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Research Article

Photoperiodic Induction of Diapause Character and Sorbitol Level in the Silkworm Eggs of APM1, MU303 and PM Strains/Races

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ABSTRACT

Embryonic diapause is the suspended animation responsible for the onset of Voltinism in the silkworm. The embryo is completely arrested during the developmental event to overcome unfavourable environmental conditions. This further results in the synthesis of sorbitol from glucose and examines the differences between sorbitol levels in the diapause-induced silkworm eggs of APM1, MU303 and PM strains by exposure to photoperiodic regime of 16L:08D, 14L:10D and 12L:12D. The results obtained revealed the fact that the sorbitol level is high during the 6th day in all the selected strains at their 7th and 8th generation. This indicates that the enhancement of the sorbitol is independent of the induced diapause in all three strains of the mulberry silkworm. Further, it is also clear that the enhancement of sorbitol concentration is devoid of a photoperiodic regime.

Keywords: *Sorbitol, Silkworm Eggs, Photoperiodism, Diapause character.*

INTRODUCTION

Embryonic diapause in insects is often considered one of the promising substantial alternative developmental pathways that aid in overcoming unfavourable environmental conditions (Rudramuni *et al*., 2021). In silkworm *Bombyx mori* L, the diapause induces the alteration in the Voltinism, resulting in the onset of univoltine, bivoltine and multivoltine generation (Rudramuni *et al.*, 2021). The commencement of embryonic diapause in silkworms is an overwintering mechanism (Yamashita & Hasegawa, 1985; Yamashita & Yaginuma, 1991; Xu *et al*., 1995a, b; & Shiomi *et al.*, 2015) where the embryonic development completely ceases (Horike & Sonobe, 1999; & Sasibhushan *et al.*, 2013). There are a series of physiological and biochemical alteration that takes place in the silkworm eggs, which is essential in the survivability of the embryo. The most important change is the reduction of glucose to form sorbitol.

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Sorbitol is alcohol synthesized from glycogen (Chino, 1957) catalyzed by glycogen phosphorylase-a during the retardation of embryogenesis in response to the onset of embryonic diapause. Glycogen phosphorylase-a is a key enzyme in this process which is observed to be abundant in diapausing eggs but absent in non-diapausing eggs. Recent studies revealed the capacity of sorbitol to arrest embryonic development in the silkworm (Horie *et al*., 2000) but do not affect the development of embryos from diapause eggs (Iwata *et al.*, 2005). When newly laid non-diapause eggs are exposed to low temperatures, sorbitol accumulates, and the extent of accumulation is closely dependent on exposure temperatures. In this case, glycogen is also used as an initial substrate for the formation of sorbitol. Thus, in non-diapause eggs, sorbitol production is a biochemical adaptation against low-temperature stress (Furasawa & Shikata, 1982).

These facts make sorbitol an ideal metabolic entity to assess the embryonic diapausing state in the silkworm (Yamashita et al., 1981) under normal conditions. Consequently, in the present study, we have estimated the concentration of sorbitol in the embryonic diapause-induced eggs derived from three different silkworm races viz. PM, MU303 and APM1. The purpose of the present study was to establish a comparative account of the impact of induced diapause in terms of sorbitol level in these three strains of silkworms.

MATERIALS AND METHODS

Collection of silkworm eggs:

The present study was conducted in three different races of silkworms *viz.*APM1, MU303 and PM. A total of 3 Disease Free Layings of APM1 were bought from APSSRDI, Kirikere, Hindupur, Andhra Pradesh. The Disease Free Layings of MU303 were collected from the germplasm of the DOS in Sericulture University of Mysore, Mysuru and the Pure Mysore was collected from CSR&TI, cold storage, and Mysuru.

Induction of embryonic diapause by photoperiodic regime:

The combination of three photoperiodic regimes *viz.* 16L: 08D, 14L: 10D and 12L: 12D were chosen for the present investigation. The photoperiodic combinations are imposed in the BOD incubator in three replicates and maintained as a control, respectively.

Estimation of sorbitol concentration:

Two vials containing 1 ml of an aliquot of embryonic stages of silkworm eggs acidified with 0.1 ml of 10 N sulphuric acid and 0.5 ml of 0.1 M periodic acid and, after five minutes, 0.5 ml of 1 M sodium arsenite were added. After 10 minutes, 3 ml of distilled water was added. To this solution, 0.5 ml was transferred into a test tube, and 5 ml of chronotropic acid reagent, and the solution was shaken. The test tubes were kept in a boiling water bath for 30 minutes at 110^0C and cooled. The optical density of the colour was measured at 570 nm against blank. The sorbitol content will be represented as mg of sorbitol per gram of wet weight of the tissue.

RESULTS AND DISCUSSION

A magnitude of change in the sorbitol has been reported in all three strains/races of silkworms on the 7^{th} and 8^{th} generation. Generally, in the 7^{th} generation of the eggs of APM1, the highest concentration of sorbitol in the eggs was reported on the 6^{th} day of the 8^{th} generation when exposed to 16L: 08D, 14L: 10D and 12L: 12, respectively (Fig 1). However, the sorbitol content was higher in the eggs treated with 16L: 08D compared to the rest of the photoperiodic regime and control (Fig 1a). In contrast, in the 7^{th} generation of MU303, the sorbitol level was observed to be more on the 5^{th} day of the eggs treated with 16L:08 D (Fig 2a), followed by the 6^{th} day of eggs treated with 14L:10D and 12L: 12D respectively (Fig2b and 2c). Further, the highest sorbitol level in the 7^{th} generation in PM race was reported on the 6^{th} day of eggs treated with 16L: 08D, 14L: 10D and 12L: 12D (Fig 3). However, the highest sorbitol content was in the eggs treated with 16L:08D

compared to the other two photoperiodic regimes (Fig 3a).

Similarly, the sorbitol level was observed to be more significant on 6th-day eggs of 8th-generation APM1 when treated with 16L:08D, 14L:10D and 12L:12D (Fig 4), with the highest in 16L:08D compared to that of the other two photoperiodic regimes (Fig 4a). In addition, the eggs of MU303 on the 8th generation treated with 16L: 08D were shown the highest sorbitol content on the 6th day (Fig 5a). The other two photoperiodic regimes also showed the same result (Fig 5). Finally, the sorbitol level in the eggs on the 8th generation of PM was more on the 6th day treated with 16L: 08D, 14L:10D and 12L:12D (Fig 6). Further, the highest sorbitol level was observed in the silkworm eggs treated with 16L:08D in comparison with the rest two photoperiodic regimes (Figure 6a).

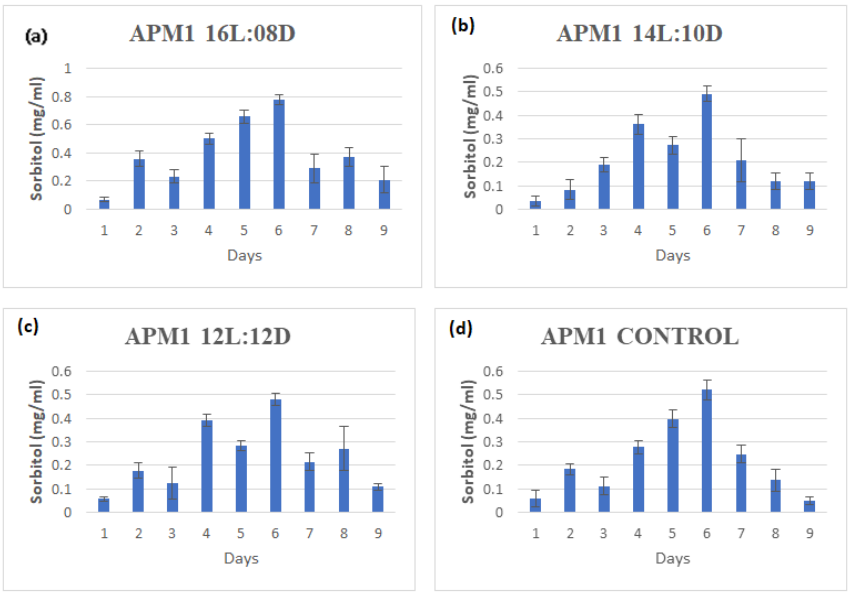

Fig 1: The Induced embryonic diapause in the sorbitol level of 7th generation of APM1 at a photoperiodic regime of (a) 16L: 08D, (b) 14L: 10D and (c) 12L: 12D with control

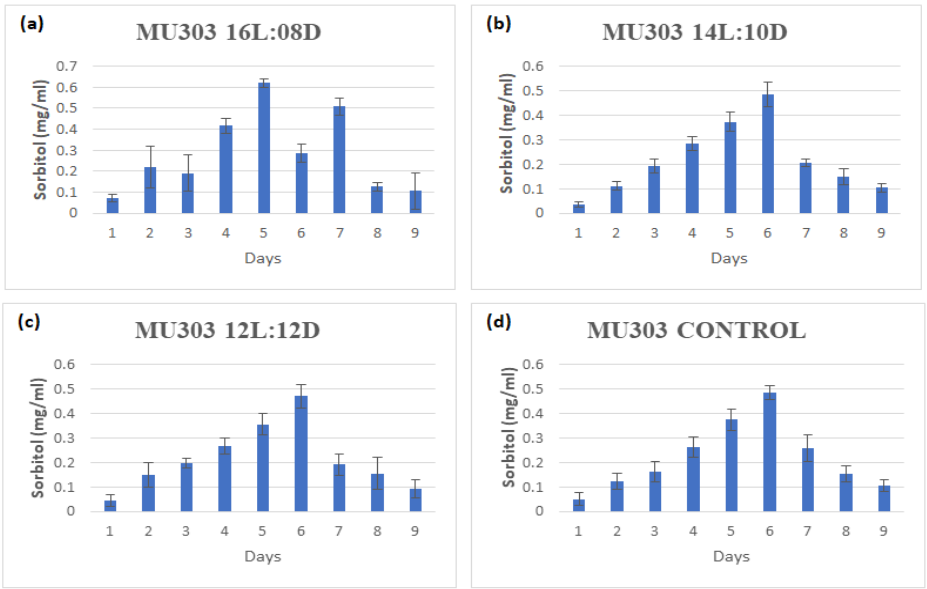

Fig 2: TheInduced embryonic diapause in the sorbitol level of 7th generation of MU303 at a photoperiodic regime of (a) 16L: 08D, (b) 14L: 10D and (c) 12L: 12D with control

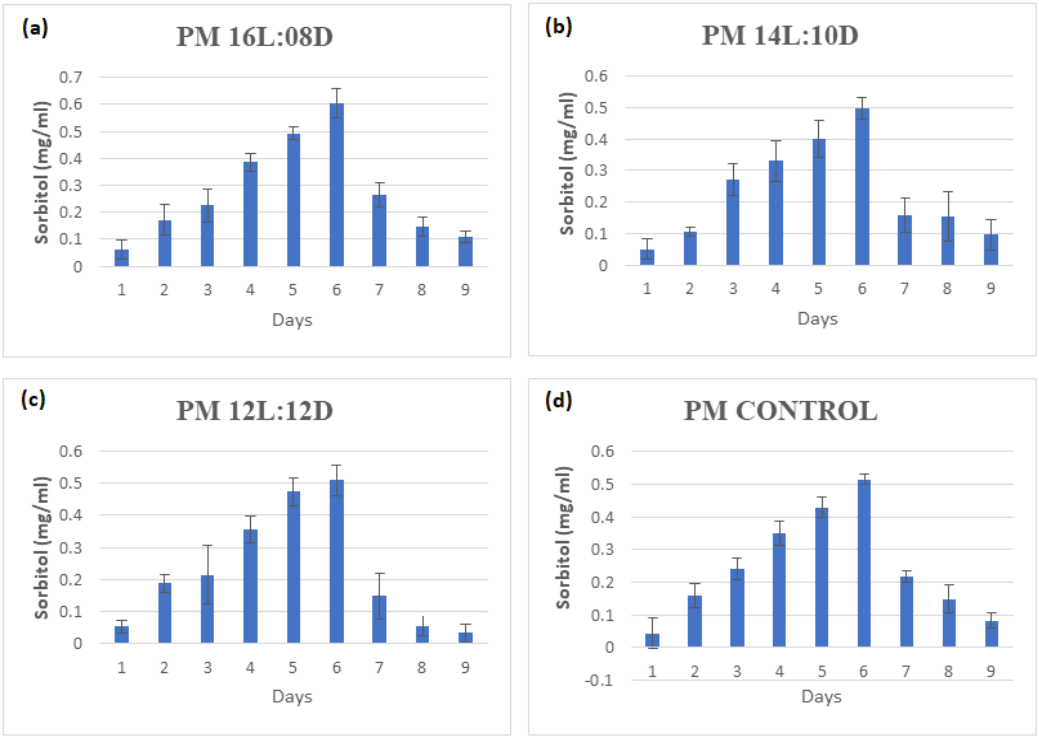

Fig 3: The Induced embryonic diapause in the sorbitol level of 7th generation of PM at a photoperiodic regime of (a) 16L: 08D, (b) 14L: 10D and (c) 12L: 12D with control

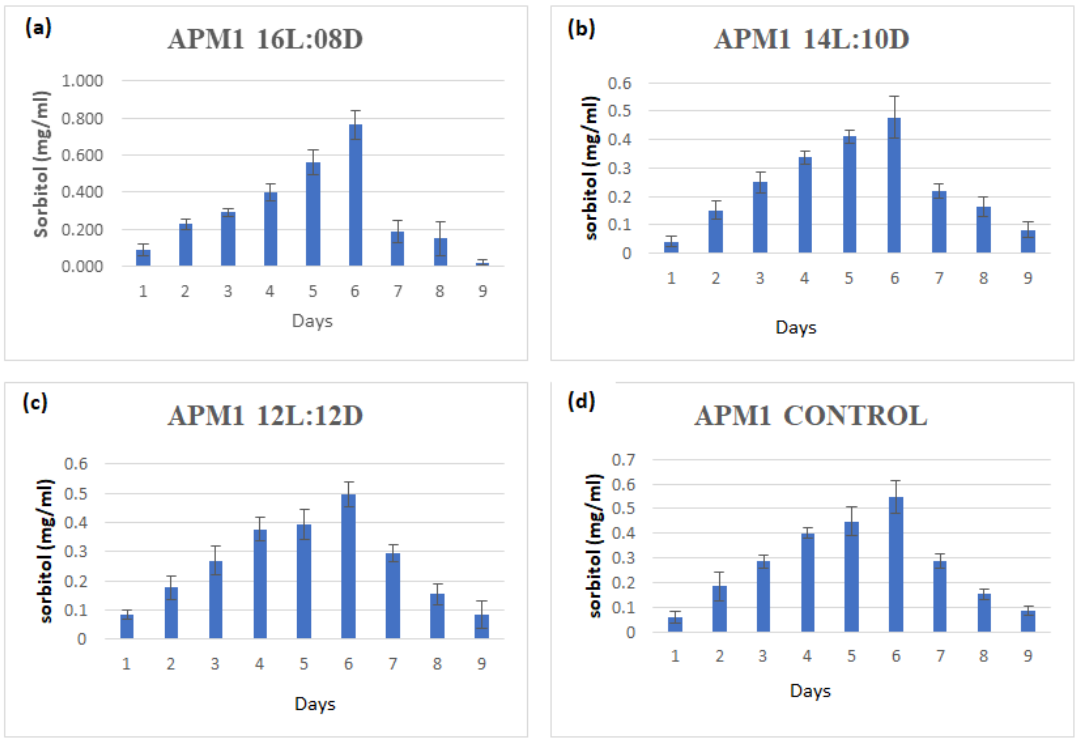

Fig 4: The Induced embryonic diapause in the sorbitol level of 8th generation of APM1 at a photoperiodic regime of (a) 16L: 08D, (b) 14L: 10D and (c) 12L: 12D with control

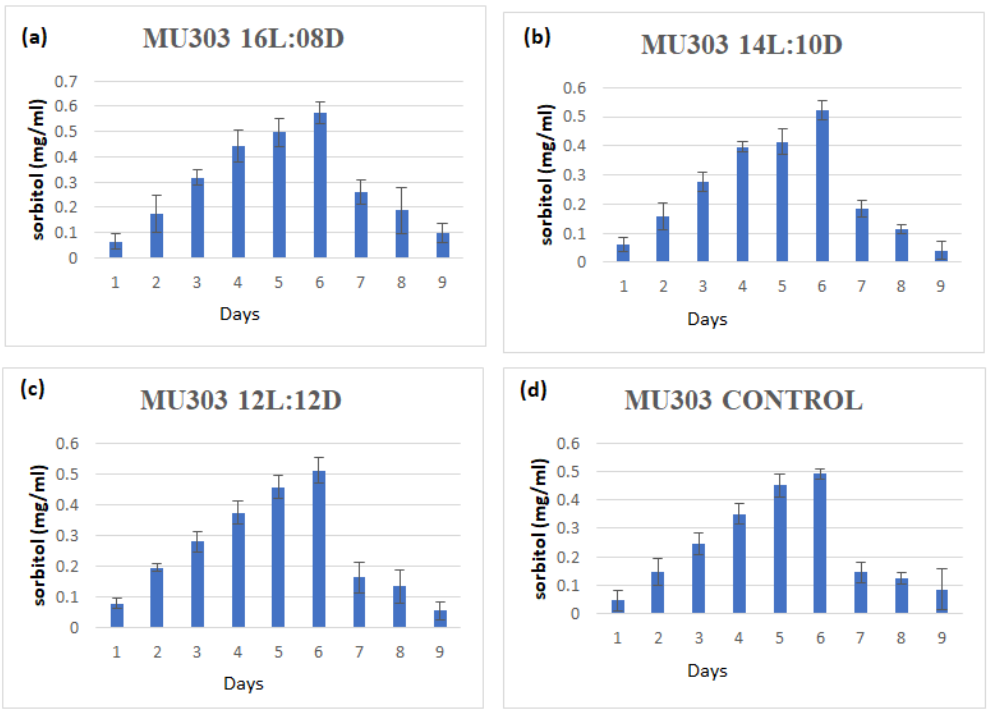

Fig 5: The Induced embryonic diapause in the sorbitol level of 8th generation of MU303 at a photoperiodic regime of (a) 16L: 08D, (b) 14L: 10D and (c) 12L: 12D with control

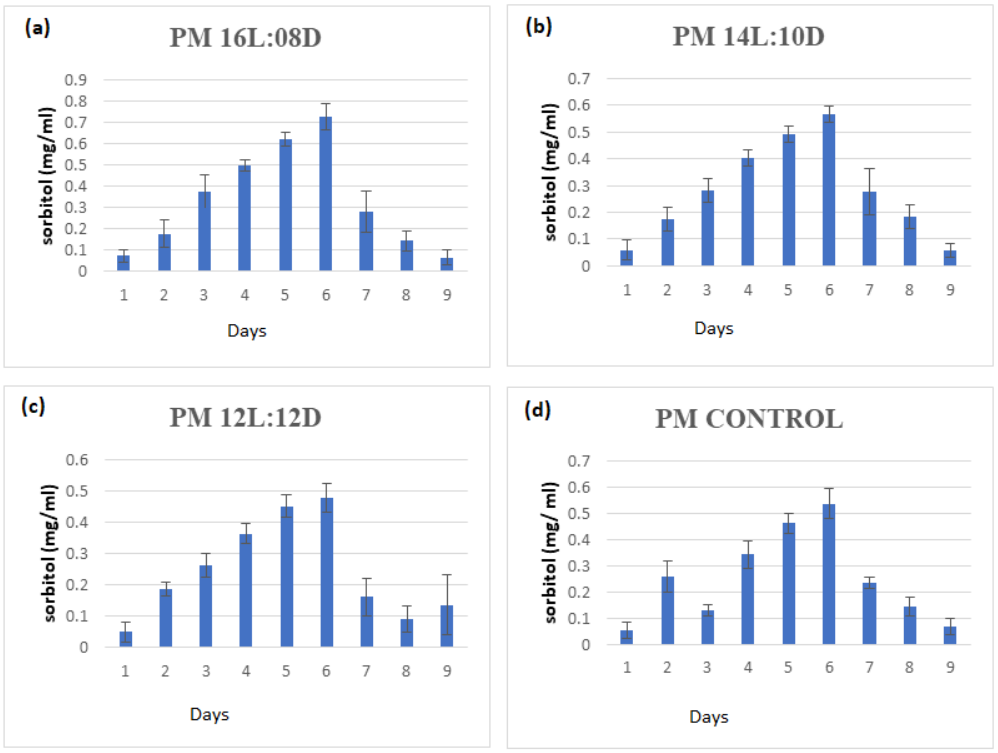

Fig 6: The Induced embryonic diapause in the sorbitol level of 8th generation of PM at a photoperiodic regime of (a) 16L: 08D, (b) 14L: 10D and (c) 12L: 12D with control.

CONCLUSION

Overall, our results revealed the enhancement of sorbitol levels on the 6th day of eggs in their 7th and 8th generation. However, the eggs of MU303 treated with 16L: 08D at their 7th generation is an exception to this statement. In addition, the overall highest sorbitol level was in the 8th generation of PM eggs treated with 16L: 08D representing the maximum effect of induced embryonic diapause in this race of

silkworms. Altogether, it is confirmed from the present study that the induced embryonic diapause is independent of the photoperiodic regime as well as the races/strains of the silkworm, and the sorbitol level is maximum at the 6th day only in all diapause eggs.

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Conflict of Interest: There is no conflict of interest between authors.

Author's Contribution: The research was performed with teamwork.

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