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Influence of temperature on growth and development of bivoltine silkworm breeds and hybrids: A comprehensive review

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Abstract

Silkworms, being poikilothermic organisms, are highly sensitive to temperature fluctuations, which greatly influence their physiological processes and overall development. Seasonal temperature variations significantly impact the phenotypic expression of genotypic traits, affecting critical crop parameters such as cocoon weight, shell weight and the cocoon shell ratio. Temperature is a key factor that directly regulates silkworm growth, development and silk yield. While optimal temperature promotes healthy development, exposure to extreme temperature leads to stunted growth, increased disease susceptibility and reduced cocoon quality. This review delves into the effects of temperature on silkworm biology, including growth and development, reproductive potential, nutritional indices and post-cocoon characteristics. In addition, it also explores the evolution and performance of thermotolerant bivoltine silkworm breeds and hybrids, highlighting their adaptability to varying thermal conditions. This review provides valuable insights into the thermotolerance mechanisms of silkworms, offering guidance for improving sericulture practices in regions with fluctuating temperatures. It also proposes future strategies to optimize silkworm rearing practices in response to climate variability, ensuring sustainable silk production.

Keywords: *Bombyx mori*, bivoltine silkworm, thermotolerant breeds, high temperature, post-cocoon parameters, nutritional indices, reproductive parameters

Introduction

Sericulture is the practice of rearing silkworms for silk production and it plays a crucial role in the rural economies of several countries, including India, where it supports the livelihoods of many farmers. India leads the world in silk consumption and ranks second in raw silk production after China. Notably, India is the only country that produces all four of the world's economically important silks *viz.*, mulberry, Tasar, muga and ERI. The success of the sericulture industry depends on several factors, among which environmental conditions, particularly abiotic factors like temperature, play a critical role in the growth and productivity of silkworms (Benchamin and Jolly, 1986) [1]. As a tropical country, India primarily relies on multivoltine × bivoltine hybrids for silk production. However, their raw silk often falls short of international standards. Bivoltine hybrids, known for superior silk quality and productivity due to their heavier cocoon and shell weight, are pivotal to enhance India's global competitiveness in silk production (Datta & Pershad, 2002) [2]. Yet, challenges such as temperature fluctuations, poor leaf quality and inadequate summer management hinder the year-round rearing of high-yielding bivoltine hybrids in tropical climates. The domesticated mulberry silkworm (*Bombyx mori* L.) thrives under controlled environmental conditions, with an optimal temperature range of 23–28 °C for its growth and development. Deviations from this range induce physiological stress, reducing survival rates, cocoon yield and silk quality.

The increasing impact of climate change, particularly rising global temperatures, has intensified the vulnerability of silkworms to thermal stress, posing significant challenges to sericulture worldwide. These challenges, compounded by climate change, underscore the need to focus on the heritability of silk traits and develop thermotolerant breeds resilient to such stress. Ensuring stable cocoon production in high-temperature regions is crucial for the successful adoption of bivoltine races, improving silk quality and ensuring the sustainability of sericulture. To address these challenges, breeding programs have developed thermotolerant bivoltine silkworm breeds and hybrids with genetic adaptations that enable them to thrive in higher temperatures, ensuring stable productivity even under heat stress. Understanding the relationship between temperature and silkworm biology is critical for optimizing rearing practices and improving silk yield, particularly in tropical and subtropical regions. Research indicates that late-age silkworms prefer relatively lower temperature compared to chawki worms. Moreover, temperature fluctuations during larval development stages are more favourable for growth and development than constant temperature (Suresh Kumar & Harjeet Singh, 2011) [3]. Therefore, identifying the optimal temperature range, along with other critical parameters, is essential to ensure silkworms that can withstand adverse climatic conditions throughout their developmental stages.

This review aims to provide a comprehensive understanding of how temperature influences silkworm growth, development, reproductive performance, nutritional indices and post-cocoon parameters. It also highlights the evolution and performance of thermotolerant bivoltine silkworm breeds and hybrids while exploring future prospects for sericulture in the context of rising global temperatures and the challenges of sustaining productivity.

Role of Temperature on Growth and Development of Silkworm

Silkworms, as poikilothermic organisms, are particularly sensitive to temperature fluctuations, which affect vital physiological processes such as silk gland function, water balance, respiration and nutrient absorption (Sangeetha *et al.*, 2017) [4]. When exposed to temperature stress, protein synthesis is disrupted, leading to cellular abnormalities and potential cytotoxicity due to unfolded proteins (Feder *et al.*, 1996) [5]. Optimal temperature requirements vary across developmental stages. Early instar larvae show higher resilience to elevated temperatures, which promotes survival and cocoon quality (Sekarappa *et al.*, 2009) [6]. In contrast, higher temperatures during the late larval stages accelerate growth but shorten the larval period, while lower temperatures slow growth and extend the lifespan (Benchamin *et al.*, 1983; Tazima & Ohuma, 1995) [7, 8]. The role of temperature in silkworm rearing is well-documented. Research by Pandey & Tripathi (2006) [9] found that increasing temperature from 24 °C to 36 °C shortened the larval duration, affecting the later stages. Datta *et al.* (2001) [10] and Rahmathulla *et al.* (2012) [11] emphasized that the optimal temperature for silkworm growth and productivity lies between 22 °C and 28 °C, where cocoons of the highest quality are produced. Similarly, Venugopal Reddy *et al.* (2015) [12] demonstrated that larvae and silk glands thrive at 25 °C to 26 °C with 80–85% relative humidity. The optimum temperature required for rearing silkworms of different developmental stages of silkworm is shown in table 1.

Table 1: Optimum temperature requirements of silkworm during various developmental stages

Oviposition	Incubation	I instar	II instar	III instar	IV instar	V instar	Mounting & Spinning	Cocoon preservation	Male moth preservation
25±1 °C	25±1 °C	27-28 °C	26-27 °C	25-26 °C	22-25 °C	22-25 °C	24-26 °C	23-26 °C	7 °C

The acclimatization of silkworms to seasonal temperature variations has been studied extensively (Dingley & Maynard Smith, 1968) [13]. Several researchers have explored how temperature impacts key rearing parameters (Tanaka, 1973; Vijaya Kumari *et al.*, 2001; Suresh Kumar *et al.*, 2001; Pal *et al.*, 2014; Siddiqui *et al.*, 2005) [14, 10, 15, 17]. In response to heat stress, bivoltine breeds, known for superior silk quality, exhibit compensatory mechanisms such as the upregulation of heat shock proteins (HSPs), which protect cells from protein denaturation (Lindquist, 1986) [19]. Studies have confirmed that *B. mori* increases HSP production under heat stress, boosting its thermotolerance (Keshan *et al.*, 2014; Li *et al.*, 2012; Zhao and Jones, 2012; Huang *et al.*, 2007; Manjunatha *et al.*, 2010; Sosalegowda *et al.*, 2010; Rahmathulla *et al.*, 2011;, Tanjung *et al.* 2017) [25, 23, 24, 27]. Extreme temperature, however, can negatively affect gene expression and overall silkworm growth, with high temperature downregulating immune-related genes and increasing vulnerability to pathogens (Gua *et al.*, 2018).

Temperature stress can disrupt metabolic processes, hormonal balance and physiology, resulting in reduced productivity (Mukherjee *et al.*, 1995) [30]. As sericulture expanded into tropical and subtropical regions, breeding programs focused on developing thermotolerant silkworm strains to cope with these conditions. Research has shown that resistance to high temperature is heritable (Suresh Kumar *et al.*, 1999) [31], with thermotolerant hybrids demonstrating better pupation rates and resilience to heat stress. For instance, breeds like CSR2 exhibit high catalase activity and thermotolerance at temperature between 35 °C and 40 °C, making them ideal for hot climates (Pezhman & Kumar, 2011) [32]. Lakshmi *et al.* (2011) [33] and Rahmathulla (2011) [27] also reported the economic benefits of such hybrids, which perform better in heat-prone areas. High temperature exceeding 30 °C negatively impact cocoon traits and increase mortality, especially in non-feeding stages (Mathur *et al.*, 2004; Hussain *et al.*, 2011) [34, 35]. Devi and Karuna (2012) [36] further confirmed that temperature above this threshold cause cellular damage in silkworms. Recent research by Sun *et al.* (2022) [37] found that elevated temperatures disturb gut microbial balance, digestive enzyme activity and nutrient absorption, underscoring the importance of maintaining optimal conditions to ensure silkworm health and productivity.

Role of Temperature on Reproductive Potential of Silkmoth

Temperature plays a pivotal role in the reproductive performance of silkworms, affecting egg production, fecundity and overall reproductive success. As exothermic organisms, silkworm development follows a sigmoidal pattern with temperature, showing a near-linear relationship within an optimal range of 25–28 °C (Rahmathulla, 2012; Saha *et al.*, 2013) [11]. Deviations from this range negatively impact reproduction, leading to reduced egg production and fertility issues (Takten, 1973) [38]. Continuous exposure to high temperature has been shown to cause sterility and lower oviposition rates (Biram *et al.*, 2009; Verma *et al.*, 2011; Dinesh and Balkhande, 2013; Keshan *et al.*, 2014) [39, 40, 20, 88]. Figure 1 shows the effect of different temperatures on oviposition.

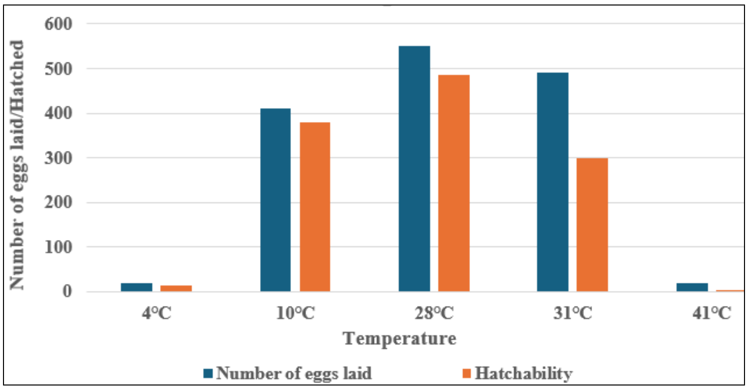

Fig 1: Egg laying capacity of *Bombyx mori L.* At different temperature for 4 hours mating duration (Source: Kayode, 2022) [21].

High temperature also affect the reproductive organs, damaging male accessory glands and female reproductive tracts, which impairs sperm viability and reduces hormone production critical for oviposition (Ouyang & Wu, 1993; Katsuki & Miyatake, 2009) [42, 43]. Thermotolerant breeds often show reduced reproductive performance, a trade-off commonly observed in species like *Bicyclus anynana* and *Helicoverpa armigera* (Mironidis & Savopoulou-Soultani, 2010; Janowitz & Fischer, 2011) [4, 45]. Dahi *et al.* (2016) [46] found that different temperatures (20 °C, 23 °C and 26 °C) significantly affect silkworm egg yield, with eggs being particularly sensitive to high temperature. Incubation above 25 °C accelerates embryonic development but leads to embryo death due to improper yolk absorption (Vemananda Reddy *et al*., 2003) [47]. Environmental conditions during embryonic development influence the diapause characteristics of eggs. Temperature requirement during different types of acid treatment to break the diapause is given in table 2.

Table 2: Temperature requirement during different types of acid treatment

Cold acid treatment	Hot acid treatment	Short term chilling and treatment	Long term chilling and treatment
25 °C	46 °C	47 °C	48 °C

Influence of Temperature on Nutritional Indices

Temperature significantly affects the nutritional and physiological aspects of silkworms, particularly in food consumption efficiency and gene expression. The adaptability of insects, including silkworms, to environmental conditions can be better understood by studying their food consumption and utilization (Benchamin & Jolly, 1986) [1]. These effects vary with the genetic makeup of the silkworms, influencing how nutrients are utilized and impacting gene regulation mechanisms (Ogunbanwo & Okanlawon, 2009; Ramesha *et al*., 2010) [48, 49]. Scriber and Slarisky (1981) [50] noted that fluctuating temperatures could sometimes enhance growth more than constant conditions, though high temperatures tend to degrade mulberry leaf quality, critical for silkworm nutrition. Temperature increases from 20 °C to 30 °C reduce the efficiency of leaf-to-silk conversion, affecting food intake and silk production (Rahmathulla *et al*., 2004) [51]. Cooler temperatures, especially during the late larval stages, have been found to improve mulberry leaf consumption and silk conversion efficiency (Shen, 1986) [52]. Research by Narayana *et al*. (1985) [53] revealed that optimal temperatures between 23 °C and 25 °C improved key nutritional indices like food intake and digestibility, enhancing physiological performance. Similarly, Dahi *et al*. (2016) [46] showed that food utilization efficiency, including the conversion of ingested and digested food into cocoon mass, varied with temperature. As temperature rises, indices like consumption index (CI), approximate digestibility (AD) and growth rate (GR) increase due to enhanced physiological activity (Basavaraju *et al*., 1998) [54]. However, higher temperatures can accelerate gut passage and reduce conversion efficiency, leading to faster but less efficient growth (Muniraju *et al*., 2004) [55]. These findings emphasize the complex interaction between temperature and silkworm physiology, where rising temperature can boost certain nutritional processes but compromise others, such as conversion efficiency.

Influence of Temperature on Spinning and Post-Cocoon Parameters

As ectothermic organisms, silkworms are highly sensitive to external temperatures during cocoon spinning, which directly influences silk quality. Higher temperature increase the spinning rate, while lower temperature can inhibit spinning altogether (Sehnal *et al*., 1990) [56]. At elevated temperature, silkworms spin longer cocoons, which help dissipate heat through a greater surface area to volume ratio, maintaining suitable conditions for metamorphosis (Horrocks *et al*., 2013) [57]. Faster spinning at higher temperature also results in stiffer and thinner fibres, as confirmed by SEM analysis, where fibers spun at 35 °C were found to be 25% thinner than those at 15 °C (Offord *et al*., 2016) [58]. These thinner fibers enhance stiffness but may reduce flexibility, impacting the mechanical properties of the silk (Mortimer *et al*., 2015) [59].

Post-cocoon parameters, such as cocoon size and quality, are crucial for reeling efficiency. For silk reelers, cocoon parameters are especially important as they impact the quality, quantity and efficiency of the reeling process (Rahmathulla, 2012) [11]. Irregular cocoons result in thread breakage and inconsistent silk filament quality, affecting production (Takabayashi, 1997) [60]. Studies have shown significant variations in cocoon morphology between hybrids, leading to differences in filament size and quality (Nakada, 1993; Rahmathulla *et al*. 2004; Gowda & Reddy, 2007; Manisankar *et al*., 2008) [51, 62]. Maintaining moisture content below 20% in the cocoon layer improves reelability (Akahane & Subouchi, 1994) and optimal post-cocoon conditions are achieved at 18–28 °C, where silkworms produce better quality silk (Ram *et al*., 2016) [65]. Silk fibroin remains stable up to 100 °C, but prolonged exposure causes slight discoloration, and decomposition accelerates above 130 °C, with the silk disintegrating at 170 °C (Sericulture manual, 1972). Temperature also affects cocoon shape and size, impacting reeling efficiency. At temperatures above 22–25 °C, cocoons become loose and wrinkled, complicating reeling, while lower temperatures produce larger, denser cocoons, with extended spinning durations.

Evolution of Thermotolerant Bivoltine Silkworm Breeds/Hybrids

Significant progress has been made in developing thermotolerant bivoltine silkworm breeds and hybrids to withstand temperature stress. Hybridization of multivoltine and bivoltine strains, combined with selective breeding for traits such as antioxidant enzyme activity, heat shock protein (HSP) expression and enhanced cellular stress responses, has improved the survival, productivity and adaptability of these breeds under high temperature. In tropical regions, where silkworm rearing faces fluctuating seasonal conditions and high summer temperatures, thermotolerant breeds are essential. Understanding the genetic basis and variability of qualitative and quantitative traits under heat stress is crucial for selecting thermotolerant parental lines for breeding (Kumar *et al*., 2001).

Key developments include CSR18 and CSR19, evolved by exposing the B20 × BCS12 hybrid to 36 °C and 85% relative humidity (Suresh Kumar *et al*., 2002) [69] and ND5 × CSR17, developed under similar conditions (Raghavendra Rao *et al*., 2005) [69]. Other thermotolerant hybrids include SR1 × SR4, SR2 × SR5 and Chamaraja (CSR50 × CSR51), all bred under high-temperature conditions (Sudhakara Rao *et al*., 2006; Dandin *et al*., 2006) [71, 78]. Suresh Kumar *et al*. (2006) [73] also developed CSR46 and CSR47 using CSR17 × CSR18 and CSR4 × CSR19, employing repeated backcrossing up to the F5 generation and alternating rearing temperatures in the F6 generation. Further development include, SD7 × SD12, a thermotolerant hybrid, was created from parental breeds SD7 and SD12, known for producing white, oval, and dumbbell-shaped cocoons (Rao *et al*., 2005) [73]. Kumari *et al*. (2011) [75] identified thermotolerant bivoltine strains BD2-S, SOFBR and BO2, which exhibited significant genetic differences under thermal stress. Breeds TT2, TT3, TT6 and TT7 showed increased survival, indicating successful introgression of thermotolerance traits from parent strains CSR27, SK3, CSR16 and CSR26. SSR markers were used to identify donor parents (Moorthy *et al*., 2011) [76]. More recently, double hybrid WB-DH1 (WB1.3 × WB7.5) was developed through molecular interventions using SSR markers, demonstrating its suitability for rearing in Eastern and Northeastern India across both favourable and unfavourable seasons (Chandrakanth *et al*., 2021) [77].

Performance of thermotolerant bivoltine silkworm breeds/hybrids

The performance of thermotolerant bivoltine silkworm breeds and hybrids is crucial for successful rearing under fluctuating environmental conditions. Several studies have demonstrated their adaptability and superior traits compared to non-thermotolerant breeds. Vijaya Kumari *et al*. (2001) [15] found that CSR2 × CSR5 performed better at 25 °C, with the highest ERR and shell ratio. Sudhakara Rao *et al*. (2001) [78] reported that A105 × J2 and B × NB4D2 outperformed controls in fecundity, cocoon yield, filament length and raw silk percentage. Similarly, CSR18 × CSR19 showed better adaptability at 36 °C, with a pupation rate of 88.25% and shell ratio of 20.80% (Suresh Kumar *et al*., 2002) [69]. Naseema Begum *et al*. (2003) [80] found that hybrid G × CSR12 performed well at 31 °C, with high evaluation index values for cocoon weight and filament length. Hybrids ND5 × CSR17 and SD7 × SD12 also demonstrated improved survival and cocoon traits under high temperature (Raghavendra Rao *et al*., 2005; Rao *et al*., 2005) [69]. Lakshmi & Chandrashekaraiah (2006) [74] identified highly thermotolerant breeds such as APS19, APS31, APS6 and APS24, with survival rates between 89% and 93%.

The cocoon colour sex-limited hybrid Nandi (CSR8 × CSR2) also showed higher pupation rate under high temperature compared to CSR2 (Suresh Kumar *et al*., 2007). Thermotolerant hybrids SR1 × SR4 and SR2 × SR5 exhibited superior survival and cocoon quality in high-temperature conditions (Sudhakara Rao *et al*., 2007) [78]. Ramesh Babu *et al*. (2007) [83] identified potential thermotolerant lines such as APS5, APS9, APS11 and APS31. Gowda and Reddy (2007) [62] evaluated CSR2 × CSR4, FC1 × FC2 and CSR18 × CSR19 under varying temperatures, showing that high temperature (30 °C) negatively impacted key traits like cocoon weight and filament length. Hussain *et al*. (2011) [35] found that Pak-4 was the best-performing inbred breed under thermal stress. Hybrids like HTO5 × HTP5 and P5 × NB18 also showed economic merit under high temperature (Lakshmi *et al*., 2011; Gangwar, 2011) [33]. Ramesh *et al*. (2012) [85] identified HL1 and HL7 as top performers under high-temperature conditions. Chandrakanth *et al*. (2015) [86] identified SK4C and BHR3 as potential thermotolerant breeds. Verma *et al*. (2017) [88] confirmed CSR18 × CSR19 as a robust hybrid with high heterosis and tolerance to heat stress. Chandrakanth *et al*. (2018) [88] identified HTH3 × HTH6 and HTH4 × HTH9 as tolerant hybrids, based on minimal reductions in pupation rate. Using marker-assisted selection (MAS), Sivaprasad *et al*. (2018a) developed the hybrid TT21 × TT56 for summer rearing, yielding 75 kg/100 dfls with a 21.67% shell ratio at the farmer level.

Recent studies on thermotolerant bivoltine silkworm breeds have focused on their potential tolerance to muscardine disease. These investigations assessed the performance of various breeds under fungal stress, exploring their dual tolerance to both high-temperature and muscardine disease. Keerthana *et al*. (2019a, 2019b) [90-91] inoculated ten thermotolerant breeds (B1-B8, APS12, APS45) and control CSR2 with different dilutions of *Beauveria bassiana*. B4 performed best at 10^{-3} and 10^{-4} spore dilutions, showing superior cocoon yield, ERR and filament traits, while B1 excelled in cocoon and filament traits, and B8 in shell ratio and filament length. Sreejith (2019) further evaluated these breeds under high-temperature and fungal stress. B2 and B4 performed best for larval and cocoon traits, with B1, APS12 and APS45 showing better survival under both stressors. Jayashree *et al*. (2020) [93] tested single-cross hybrids, identifying B1 × B8 and B1 × B4 as superior for cocoon yield, shell weight and filament length, demonstrating resilience under both stresses. Sahana *et al*. (2021) [95] confirmed that B1 and B4 consistently performed well under high-temperature and fungal conditions, making them promising for dual stress tolerance.

Chandrakala *et al*. (2022) [95] found that crosses involving B1, B4 and B8 with CSR4 produced superior progeny for cocoon traits across six generations. Manjunatha *et al*. (2023) [96] identified B1 and B4 as the most tolerant, with B1 showing longer larval duration, higher fifth instar weight and improved cocoon yield, while B4 recorded the highest ERR and shell ratio. Recent studies by Sruthi *et al*. (2024) and Manjunatha *et al*. (2024) [95] have also investigated the enzyme response in these thermotolerant breeds. Thrilekha *et al*. (2024a) [99] evaluated new bivoltine silkworm foundation crosses under high-temperature and B. bassiana inoculation. B1 × B2 and B1 × B4 outperformed commercial crosses, showing potential for breeding thermotolerant and muscardine-resistant lines. In a follow-up study, Thrilekha *et al*. (2024b) [100] developed seven double hybrids from these crosses. Two hybrids, (B1 × B2) × FC1 and

(B1 × B4) × FC1, outperformed FC2 × FC1 in economic key traits, showing enhanced dual tolerance to both high temperature and muscardine disease, making them promising for stress-resilient hybrids.

Future Strategies

India, the second-largest silk producer globally, faces challenges in tropical regions due to fluctuating temperatures. While thermotolerant silkworms introduced during summer have improved productivity, they have yet to match the yields of traditional bivoltine hybrids, highlighting the need for high-yielding thermotolerant races. Continuous breeding programs are focusing on developing genotypes that can withstand environmental stress, with an emphasis on advanced genetic transformation techniques, including the development of transgenic silkworms with enhanced commercial traits. To address research gaps, further study is required on the long-term adaptability of silkworms to climate change. Genomic tools like CRISPR and transcriptomics offer promising avenues for identifying key thermotolerance genes. Integrating molecular and conventional breeding approaches will improve heat resilience and disease tolerance in new hybrids (Zhao *et al*., 2012) [23]. Thermotolerant silkworms are crucial for stabilizing silk yield and quality under climate stress, which could enhance economic viability, expand cultivation areas and improve farmer income in regions like India.

Conclusion

This review emphasizes the critical role of temperature on the growth, development and physiology of bivoltine silkworms. High temperature can cause metabolic stress, reduce silk production and increase susceptibility to diseases. However, advances in breeding have led to the development of thermotolerant strains that demonstrate improved survival, silk yield and metabolic efficiency under heat stress. As climate change increasingly affects agriculture, thermotolerant breeds are essential for sustaining sericulture, particularly in regions where it is integral to farmer livelihoods. Continued research and breeding efforts utilizing both traditional and molecular techniques are vital for strengthening thermotolerance in bivoltine silkworms. Proactive investment in research and its practical application can help the sericulture industry adapt to the challenges posed by climate change, securing its future in hotter, more unpredictable environments.

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