Impact of photoperiod on circadian sucrose and sucrase rhythms in the digestive system of silkworm, \textit{Bombyx mori}.

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Abstract: The impact of photoperiod on circadian sucrose and sucrase rhythms were analyzed in the digestive system of \textit{Bombyx mori} under 12 hr light-dark cycle (LD), continuous light (LL) and continuous dark (DD). The rhythmic changes were interpreted as synthetic cycles in gut wall and release or uptake cycles in gut lumen. The gut wall comprised 6 sucrose synthetic cycles (SS cycles) under LD, LL and 5 under DD. The 24 hr rhythm of LD and LL was clock shifted to 28.8 hr under DD. In gut content, the sucrose rhythm showed 7 sucrose uptake cycles (SU cycles) under LD, 6 under LL and 5 under DD and the 24 hr rhythm of LD and LL and its 24-hr rhythm is advanced to 18.2 hr. In the gut wall sucrase rhythm maintained 7 SES cycles under LD and DD and 9 cycles under LL and its 24-hr rhythm is advanced to 18.2 hr. In the gut lumen 5 SER cycles under LD, 8 under LL and 6 under DD and its rhythm is advanced to 15 hr under LL and 20 hr under DD. Further analysis of data showed that LD favoured both synthesis and uptake of sucrose while LL, favoured the sucrose synthesis and its release.

Keywords: \textit{Bombyx mori}, Circadian sucrose rhythm, Circadian sucrase rhythm, Gut, Photoperiod.

INTRODUCTION

Sucrose is a non-reducing disaccharide and the product of photosynthesis in all plants (Sumida and Ueda, 2007). It is the principal dietary sugar of the silkworm, \textit{Bombyx mori}, as the mulberry leaf, the sole food of this insect, contains significant amount of sucrose; about 72 mg/g dry weight (Kuribayashi et al., 1990). The fresh mulberry leaves are the rich source of sucrose and its strength stimulates feeding in the silkworm larvae compared to other dietary carbohydrates such as pectin, xylan and starch (Sumida and Ueda, 2007). The dietary sucrose represents the chief energy source for metabolism in insects and its excess concentration contributes to the accumulation of glycogen and trehalose their tissues by inactivating glycogen phosphorylase (Thompson and Redak, 2000; Thompson et al., 2001; Thompson et al., 2002; Sumida and Ueda, 2007). Further, it functions as a cryoprotectant and membrane stabilizer and regulates the water flux across the gut wall (Thompson, 2003). Sucrase is a carbohydrase that hydrolyses sucrose to glucose and fructose and it is widely distributed in the different regions of the digestive system (Kanekatsu et al., 1992). In \textit{Bombyx mori}, it occurs in three distinct forms with different molecular weights and pH ranges. Sucrase-I (MW: 31000; pH 6.5) is produced by the columnar midgut cells and has a large affinity to sucrose while, sucrase- II (MW: 66000; pH 6.5) and sucrase- III (MW: 96000; pH 6.6), are produced by the goblet cells, predominantly in the posterior part of the midgut.

Together with maltase, lactase and glucoamylase, sucrase- II forms a complex and hydrolyses different sugars (Kanekatsu et al., 1992). Though, sucrases have been extensively studied with reference to their properties, characterization and developmental changes their rhythmic nature in the gut has not been established (Sumida et al., 1990; Kanekatsu et al., 1992; Sumida et al., 1994). Further, the availability of large volume of data on circadian clock mechanism, circadian genes and their products in Drosophila and \textit{Bombyx mori} (Shimizu et al., 2001; Williams and Sehgal, 2001; Hall, 2003; Sharma, 2003; Satyanarayana et al., 2004; Iwai et al., 2006; Peschel et al., 2009), suggest that many physiological and biochemical processes in insects are under the control of light-sensitive endogenous circadian clocks, that are fine tuned to work in a time schedule of 24 hr (Fonagy, 2009; Hirayama and Sessone-Corsi, 2009). More importantly, the recent reports on circadian physiology of \textit{B. mori}, emanated from our laboratory (Sailaja and Sivaprasad, 2010a, 2010b; Sailaja and Sivaprasad, 2011; Sailaja et al., 2011; Sivaprasad and Sailaja, 2011; Bhuvaneswari and Sivaprasad, 2012a,b) provides impetus for similar investigations. The present study aims at analyzing circadian changes in the profiles of sucrose and sucrase in the digestive system of \textit{B. mori} under altered photoperiodic conditions.

MATERIALS AND METHODS

The Pure Mysore x CSR, hybrid variety of the silkworm...
Circadian sucrose rhythms

Gut wall: Under LD, the sucrose rhythm of the gut wall showed 6 peaks and 7 troughs during the 24 hr free running period of the rhythm (Fig.1A). The first peak occurred early at 07 hr with a sucrose value ~15 mg/g wet wt. of tissue, while the subsequent peaks occurred at 09 hr (~13 mg), 14 hr (~23 mg), 18 hr (~19 mg), 20-23 hr (~27 mg) and next day again at 02 hr (~24 mg). Troughs occurred at 06 hr (~11 mg), 08 hr (~11 mg), 10 hr (~09 mg), 16 hr (~18 mg), 19 hr (~15 mg), 00 hr (~15 mg) and next day at 04:05 hr (~18 mg). Under LL, the sucrose rhythm showed 6 peaks and 7 troughs during the 24 hr free running period. While the peaks occurred at 07 hr (~19 mg), 09-10 hr (~16 mg), 14 hr (~16 mg), 18 hr (~26 mg), 23 hr (~22 mg) and next day at 02 hr (~18 mg), the troughs occurred at 06 hr (~14 mg), 08 hr (~10 mg), 11-13 hr (~11 mg), 15 hr (~11 mg), 19 hr (~14 mg) and next day at 01 hr (~10 mg) and at 06 hr (~12 mg). Under DD, the rhythm showed 5 peaks and 6 troughs during the 24 hr free running period. While the peaks occurred at 07-11 hr (~14 mg), 15 hr (~24 mg), 21 hr (~34 mg) and next day at 03 hr (~13 mg) and at 05 hr (~18 mg), troughs occurred at 06 hr (~9 mg), 12-13 hr (~10 mg), 17 hr (~17 mg) and next day at 01 hr (~10 mg), 04 hr (~10 mg) and at 06 hr (~15 mg). At the same, the intervals between peaks and troughs varied from one photoperiodic condition to the other. The interval between peaks was about 2.7 hr under LD, 3.0 hr under LL and 3.6 hr under DD and that between troughs was 3.1 hr under LD and LL and 3.8 hr under DD. The combined mean interval of peaks and troughs was roughly about 2.9 hr under LD, 3.1 hr under LL and 3.7 hr under DD (Tables 1A, B and 5).

Gut Content: Under LD the sucrose rhythm of the gut content showed 7 peaks and 7 troughs during the 24 hr free running period of the rhythm (Fig. 1B). The peaks appeared at 07 hr (~37 mg), 10 hr (~25 mg), 13 hr (~45 mg), 15 hr (~41 mg), 17 hr (~48 mg), 20 hr (~88 mg) and next day at 02 hr (~55 mg), while the troughs were recorded at 06 hr (~13 mg), 08 hr (~13 mg), 12 hr (~17 mg) 14 hr (26 mg), 16 hr (~37 mg), 19 hr (~37 mg) and next day at 03 hr (~33 mg). Under LL, the sucrose rhythm showed 6 peaks and 6 troughs during the 24 hr free running period. The peaks occurred at 07 hr (~39 mg), 10 hr (26 mg), 13 hr (~48 mg), 21 hr (~65 mg), 23 hr (~46 mg) and next day at 02 hr (~34 mg), while the troughs appeared at 09 hr (~25 mg), 11 hr (~16 mg), 17 hr (~31 mg), 22 hr (~34 mg) and next day at 01 hr (~20 mg) and 06 hr (~22 mg). Under DD, the sucrose rhythm showed 5 peaks and 6 troughs during the 24 hr free running period. Peaks appeared at 07-08 hr (~33 mg), 10 hr (~26 mg), 13 hr (~45 mg), 17 hr (~56 mg) and at 20 hr (~78 mg), and the troughs were recorded at 06 hr (~11 mg), 09 hr (~18 mg), 11 hr (~18 mg), 14 hr (~36 mg), 18 hr (~37 mg) and next day at 03 hr (~21 mg). The interval between peaks was about 2.7 hr under LD, 3.2 hr under DD, and 3.6 hr under LL. The periodic intervals between peaks were: 2.7, 3.2 and 3.6 hr respectively under LD, LL and DD conditions. After third moult, the larvae were divided into three batches and reared separately under three different photoperiodic conditions viz., 12 hr light and 12 hr dark cycle (LD), continuous light (LL) and continuous dark (DD), but fed uniformly five times a day as usual.
Fig. 1. Phase response curves (PRCs) of the 24 hr circadian sucrose rhythms in the gut wall (A) and gut content (B) of fifth instar larva of Bombyx mori, under 12 hr light: 12 hr dark cycle (LD), continuous light (LL) and continuous dark (DD) conditions. The values expressed in mg glucose/g wet weight of tissue, represent the 24 hr (from 6 AM on day 5 to 6 AM on day 6) free running time of the circadian rhythm (P values: <0.001).

Circadian sucrase rhythms

Gut wall: Under LD the rhythm of sucrase activity showed 7 peaks and 7 troughs in the gut wall during the 24 hr free running period of the rhythm (Fig. 2A). Peaks appeared at 08 hr (1.03 µ moles), 15 hr (1.18 µ moles), 17 hr (2.26 µ moles), 21 hr (0.96 µ moles), 00 hr (1.29 µ moles) and next day at 02 hr (1.08 µ moles) and 06 hr (0.98 µ moles), and troughs appeared at 06 hr (0.82 µ moles), 13 hr (0.37 µ moles), 16 hr (0.81 µ moles), 19 hr (0.71 µ moles), 23 hr (0.49 µ moles) and next day at 01 hr (0.88 µ moles) and 04 hr (0.38 µ moles). Under LL the rhythm showed 9 peaks and 8 troughs during the 24 hr free running time. The peaks appeared at 06 hr (1.06 µ moles), 10 hr (1.03 µ moles), 14 hr (1.96 µ moles), 18 hr (1.05 µ moles), 20 hr (0.95 µ moles), 00 hr (2.54 µ moles) and next day at 02 hr (1.31 µ moles), 04 hr (1.0 µ moles) and 06 hr (0.95 µ moles), while the troughs appeared at 09 hr (0.65 µ mole), 13 hr
Fig. 2. Phase response curves (PRCs) of the 24 hr circadian sucrase rhythms in the gut wall (A) and gut content (B) of fifth instar larva of Bombyx mori, under 12 hr light: 12 hr dark cycle (LD), continuous light (LL) and continuous dark (DD) condition. The values expressed in µ moles of glucose/mg sucrose/hr, represent the 24 hr (from 6 AM on day 5 to 6 AM on day 6) free running time of the circadian rhythm (P values: <0.001).

Gut wall: Under LD, the sucrase activity showed 6 peaks and 6 troughs during the 24 hr free running period. The peaks appeared at 16 hr (0.33 µ moles), 16-17 hr (~0.68 µ moles), 19 hr (0.85 µ moles), 22 hr (0.52 µ moles), and next day at 01 hr (0.44 µ moles), 03 hr (0.58 µ moles) and at 05 hr (0.54 µ moles). Under DD, the rhythm showed 7 peaks and 6 troughs during the 24 hr free running period. The peaks appeared at 06 hr (0.52 µ moles), 08-09 hr (0.59 µ moles), 14 hr (1.68 µ moles), 17 hr (1.51 µ moles), 19 hr (1.33 µ moles), 00 hr (2.90 µ moles) and next day at 06 hr (1.76 µ moles), the troughs appeared at 07 hr (0.34 µ moles), 11 hr (0.37 µ moles), 16 hr (0.82 µ moles), 18 hr (0.53 µ moles), 23 hr (0.48 µ moles) and next day at 05 hr (0.55 µ moles). The interval between peaks was about 3.1 hr under LD, 2.7 hr under DD and that between troughs was about 3.1 hr under DD, 2.4 hr under LL and 3.7 hr under DD. The combined mean interval of peaks and troughs was about 3.1 hr under LD, 2.6 hr under LL and 3.5 hr under DD (Tables 3A, B and 6).

Gut content: Under LD, the sucrase activity showed 5 peaks and 5 troughs in the gut content during the 24 hr free running period of the rhythm (Fig. 2B). Peaks appeared at 07 hr (0.038 µ moles), 16 hr (0.045 µ moles), 21 hr (0.024 µ moles) and next day at 04 hr (0.021 µ moles) and 06 hr (0.029 µ moles) and troughs appeared at 06 hr (0.016 µ moles), 09 hr (0.003 µ moles), 18-19 hr (0.005 µ moles), 23 hr (0.004 µ moles) and next day at 05 hr (0.015 µ moles). Under LL the sucrase activity showed 8 peaks and 8 troughs in the digestive juice during the 24 hr free running period of rhythm. Peaks appeared at 07 hr (0.018 µ moles), 09 hr (0.012 µ moles), 14 hr (0.020 µ moles), 17 hr (0.027 µ moles), 21 hr (0.018 µ moles), 23 hr (0.015 µ moles) and next day at 01 hr (0.014 µ moles) and 06 hr (0.025 µ moles) and the troughs occurred at 06 hr (0.016 µ moles), 08 hr
Fig. 3. Circadian changes in sucrose profiles and sucrase activity in the gut wall of the fifth instar larva of Bombyx mori, under (A) 12 hr light: 12 hr dark cycle (LD), (B) continuous light (LL) and (C) continuous dark (DD) conditions. The values expressed in mg glucose per gm wet weight of tissue in case of sucrose and moles of glucose formed/ mg sucrose/ hr in case of sucrase, represent the 24 hr (6 AM on day-5 to 6 A.M on day 6) free running time of the circadian rhythm. (P values: <0.001).
Fig. 4. Circadian changes in sucrose profiles and sucrase activity in the gut content of the fifth instar larva of Bombyx mori, under (A) 12 hr light: 12 hr dark cycle (LD), (B) continuous light (LL) and (C) continuous dark (DD) conditions. The values expressed in mg glucose per ml of tissue in case of sucrose and µ moles of glucose formed/mg sucrose/hr in case of sucrase, represent the 24 hr (6 AM on day-5 to 6 AM on day-6) free running time of the circadian rhythm. (P values: <0.001).
Table 1 (A and B). Interval between peaks (A) and troughs (B) in the levels of sucrose in the gut wall of the fifth instar larva of *B. mori* during the free running time of the circadian rhythm under 12 hr light/dark cycle (LD), continuous light (LL) and continuous dark (DD) conditions.

### (A)

| Photo period | No. of peaks | Interval between peaks in hours | Mean interval in hours |
|--------------|--------------|---------------------------------|------------------------|
|               | 1-2 | 2-3 | 3-4 | 4-5 | 5-6 |               |
| LD            | 6   | 2   | 5   | 4   | 2   | 3           | 2.7        |
| LL            | 6   | 2   | 4   | 4   | 5   | 3           | 3.0        |
| DD            | 5   | 4   | 6   | 6   | 2   | -           | 3.6        |

### (B)

| Photo period | No. of troughs | Interval between troughs in hours | Mean interval in hours |
|--------------|----------------|-----------------------------------|------------------------|
|               | 1-2 | 2-3 | 3-4 | 4-5 | 5-6 | 6-7 |               |
| LD            | 7   | 2   | 2   | 6   | 3   | 5   | 4           | 3.1        |
| LL            | 7   | 2   | 3   | 2   | 4   | 6   | 5           | 3.1        |
| DD            | 6   | 6   | 4   | 8   | 3   | 2   | -           | 3.8        |

Source: Fig. 1 A.

Table 2 (A and B). Interval between peaks (A) and troughs (B) in the levels of sucrose in the gut content of the fifth instar larva of *B. mori* during the free running time of the circadian rhythm under 12 hr light/dark cycle (LD), continuous light (LL) and continuous dark (DD) conditions.

### (A)

| Photo period | No. of peaks | Interval between peaks in hours | Mean interval in hours |
|--------------|--------------|---------------------------------|------------------------|
|               | 1-2 | 2-3 | 3-4 | 4-5 | 5-6 | 6-7 |               |
| LD            | 7   | 3   | 3   | 2   | 2   | 3   | 6           | 2.7        |
| LL            | 6   | 3   | 3   | 8   | 2   | 3   | -           | 3.2        |
| DD            | 5   | 2   | 3   | 4   | 3   | -   | -           | 2.4        |

### (B)

| Photo period | No. of troughs | Interval between troughs in hours | Mean interval in hours |
|--------------|----------------|-----------------------------------|------------------------|
|               | 1-2 | 2-3 | 3-4 | 4-5 | 5-6 | 6-7 |               |
| LD            | 7   | 2   | 4   | 2   | 2   | 3   | 8           | 3.0        |
| LL            | 6   | 2   | 6   | 5   | 3   | 5   | -           | 3.5        |
| DD            | 6   | 3   | 2   | 3   | 4   | 9   | -           | 3.5        |

Source: Fig. 1 B.

DISCUSSION

In silkworm, the gut acts as an organ of synthesis, storage, secretion and absorption of dietary nutritive materials including sucrose. This disaccharide is digested by the group of sucrases produced in the glandular epithelium of the midgut and released into the gut lumen and the end products of digestion (glucose and fructose) are absorbed back into the midgut cells. The glucose moieties so absorbed, are converted to storage carbohydrates, namely, glycogen and trehalose (Thompson and Redak, 2000; Thompson et al., 2001; Thompson et al., 2002). The present study on the circadian profiles of sucrose and sucrase activity confirms that their levels are modulated in a circadian pattern under the impact of light signals, similar to those in other biochemical constituents like proteins, free amino acids, carbohydrates, amylase, trehalose, trehalase and protease in different tissues of *B. mori* (Sailaja and Sivaprasad, 2010 a,b; Sailaja and Sivaprasad, 2011; Sailaja et al., 2011; Sivaprasad and Sailaja, 2011; Bhuvaneswari and Sivaprasad, 2012a,b). The circadian sucrose and sucrase data, presented as peaks and troughs in PRC’s (Figs. 1-4 and Tables 1-6) were analyzed in terms of the number of sucrose synthesis cycles (SS cycles) in the gut wall and number...
Table 3 (A and B). Interval between peaks (A) and troughs (B) in the activity levels of sucrase in the gut wall of the fifth instar larva of B. mori during the free running time of the circadian rhythm under 12 hr light/dark cycle (LD), continuous light (LL) and continuous dark (DD) conditions.

| Photo period | No. of peaks | Interval between peaks in hours | Mean interval in hours |
|--------------|--------------|---------------------------------|------------------------|
|              | 1-2 2-3 3-4 4-5 5-6 6-7 7-8 8-9 |                                 |                        |
| LD           | 7 7 2 4 3 2 4 - - | -                               | 3.1                    |
| LL           | 9 4 4 4 2 4 2 2 2 | 2                               | 2.7                    |
| DD           | 7 2 5 3 2 5 6 - - | -                               | 3.3                    |

(A)

Source: Fig. 2 A.

Photo period | No. of troughs | Interval between troughs in hours | Mean interval in hours |
|--------------|----------------|-----------------------------------|------------------------|
|              | 1-2 2-3 3-4 4-5 5-6 6-7 7-8 |                                 |                        |
| LD           | 7 7 3 3 4 2 3 - - | -                               | 3.1                    |
| LL           | 8 4 3 2 3 3 2 2 2 | 2                               | 2.4                    |
| DD           | 6 4 5 5 2 5 6 - - | -                               | 3.7                    |

(B)

Source: Fig. 2 B.

Similarly, the number of peaks in sucrase activity was interpreted as sucrase enzyme synthetic cycles (SEScycles) in the gut wall and sucrase release cycles (SER cycles) in the gut content. In both the compartments of the digestive system (gut wall and gut lumen), the height of peaks was interpreted in terms of intensity of synthetic/ release cycles and the mean peak value (average of all peaks) in terms of the average levels of sucrase or sucrase activity maintained during the 24 hr free running time of the rhythm.

Circadian sucrose rhythm

Gut wall: The sucrase maintains a glucose gradient between the two compartments (gut wall and gut lumen) of digestive system by appropriately altering its synthesis and release (Thompson, 2003). Accordingly, the sucrase rhythm maintains 6 SS cycles under LD and LL with duration of 4.0 hr each and 5 cycles under DD each with duration of 4.8 hr. Thus, DD condition modulates sucrose synthesis by extending the duration of each cycle by 48 min (from 4.0 hr to 4.8 hr). Due to extension of the duration of all SS cycles in a day, the normal 24 hr free running time of sucrose rhythm under LD and LL is clock-shifted to 28.8 hr under DD (Table 5). Further analysis of the intensity of peaks; in terms of the height indicates that sucrose synthesis is high during scotopic phase compared to photic phase of the day. Evidently, the active synthetic phases occurred thrice (at 14-15 hr, 20-23 hr and 02 hr) under LD, twice each under LL (at 18 hr and 23 hr) and DD (at 14-16 hr and 21 hr). The photoperiod not only altered the number of peaks (Iwai et al., 2006) but also the mean peak value (MPV) of the sucrose during the free running time of the rhythm. Significantly, MPVs maintained higher levels under both LD and DD (~20.6 mg) and moderate levels under LL (19.4 mg). Our findings confirm that dark cues modulate the circadian rhythms by appropriately altering the levels of different biochemical constituents in B. mori and other insects (Fonagy, 2009; Weng et al., 2009; Bhuvaneswari and Sivapradas, 2012a,b).

Gut content: The mulberry leaf is the rich source of sucrose for the silkworm, B. mori, in its gut. It senses the
Table 5. Comparative analysis of the phase response curves of the sucrose rhythm in the gut wall and gut content of the fifth instar larva of *Bombyx mori*, under 12hr light / dark cycle (LD), continuous light (LL) and continuous dark (DD) conditions.

| Parameter                              | LD | LL | DD | LD | LL | DD |
|----------------------------------------|----|----|----|----|----|----|
| No. of peaks                           | 6  | 6  | 5  | 7  | 6  | 5  |
| No. of troughs                         | 7  | 7  | 6  | 7  | 6  | 6  |
| Mean interval b/w peaks (hr)           | 2.7| 3.0| 3.6| 2.7| 3.2| 2.4|
| Mean interval b/w troughs (hr)         | 3.1| 3.1| 3.8| 3.0| 3.5| 3.5|
| Combined mean interval b/w peaks and troughs (hr) | 2.9| 3.1| 3.7| 2.9| 3.4| 3.0|
| Probable no. of SS/SU cycles           | 6  | 6  | 5  | 7  | 6  | 5  |
| Approximate time taken for each SS/SU cycles (hr) | (24/6=4) | (24/6=4) | (24/5=4.8) | (24/7=3.4) | (24/6=4) | (24/5=4.8) |
| Free running time of rhythm (hr)       | 24 | 24 | 28.8| 24 | 28 | 34 |
| Mean peak value                        | 20.1| 19.4| 20.6| 48.3| 42.9| 47.5|

Source: Fig. 1A and B; SS cycles: Sucrose synthetic cycles; SU cycles: Sucrose uptake cycles

Table 6. Comparative analysis of the phase response curves of the sucrase rhythm in the gut wall and gut content of the fifth instar larva of *Bombyx mori*, under 12hr light / dark cycle (LD), continuous light (LL) and continuous dark (DD) conditions.

| Parameter                              | LD | LL | DD | LD | LL | DD |
|----------------------------------------|----|----|----|----|----|----|
| No. of Peaks                           | 7  | 9  | 7  | 5  | 8  | 6  |
| No. of Troughs                         | 7  | 8  | 6  | 5  | 8  | 6  |
| Mean interval b/w Peaks (hr)           | 3.1| 2.7| 3.3| 4.6| 2.9| 3.8|
| Mean interval b/w Troughs (hr)         | 3.1| 2.4| 3.7| 4.2| 2.5| 3.8|
| Combined mean interval b/w Peaks and troughs (hr) | 3.1| 2.6| 3.5| 4.4| 2.7| 3.8|
| Probable no. of SES/SER cycles         | 7  | 9  | 7  | 5  | 8  | 6  |
| Approximate time taken for each SES/SER cycles (hr) | (24/7=3.4) | (24/9=2.6) | (24/7=3.4) | (24/5=4.8) | (24/8=3) | (24/6=4) |
| Free running time of Rhythm (hr)       | 24 | 18.2| 24| 24 | 15 | 20 |
| Mean Peak value                        | 1.25| 1.32| 1.47| 0.030| 0.019| 0.030|

Source: Fig. 2A and B; SES cycles: Sucrase enzyme synthetic cycles; SER cycles: Sucrase enzyme release cycles

Environmental cues and stimulates the release of sucrases from the gut wall. The sucrases so released digest the sucrose and contributes to the formation of trehalose, the insect blood sugar (Sumida and Ueda, 2007). The uptake of sucrose, in this fashion, occurs at regular intervals, referred to as sucrose uptake cycles (SU cycles) in the present report. In the gut content sucrose rhythm maintained 7 SU cycles with a duration of 3.4 hr each under LD, 6 SU cycles under LL with a duration of 4.0 hr each and 5 SU cycles under DD with a duration of 4.8 hr.
Sucrase activity remains higher in the gut lumen due to the stimulation and activation brought about by the concentration of sucrose derived from the ingested food (Sumida et al., 1990). The deficiency of this enzyme in gut content disturbs the sugar metabolism leading to slow growth rate, small body size, under developed silk gland and small cocoon size in B. mori (Sashindran Nair et al., 2004; Sumida and Ueda, 2007; Narayanaswamy and Shankar, 2010). The sucrase rhythm in the gut content, represented as sucrase enzyme release cycles (SER cycles) showed 5 cycles under LD, with a duration of 4.8 hr each, 8 cycles under LL with a duration of 3.0 hr each and 6 cycles under DD with a duration of 4.0 hr each. Significantly, the duration of each SER cycle was reduced by 1.8 hr (from 4.8 to 3.0 hr) under LL and by 48 min (from 4.8 to 4.0 hr) under DD. Consequently, the 24 hr sucrase rhythm under LD, has been rescheduled to operate at 15 hr under LL and 20 hr under DD (Table 6). Within the free running time of the rhythm, sucrase recorded active levels in 4 phases under all the three photoperiodic conditions, most of them coinciding with photopic phase of the day. Under LD, SER cycles occurred at 07 hr, 16 hr, 21 hr and 06 hr, while they occurred at 06-07 hr, 14-17 hr, 21-01 hr and 06 hr under LL and at 06-07 hr, 14 hr, 19 hr and 03-04 hr under DD. Surprisingly, the prevalence of higher MPVs under LD and DD (0.030 ì moles) and moderate MPVs under LL (0.019 ì moles), further substantiated the fact that dark cues ensure sustained sucrase activity, throughout the free running time of the rhythm despite the fact that light cues boost it up at regular intervals.

**Gut wall:** The glandular epithelium of the midgut wall is the major source of sucrase which digests the dietary sucrose and this process is accompanied by the transfer of monosaccharide moieties from sucrose through a sucrose and this process is accompanied by the transfer of monosaccharide moieties from sucrose through a transglucosidation process necessary for the growth and development of silkworm larvae and this enzyme is synthesized and released in response to the stimulant. Probably, because of this reason the higher activity levels throughout the free running time of the rhythm despite the fact that light cues boost it up at regular intervals.

**Sucrase rhythm versus sucrase rhythm:** A comparative analysis of PRCs of circadian sucrose and sucrase rhythms indicate inverse relationship between them during the free running period. This is true in both the compartments of digestive system (gut wall and gut content) under three photoperiodic conditions (LD, LL and DD) examined (Figs. 3 and 4). In this relationship, the peaks (higher levels) in one biochemical parameter were accompanied by troughs (lower levels) in the other. For instance, under LD, the peaks in sucrose levels of gut wall at 07-09 hr, 11-15 hr, 20-02 hr, 02-03 hr and 04-06 hr were coincided with troughs in sucrase activity. At the same time, the peaks in enzyme activity levels at 08 hr, 07 hr and at 00 hr were accompanied by troughs in its substrate levels (Fig. 3A). Similarly, under LL condition, the peaks in sucrase activity at 07 hr, 09 hr, 13 hr, 17-23 hr and 02-05 were by and large coincided with the timing of troughs in sucrose levels. At the same time, the peaks in the enzyme activity at 08 hr, 14-15 hr and 00 hr matched with corresponding troughs in its substrate concentration (Fig. 3B). Under DD, the elevated points in sucrose levels at 07-11 hr, 15-16 hr, 18-23 hr, 03 hr and 05 hr, were coincided with lower points in the sucrase activity (Fig. 3C).

The enzyme (sucrase) and the substrate (sucrose)
relationship continued in more or less similar fashion in the gut lumen, with + or - 1 hr, during the free running time of the respective rhythms. For example, under LD, the peaks in sucrose levels at 07 hr, 10 hr, 13 hr, 17 hr, 20-21 hr and at 02 hr were accompanied by troughs in sucrase activity at 08 hr, 11 hr, 13 hr, 18 hr, 22-23 hr and at 02-03 hr. Conversely, the peaks in enzyme activity at 07 hr, 10 hr, 13 hr, 16 hr, 22 hr, 01-04 hr and 06 hr were accompanied by troughs in its substrate levels at 08 hr, 11-12 hr, 14 hr, 16 hr, 23-01 hr, 03-04 hr and 06 hr respectively. Similarly under LL, the peaks in sucrose levels at 07 hr, 10 hr, 13 hr, 21 hr, 23 hr and 02 hr were accompanied by troughs in enzyme activity at 08 hr, 11 hr, 15-16 hr, 22 hr, 00 hr and 02-03 hr respectively. Under the same light condition, the peaks in sucrose activity at 07 hr, 09 hr, 14 hr, 17 hr, 21 hr, 23 hr, 01 hr, 04 hr and 06 hr were accompanied by troughs in its substrate levels at 08 hr, 09 hr, 15-16 hr, 17 hr, 22 hr, 00-01 hr, 04 hr and 06 hr respectively (Fig 4B). Likewise, under DD, the peaks in sucrase levels at 07-08 hr, 10 hr, 13 hr, 16-17 hr, 20-21 hr, 02 hr, 04-06 hr were accompanied by troughs in sucrose levels at 08-09 hr, 10-11 hr, 15-16 hr, 18 hr, 22-23 hr and at 05 hr respectively. At the same time, the peaks in sucrase levels at 07 hr, 14 hr, 17 hr, 19 hr, 03-04 hr and 06 hr were accompanied by troughs in its substrate (sucrose) levels at 09 hr, 19 hr, 22-01 hr, 03 hr and 06 hr respectively.

In-depth analysis of free running time of sucrose and sucrase rhythms showed a time dependent and light-sensitive rhythmic changes in their levels, much like carbohydrate and amylase rhythms reported in our previous investigations (Bhuvaneswari and Sivaprasad, 2012 b). Such changes were more pronounced in the cellular compartment (gut wall) of the gut than in its luminal compartment (gut lumen). Interestingly in the present study, higher sucrase activity levels and lower sucrose levels were observed during the photic phase, while lower sucrase levels and higher sucrose levels were recorded during scotic phase of the day (Fig 3. ABC). For instance, the sucrose rhythm maintained higher MPVs (20.6 mg) throughout its free running time in total darkness and lower MPVs (19.4 mg) under continuous light condition. Further, higher sucrase activity levels and lower sucrose levels were observed at 06-17 hr under LD, 06-15 hr under LL and 01-06 hr under DD. Similarly, lower sucrase activity levels and higher sucrose levels were observed at 19-06 hr under LD, 16-06 hr under LL and 18-22 hr under DD. Obviously, in B. mori dark condition stimulates sucrase synthesis and its accumulation in gut wall cells, while the light condition enhances its breakdown and mobilization of its products as observed in some other insects (Kostal and Shimada, 2001; Saunders, 2002; Syrova et al, 2003).

In the gut content, on the other hand, the sucrose and sucrase rhythms are governed by the sucrose content present in the dietary mulberry leaf, which in turn reinforces its own levels by simulating release of sucrose from the gut wall cells (Kanekatsu et al., 1992; Sumida and Ueda, 2007; Manjula et al., 2010). Thus, the sucrase levels in the gut content represent those derived two sources; mulberry leaf and gut wall. The sucrase activity in the gut lumen is modulated in a light- and dose-dependent fashion like that of amylase activity (Bhuvaneswari and Sivaprasad, 2012 b). For example, its activity continued throughout the day under LD condition, due to the availability at least 12 hr light and adequate sucrose from the diet (Fig. 4A). In the presence of continuous light (LL) the sucrase activity was further boosted up, with the result the mean sucrose levels slightly declined during the free running time of its rhythm (Fig 4B). Contrary to this, the sucrase activity was retarded under continuous dark, resulting in the accumulation of undigested sucrose in the gut lumen, which manifested in the form of higher MPVs in its rhythm (Fig. 4 C). The present study demonstrates that the circadian sucrose and sucrase rhythms of the digestive system in B. mori are modulated by internal and external factors; internally by the concentrations of sucrose and sucrases themselves and externally by the photoperiod.

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