008). Therefore, it is important to know into which organ or fraction (e.g. soluble sugars, structural components) new photosynthates are partitioned. Although several researchers have predicted the partitioning of photosynthate in sugarcane (Bonnett et al., 2006; Inman-Bamber et al., 2008), it has not been measured directly. ^{13}C analysis is useful for measuring the partitioning of photosynthates, and other data, such as those on photosynthesis and carbon budgeting, can be added for various analyses (Sasaki et al., 2005, 2007).

We analyzed the effects of the temperature lowered

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Table 1. Mean daytime and night-time temperatures during the 4-week treatment period in sugarcane.

Treatment	2005		2006	
	Daytime	Night-time	Daytime	Night-time
	°C		°C	
Control	33.7±0.4	26.2±0.3	30.9±0.6	23.8±0.4
LNT	33.7±0.4	15.3±0.2	30.9±0.6	15.5±0.2
LDT	26.6±0.5	26.2±0.3	24.0±0.7	23.8±0.4

Values are means of data for 4 weeks±SE. LNT: lowered night-time temperature; LDT: lowered daytime temperature.

in the daytime or night-time on sugar assimilation in sugarcane in terms of carbon budget, the partitioning of new photosynthates and other characters related to sugar yield.

Materials and Methods

1. Cultivation of plants

Single-bud stem sections of sugarcane (*Saccharum officinarum* L. cv. NiF8) were placed in vermiculite in a greenhouse at the University of Tokyo on 21 April 2005 and 28 April 2006. On 30 May 2005 and 30 May 2006, rooted seedlings were transplanted singly into 3.8-L plastic pots filled with field soil (black volcanic ash soil). All tillers were removed as they emerged, leaving just one stem. Fertilizer in the form of 300 mL Hoagland's solution (6 mM $Ca(NO_3)_2$, 12 mM KNO_3, 2 mM KH_2PO_4, 2 mM $MgSO_4$, 25 μM H_3BO_3, 10 μM $MnSO_4$, 2 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM H_2MoO_4 and 0.1 mM $FeC_6H_5O_7$) was applied to each pot and renewed once a week.

2. Temperature- treatments

Three temperature treatments–control, lowered night-time temperature (LNT), and lowered daytime temperature (LDT)–were applied for 4 wk from 27 August in 2005 and 28 September in 2006. There were eleven plants in 2005 and eight plants in 2006 in each treatment group. Control plants were grown in the greenhouse the optical transmittance of which is 83.6%. The plants under LDT treatment (LDT plants) were placed outside from 0600 to 1800, and the plants under LNT treatment (LNT plants) were placed in an air-conditioned room from 1800 to 0600. Before and after the temperature treatment, LDT and LNT plants were put in the greenhouse along with the control plants. In 2005, the average night-time temperature for LNT plants was 15.3ºC, whereas that for control and LDT plants was 26.2ºC; the average daytime temperature for LDT plants was 26.6ºC, whereas that for control and LNT plants was 33.7ºC (Table 1). In 2006, the average night-time temperature for LNT plants was 15.5ºC, whereas that for control and LDT plants was 23.8ºC; the average daytime temperature for LDT plants was 24.0ºC, whereas that for control and LNT plants was 30.9ºC (Table 1).

3. Whole-plant photosynthetic rate

Whole plant photosynthetic rate was measured before the temperature treatment on 22-26 August in 2005 and 25–28 September in 2006. Four plants were used for measurement in each year. Photosynthetic rate per unit leaf area was measured with a Portable Photosynthesis System (CIRAS-1, PP Systems, Hertfordshire, UK) at a CO_2 concentration of 360 μL L^{-1}, a photosynthetic photon flux density (PPFD) of 1400 μmol m^{-2} s^{-1} under artificial light and a leaf temperature of 17ºC, 22ºC, 27ºC, 32ºC or 37ºC. Furthermore, to examine the influence of acclimatization, the photosynthetic rates per unit leaf area at daytime temperature under each treatment (LNT and control : 31ºC, LDT : 24ºC) on 20-22 October, 3 weeks after the start of the temperature treatments. Four plants were used for measurement in each treatment. Photosynthetic rate per unit leaf area was measured from 0900 to 1500. The rate per unit leaf area was measured at the uppermost fully expanded leaf (1st leaf), two leaves below the 1st leaf (3rd leaf), four leaves below the 1st leaf (5th leaf) and two leaves above the 1st leaf (–2nd leaf). The photosynthetic rates per unit leaf area of other leaves (–1st, 2nd, 4th) were estimated from the values of the neighbouring leaves. The leaf photosynthetic rate was estimated as the multiplication of the photosynthetic rate per unit leaf area and leaf area. The whole-plant photosynthetic rate was thus estimated as the sum of individual leaf photosynthetic rates.

4. Whole-plant respiration rate

The schedule and number of replications for measurement of whole-plant respiration rate were the same as those for the measurement of whole plant photosynthesis rate. In both years, the whole-plant respiration rate was measured in an open chamber system with an infrared gas analysis system (SPB-H5, ADC BioScientific Ltd., Herts, England) (Fig. 1). Since the sugarcane plant is too big, the whole plant was divided into several pieces and Vaseline was then put on to their transverse section. In a preliminary experiment with sugarcane, we confirmed that whole-plant respiration rate did not change before and after dividing. The plants were placed in a 22.3 L chamber the temperature of which was controlled with a water bath (Fig. 1). Air decarboxylated by a soda lime column was pumped into the chamber at 2 L min^{-1}. A small portion of the air was injected into the SPB-H5 and the CO_2 concentration in the flow was measured. Respiration rate was calculated from the difference between the CO_2 concentration with and without the plant. Respiration rate was measured in dark at 17ºC, 22ºC, 27ºC, 32ºC and 37ºC before the temperature treatment, and at night-time temperature 3 wk after the beginning of temperature treatment (LDT and control : 24ºC, LNT : 16ºC). The whole plant respiration rate was

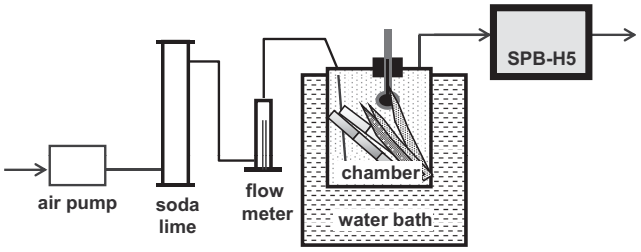

Fig. 1. System used to measure whole-plant respiration rate in sugarcane. Air decarboxylated with a soda lime column was pumped into the chamber at 2 L min^{-1}. A small portion of the air was injected into the SPB-H5, and the CO_2 concentration in the flow was measured. Respiration rate was calculated from the difference between the CO_2 concentration with and without the plant.

measured from 2100 to the next 0300.

5. Growth and sugar analysis

On 25 August 2005, before the temperature treatment and on 24 and 25 September 2005, 4 wk after the beginning of the temperature treatments, five plants were harvested. The stalk was excised at the soil surface, its leaves were removed. Then, the stem from the ground to the base of the uppermost leaf was used for evaluation of length and fresh weight. The internode below the node connected to the uppermost expanded leaf was designated the top internode. The upper (second from top), middle (central) and lower (second from bottom) internodes were weighed. Thereafter, each internode was juiced with a garlic crusher and the juice was stored at –80ºC for sugar analysis. Sucrose and hexose concentrations in juice were determined enzymatically according to the method reported by Nakamura and Yuki (1992). The sugar concentration (w/v) of whole-stalk was calculated as the mean value of the three parts (upper, middle and lower). The sugar yield of the whole-plant was calculated from sucrose concentration in juice and stem fresh weight.

6. Feeding of ^{13}C and sampling

On 10 September 2005, 2 wk after the beginning of the temperature treatments, $^{13}CO_2$ was fed to six plants in each treatment group. The second-highest expanded leaf (2nd leaf) of each plant was covered with a transparent bag made of 0.10-mm polyvinylchloride film that neither transmitted nor absorbed air or CO_2; the leaf took up $^{13}CO_2$ gas liberated from 500 mg of $Ba^{13}CO_3$ powder mixed with 5 mL of 7.3 M H_3PO_4 inside the bag. To allow plants to absorb all the liberated $^{13}CO_2$, we sealed the bag by tying its mouth with rubber plates fastened together with clips for 6 h. Two plants in each treatment were harvested immediately after the feeding of $^{13}CO_2$ to confirm that they took up the $^{13}CO_2$. The other plants were harvested 2 wk after feeding of the $^{13}CO_2$. The plants were divided into leaf blades, leaf sheaths, stems and roots. Leaf blades, leaf sheaths and stems were frozen in liquid nitrogen and stored at –80ºC, and then freeze-dried. Roots were carefully cleaned under running water to remove soil and other matter, and then freeze-dried.

7. Extraction of structural and non-structural carbohydrates

The freeze-dried plant materials were weighed and then ground to a fine powder. Samples (approximately 500 mg) were incubated in 80% ethanol at 80ºC for 1 hr, and then centrifuged at 3000×*g* for 10 min, after which the ethanol-soluble fraction was decanted.

The ethanol-soluble fraction was dried in a centrifugal dryer in a vacuum (CVE-200D, EYELA, Tokyo, Japan) and then further fractionated with a mixture of 2 mL distilled water and 2 mL chloroform. The aqueous phase was passed through a cation-exchange resin (Dowex-50, Dow Chemical, Midland, Michigan, USA) to remove amino acids. The efflux was collected and used as the soluble sugar fraction. Distilled water was added to the ethanol-insoluble fraction, which had been dried in a centrifugal dryer in a vacuum, and the suspension was boiled for 4 hr. Twenty units of glucoamylase in 0.5 mL of 100-mM acetate buffer (pH 4.6) was added to the suspension, which was then incubated for 2 hr at 60ºC to digest the starch into glucose. After centrifugation of the suspension at 3000×*g* for 10 min, the water-soluble fraction was collected, then passed through Dowex-50 and a nitrocellulose filter (Advantec, Tokyo, Japan) to remove proteins. The filtrate was collected and used as the insoluble sugar fraction (i.e., starch). The water-insoluble fraction (structural components) was washed twice with distilled water and dried in an electric oven at 80ºC.

8. Measurement of ^{13}C content

The total carbon and ^{13}C contents were determined with an elemental analyzer (NC2500, Thermoquest, San Jose, CA, USA) and a mass spectrometer (Delta Plus System, Thermoquest). Each fraction was dried completely and its ^{13}C content was determined. The ^{13}C content in each organ was calculated by equation 1:

$$^{13}C\ \text{content} = (\text{total carbon atom content}) \times (^{13}C\ \text{atom excess}\ \%) \times 13 \quad (1)$$

where '^{13}C atom excess %' is the difference in the ratio of $^{13}C/(^{12}C+^{13}C)$ between the plants supplied with $^{13}CO_2$ and those supplied with ordinary $^{12}CO_2$. The ^{13}C content was expressed as a percentage of the ^{13}C that was incorporated immediately after ^{13}C feeding.

9. Statistics

For all statistical analyses we used Fisher's protected least significant difference (PLSD) test, included in

SPSS (SPSS14.0J, SPSS Inc., Chicago, Illinois, USA).

Results

To estimate the carbon budget, in 2005 we measured the gas exchange rates in a whole plant at different temperatures before the start of treatments (Fig. 2). The dark respiration rate in the plant at 17°C, which corresponded approximately to the night-time temperature of an LNT plant (see Table 1), was only about 60% of that in the plant at 27°C, which corresponded to the night-time temperature of a control plant. The photosynthetic rate in the plant at 27°C, which corresponded to the daytime temperature of an LDT plant, was only about 85% of that in the plant at 32°C, which corresponded to the daytime temperature of a control plant.

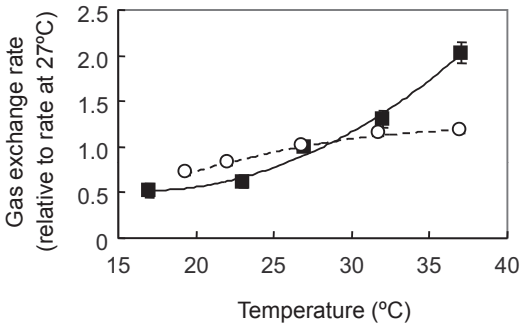

Fig. 2. Photosynthetic and respiration rates in a whole plant at different temperatures in sugarcane in 2005.
○ Photosynthetic rate; ■ Respiration rate. Bars indicate the SE of four replications.

We investigated sugar yield and related characters, including stem length, stem fresh weight and sucrose concentration, before and after the temperature treatment (Table 2). At the end of the 4-wk treatment period the control stems had grown by 21 cm, but the LNT and LDT stems had grown only slightly; this difference was significant ($P<0.05$). Stem fresh weight followed the same pattern: it increased by 58 g in control plants, but the LNT and LDT plants had significantly lighter stems ($P<0.05$). Although the difference of stem fresh weight between LNT and LDT plants seemed to big, this difference was not significant. Sucrose concentration in the juice extracted from the stem increased in all groups, but LNT plants (13.8%) and LDT plants (16.5%) each had significantly greater sucrose concentrations than the controls: temperature lowered in the daytime or night-time promoted sucrose concentration in the juice. Sugar yield in sugarcane is dependent on stem fresh weight and sucrose concentration, so although LNT plants had a significantly greater mean sucrose concentration than the controls, because of their lower stem fresh weight they did not have a higher sugar yield. In contrast, because the LDT plants had a 67% higher mean sucrose concentration than the controls, LDT plants had a significantly higher (by 20%) sugar yield ($P<0.05$). Thus, the observed sugar yields were different from the carbon gain and loss changes

Table 2. Effect of low temperature on sugar yield components in sugarcane in 2005.

	Stem length (cm)	Stem fresh weight (g)	Sucrose concentration (%)	Sugar yield (g)
Before treatment	99±6	175±4	6.2±0.6	10.8±0.7
After treatment				
Control	120±1 a	233±15 a	9.9±0.7 c	18.8±1.6 b
LNT	100±5 b	165± 7 b	13.8±0.7 b	17.5±0.7 b
LDT	102±4 b	190±14 b	16.5±0.5 a	23.4±1.8 a

The stem from the ground to the base of the uppermost leaf was used for evaluation of length and fresh weight. 'Sucrose concentration' is the percentage of sucrose (w/v) in juice. Values are means±SE of five plants. Means followed by different letters differ significantly ($P<0.05$, Fisher's PLSD). LNT, lowered night-time temperature; LDT, lowered daytime temperature.

Table 3. Partitioning of fed ^{13}C in each organ 2 wk after feeding in sugarcane in 2005.

Treatment	Leaf	Leaf sheath	Stem	Root	Whole plant
			%		
Control	12.8±0.7	9.0±2.0	55.3±3.4 a	5.8±0.4 b	82.9±2.0
LNT	13.3±1.3	10.3±1.3	45.8±1.8 b	8.3±0.7 b	77.7±2.6
LDT	14.3±0.7	7.1±0.6	41.9±1.3 b	14.1±1.6 a	77.3±0.8

The total amount of ^{13}C in a whole plant immediately after feeding was regarded as 100%. Values are means±SE of four plants. Means followed by different letters differ significantly ($P<0.05$, Fisher's PLSD). LNT, lowered night-time temperature; LDT: lowered daytime temperature.

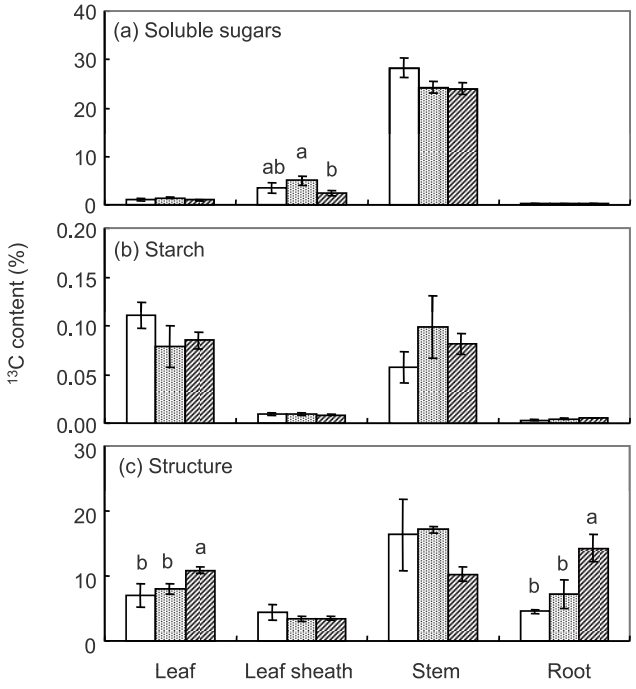

Fig. 3. Partitioning of fed ^{13}C in each fraction to different organs 2 wk after feeding in sugarcane in 2005. The total amount of ^{13}C immediately after feeding was regarded as 100%. □ Control; ▨ LNT; ▨ LDT. Bars indicate the SE of four replications. Means followed by different letters differ significantly ($P<0.05$, Fisher's PLSD).

predicted from the photosynthetic and respiration rates (Fig. 2).

Sugarcane plants were supplied with $^{13}CO_2$ 2 wk after the start of the treatments to examine the partitioning of photosynthates to different organs (Table 3). 2 wk after the feeding of ^{13}C, whole control plants had 82.9% ^{13}C. Although there were no significant differences in whole-plant $^{13}CO_2$ contents among treatments, LNT and LDT had only about 77% ^{13}C; thus, the ratio of consumed carbon to fixed carbon in these plants tended to be larger than that in control plants. Furthermore, both LDT and LNT plants had significantly less ^{13}C in the stem than did the control plants ($P<0.05$). In LDT plants significantly more fed ^{13}C was partitioned to the roots than in the other two groups ($P<0.05$).

Analysis of the partitioning of fed ^{13}C from each fraction (soluble sugars, starch and structural components) 2 wk after feeding revealed that relatively large amounts of ^{13}C were partitioned to the structural component in each organ (about 35% of the whole-plant total) (Fig. 3). The highest proportion of ^{13}C was partitioned to the soluble sugar fraction in the stem (about 26%), and there were no significant differences in ^{13}C content of this fraction in the stem among treatments. Analysis of the content in soluble sugars in stem revealed that control plants had larger proportions of glucose (12.6%) and fructose (10.2%)

Table 4. Effect of temperature treatments on proportions of soluble sugars in sugarcane stem in 2005.

Treatment	Sucrose	Fructose	Glucose
		%	
Control	77.2±2.2 c	10.2±1.0 a	12.6±1.3 a
LNT	94.1±0.6 a	3.0±0.3 c	2.9±0.3 c
LDT	87.4±1.4 b	5.9±0.6 b	6.7±0.8 b

The total amount of soluble sugars (sucrose, fructose, glucose) was regarded as 100%. Values are means±SE of five plants. Means followed by different letters differ significantly ($P<0.05$, Fisher's PLSD). LNT : lowered night-time temperature ; LDT : lowered daytime temperature.

to total soluble sugars than did LNT or LDT plants; the soluble sugar content of the stems of LNT and LDT plants was almost all sucrose (LNT: 94.1%, LDT: 87.4%) (Table 4).

As the carbon budget seemed to differ from that predicted from the pre-treatment photosynthetic and respiration rates, to confirm acclimatisation in whole plants, in 2006 we examined the effects of lowered temperature in our experimental system (Figs. 4, 5). The photosynthetic rate in LNT plants measured at 31°C after the treatment was clearly lower than that measured before the treatment, but that in the control plants was similar to that measured before the treatment (Fig. 4). The photosynthetic rate in LDT plants measured after the treatment was similar to that measured before the treatment. The respiration rate in LNT plants measured at 16°C after the treatment was similar to that measured before the treatment and to that in control plants measured at 24°C after the treatment. In LDT plants, the respiration rate measured at 24°C after the treatment was higher than that measured before the treatment though not significantly. Thus, in LDT plants, the respiration rate but not photosynthetic rate, seemed to increase after the treatment. On the other hand, in LNT plants, the photosynthetic rate, but not respiration rate, seemed to decrease after the treatment.

Discussion

Because photosynthetic and respiration rates in sugarcane are affected by temperature (Fig. 2), we hypothesised that suppression of respiration through temperature lowering in the night-time would enhance both the carbohydrate content of the whole plant and the sugar yield. However, acclimatisation to low temperatures in the daytime induced higher respiration rates at night (Fig. 5), as Yamori et al. (2005) reported in curves of respiration temperature in spinach. Energy supply by respiration may be necessary for sucrose synthesis activated by low temperature. Photosynthesis was also suppressed by acclimatization to low temperatures at night (LNT) (Fig. 4), perhaps because pyruvate phosphate dikinase, one of the

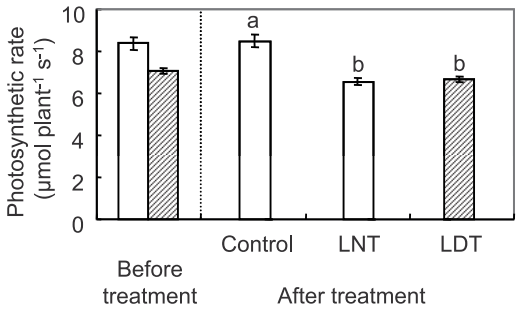

Fig. 4. Effect of the temperature lowered in the daytime on photosynthetic rates in whole plants of sugarcane in 2006. Photosynthetic rates were measured at 31ºC (□) and 24ºC (▨). The values at 31 and 24ºC before treatment were calculated from the curve of photosynthesis plotted against temperature (ref. Fig. 2). Bars indicate SE of four replications. Means followed by different letters differ significantly ($P<0.05$ by Fisher's PLSD). LNT, lowered night-time temperature; LDT, lowered daytime temperature.

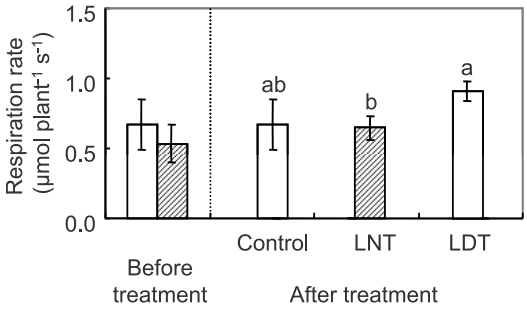

Fig. 5. Effect of temperature lowered in the night-time on whole-plant respiration rates in sugarcane in 2006. Respiration rates were measured at 24ºC (□) and 16ºC (▨). The values at 24 and 16ºC before treatment were calculated from the curve of respiration rate plotted against temperature (ref. Fig. 2). Bars are SE of four replications. Means followed by different letters differ significantly ($P<0.05$ by Fisher's PLSD). LNT, lowered night-time temperature; LDT, lowered daytime temperature.

limiting enzymes in C_4 photosynthesis, is cold labile (Hatch, 1979; Du et al., 1999), or because molecules such as ribulose-1,5-bisphosphate carboxylase/oxygenase are inactivated (Stitt and Hurry, 2002). The deterioration in the carbon budget of LNT plants is supported by the fact that less ^{13}C remained than in control plants (Table 3). A lowered daytime temperature tended to lower the photosynthetic rate (Figs. 2, 4) and acclimatisation to low temperature in the daytime increased the respiration rate at night (Fig. 5). Furthermore, although LDT plants were placed outside without shading in the daytime, less ^{13}C remained in the LDT plants than in control plants (Table 3). Although we cannot define the optimum temperature for the carbon budget from these experiments, the results indicate that a lowered temperature–whether in the daytime or at night–causes deterioration in the carbon budget.

Sugar yield depends on sucrose concentration and stem fresh weight, and a poor carbon budget is thought to suppress stem growth and sugar concentration. Sun and Chow (1949) reported that low temperatures at night suppressed stem elongation in sugarcane. Regardless of whether it occurred in the daytime or at night, a lowered temperature decreased both stem length and stem fresh weight (Table 2), but it enhanced the sucrose concentration despite the suppression of stem growth. Bonnett et al. (2006) suggested that sugarcane partitions less carbon to stored sucrose when grown under high compared with low temperature conditions. We therefore suggest that regardless of whether it occurred in the daytime or at night, a lowered temperature might partition new carbohydrate not to stem elongation, but to sucrose storage in the stem.

We used ^{13}C to clarify the partitioning of newly fixed carbon. Regardless of the temperature treatment, some ^{13}C was partitioned to structural components such as the cell membrane and cell wall (Fig. 3) (Sasaki et al., 2005, 2007). However, about 26% of fixed ^{13}C was partitioned to soluble sugars (including sucrose, fructose and glucose) in the stems. Control plants had more glucose, a growth substrate, in the stem than LNT and LDT plants (Table 4). However, LNT and LDT plants had more sucrose stored in the stem than control plants. Although the ^{13}C content of sucrose and glucose could not be separated, control plants tended to have more newly fixed photosynthates in the form of glucose in the stem, whereas LNT and LDT plants had more in the form of sucrose. These results support our suggestion that the lowered temperature might partition new carbohydrate not for use as a growth substrate, but for sucrose storage in the stem.

Several researchers have reported the effects of diurnal temperature variations on crop yield (Sato and Ikeda, 1979; Nicholls, 1997; Dhakhwa and Campbell, 1998; Gent and Ma, 1998; Peng et al., 2004; Wilkens and Singh, 2001; Lobell, 2007; Lobell and Ortiz-Monasterio, 2007), LDT and LNT treatments in this experiment corresponded to small and large variations, respectively. However, the diurnal temperature variations in these reports were almost all related to the maximum or average daytime temperature. Furthermore, the effects of diurnal temperature differences on crop yield have been discussed from the viewpoint of agriculture rather than plant physiology. Although the positive effect of a large diurnal temperature change is earlier fruit set and ripening and an increase in fruit size in tomato (Gent and Ma, 1998), and increased yield in soybean (Sato and Ikeda, 1979), these reports have never considered the changes in the carbon budget that result from the temperature change. We hypothesised that sugar yield would change because LNT and LDT would

induce better and worse carbon budgets, respectively. However, sucrose storage in the stem was accelerated neither by improvement in the budget nor by diurnal fluctuation of temperature. Bonnett et al. (2006) suggested that the sugarcane partitions less carbon to stored sucrose when grown under high compared with low temperatures such as we suggested. So how does the lowered temperature promote storage of sucrose in the stem? A low temperature increases sucrose production in *Arabidopsis* by promoting fructose-1,6-bisphosphatase and sucrose phosphate synthase (SPS), the two key regulated enzymes in sucrose synthesis that leads to a shift in carbon partitioning to sucrose (Strand et al., 1999; Stitt and Hurry, 2002). The activity of enzymes in the sucrose synthesis pathway increases during cold acclimatization in the leaves of spinach (Martindale and Leegood, 1997), winter wheat, rye and rape (Hurry et al., 1994, 1995), and in walnut wood (Margel et al., 2001). Post-transcriptional activation of SPS occurs in potato tubers (Hill et al., 1996; Deiting et al., 1998; Krause et al., 1998), apple fruits (Duque et al., 1999) and cabbage seedlings (Sasaki et al., 2001) exposed to low temperatures. Terauchi et al. (1999a, 2000) reported that lowered temperature promotes SPS activity in the stem of sugarcane. On the other hand, Inman-Bamber et al. (2008) suggested that reduced plant extension resulted in reduced demand for photo-assimilate by tops thus allowing excess assimilate to accumulate in the form of sucrose. Certainly, stem length was shorter in LNT and LDT plants than in the control plants (Table 2). Therefore, we suggest that since the stem growth is suppressed and the sucrose synthesis pathway is activated by a lowered temperature whether in the daytime or night-time, more photosynthate is partitioned into sucrose.

We conclude that a lowered temperature enhanced the sucrose concentration in the stem of sugarcane not by improvement of the carbon budget, but by promotion of partitioning of carbon into sucrose.

Acknowledgments

We measured ^{13}C by mass-spectrometry at Asia Natural Environmental Science Center of the University of Tokyo.

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**In Chinese with English summary.