1. INTRODUCTION
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In recent years, the processes of heat shock responses and the role of heat shock proteins (HSPs) have not been confined merely to "molecular chaperons" (Parsell and Lindquist, 1991), but spread over to determine their ecological and evolutionary role in the post genomic era (Sorensen and Loeschcke, 2007). It is well known that both prokaryotic and eukaryotic cells respond to unfavourable environmental conditions by increased synthesis of stress proteins such as HSPs. It is a universal phenomenon that most of the HSPs have conserved sequences from bacteria to human, but certain features of the response do vary from organism to organism (Craig, 1985). Unlike humans, plants and insects have a narrow range of tolerance to elevated temperatures and hence struggle to cope with these conditions. Consequently, the organisms that adapt over a period of time thrive whilst the others become extinct.

The cellular stress response was described in Drosophila melanogaster for the first time by Ritossa (1962) and the term "heat shock protein" was introduced by Tissieres et al., (1974) as these proteins increased in synthesis due to sudden increase in temperature. HSPs that are induced by different classes of stress viz., heat stress, cold stress, anoxia, heavy metals, UV laser irradiation and other stresses are identified based on their molecular mass ranges from 19 to 110 kDa in size and are broadly classified as large HSPs (major HSPs) and small HSPs (SmHSPs). The large HSPs are involved in major physiological processes such as cell division, transcription, protein folding, transport, membrane functions (Alique et al., 1994; Chen et al., 1996) and cytoprotective functions (Bakau and Horwich, 1988; Chirico et al., 1988; Deshaies et al., 1988; Mizzen and Welch, 1988; Palleros et al., 1991; Garrido
et al., 2001; Kregel, 2002). They can also form as large oligomeric complexes (Bentley et al., 1992; Leroux et al., 1997; Haslbeck et al., 1999), playing important roles in thermotolerance in mammalian cells (Landry et al., 1989), Drosophila (Landry and Huot, 1995), house fly (Tiwari et al., 1997), Lucilia cuprina (Tiwari et al., 1995), but not in yeast cells (Nicholl and Quainlan, 1994). SmHSPs bind specifically to cytoskeletal elements such as actin and to intermediate filaments such as desmin, vimentin and glial fibrillary acidic protein (Bennardine et al., 1992; Nicholl and Quainlan, 1994). It has also been reported that SmHSPs modulate apoptosis (Arrigo, 1998; 2005), involved in cell growth and differentiation (Mehlen et al., 1997) and play an important role in molecular mechanisms of adaptation to adverse environmental conditions (Landry et al., 1989). Recent approaches in genome wide identification of HSF (Heat shock factor) - targeting genes provide novel insights into the complex metabolic reprogramming that occurs in cells in response to stress (Hahn et al., 2004).

With the advances in proteomic and genomic research in combination with bioinformatic tools and techniques, concerted interest has been growing among scientists to determine the role of HSPs in the whole organism adaptation to heat. Even a survey on lizard species inhabiting a variety of environments, including highlands, forests, and deserts, demonstrated a remarkable diversity of constitutive HSP70 levels that were correlated with the lizard's environmental conditions (Ulmasov et al., 1992). Recently, it is revealed that the thermotolerance of an organism requires several alternative molecular mechanisms, such that HSP40, sHSPs and other still unidentified factors play an important role in this process along with HSP70 (Shilova et al., 2006).
Even to date, although some informations are available, applicable data obviously insufficient to envisage the biological importance of HSPs in silkworm. To understand the complex phenomena governing silkworm thermotolerance integrative genomic, proteomic and biotechnological approaches are required that will help to underpin the gaps in knowledge in this area which have not been documented to date in any of other organisms, including Drosophila. For example, although B. mori is derived from a wild progenitor Bombyx mandarina (Arunkumar et al., 2006), it has lost its temperature-tolerance due to continuous domestication over 5000 years. As a result of such a long period of domestication silkworm races/strains diverged and the strains (polyvoltines) grown in tropical environments became resistant to high temperatures and diseases, while the strains reared in temperate conditions (bivoltines) remained susceptible.

Current research points to the fact that the loss of tolerance to environmental insults in B. mori- unlike B. mandarina- is due to prolonged domestication, which offers opportunities for systematic reinvestigation of this phenomenon while substantial diversity remains among various silkworm strains/races. Concerted efforts have been made during the past two decades resulting in the evolution of heat-tolerant silkworm strains, following conventional breeding strategies. This process has been successful, to some degree, in the tropical environment of the Indian subcontinent. Among several breeds developed, the only bivoltine silkworm breed that performed better all through the year over three decades in the field was NB4D2, unlike other temperate breeds that were season-dependent. Many qualitatively and quantitatively superior productive/robust (thermotolerant) breeds were developed using Japanese commercial hybrids (temperate origin)
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as genetic resource material. Due to their low tolerance to the fluctuating environmental conditions in tropical climate they become unsuitable for growing year round (Nazia et al., 2005). Thus, the efforts made in the previous three decades were futile and the spread and success of silkworm rearing was mainly due to the introduction of F1 hybrids of native multivoltine as female parent (for resistance) and bivoltine as male parent (for high quality silk). Even now, it is a challenging task to develop not only stress- and disease-resistant strains, but also provide high yielding silkworm strains with improved stress tolerance.

1.1 Heat shock response and thermotolerance

The terms 'heat shock', 'acclimation' and 'hardening' are commonly used to describe the changes in an organism's living state caused by external environmental conditions and treatments (Bowler, 2005; Loeschcke and Sorensen, 2005; Lagerspetz, 2006). The thermal tolerance of economically important organisms, like silkworms, to environmental fluctuations attains significance in field-rearing conditions as performance in field/nature mainly depends on native adaptability to varied environmental conditions, which is governed by molecular mechanisms of the cell. Notably, the polyvoltine silkworm strains exhibit better survivability over bivoltine strains, which might be due to their adaptation to thermal stress. For example, a polyvoltine strain C. nichi proved to be more tolerant than the bivoltine strain NB4D2 (Joy and Gopinathan, 1995). Interestingly, in India, among bivoltines, NB4D2 exhibited better tolerance to environmental fluctuation both in laboratory and field conditions, compared to other newer bivoltine hybrids (CSR2, CSR4, NP2, KSO1, etc., Vasudha et al., 2006) - an observation also confirmed by the recent study (Firdose and
Reddy, 2009). However, the Chinese race Feng was the most tolerant strain followed by Japanese races Kuo and J-09, while another Chinese race C-54 was most susceptible (Hsieh et al., 1995). Since, the range and significance of individual adaptive reactions differ in various species under different environmental conditions, the level of tolerance in elevated temperature varies between polyvoltine and bivoltine strains/races of B. mori. This diversity could be due to the fact that the races (species) living in hot and desert conditions for many thousand years altered the molecular-biological mechanisms of adaptation, which facilitated their normal life-cycle even under extreme conditions (Evgen’ev et al., 2005).

Furthermore, Vasudha et al., (2006) demonstrated for the first time that the heat shock response in five bivoltine breeds (NB4D2, NP2, KSO1, CSR2, and CSR4) varied during different developmental stages. Of the five instars, young age silkworms that includes first, second and third instars, were relatively sensitive to any given heat shock temperatures while late age silkworms tolerated high temperatures for relatively longer periods of time. Similar results were reported by Joy and Gopinathan (1995) and thermostolerance increases as larval development proceeded sequentially in the order of first instar > second instar > third instar > fourth instar > fifth instar (Vasudha et al., 2006). Comparatively, another lepidopteran model species, Manduca sexta, exhibited 100% survival at 42°C but mortality increased as the heat shock temperature was raised to 48°C in the fifth instar (Fittingoff and Riddiford, 1990). In the case of pure mysore, a tropical multivoltine strain of B. mori in India, no mortality occurred at 42°C (for one hour) and 100% mortality was noticed at 46°C (Lohmann and Riddiford, 1992). Interestingly, a few Japanese and Chinese silkworm strains also
exhibited 100% mortality at 46°C after one-hour heat shock induction (Hsieh et al., 1995). In comparison with other insects, the threshold temperature that induce 100% mortality was 40°C in *D. melanogaster* (Lindquist, 1986), 45°C in different strains of silkworm, viz., NB4D2, NP2, KSO1, CSR2, and CSR4 (Vasudha et al., 2006), 46°C in Chinese, Japanese (Hsieh et al., 1995) and Indian silkworm strains of *B. mori* (pure mysore) (Lohmann and Riddiford, 1992; Joy and Gopinathan, 1995), 46°C in *Musca domestica* (Tiwari et al., 1997), 48°C in *Manduca Sexta* (Fittingoff and Riddiford, 1990), 48°C in *Lucilia cuprina* (Tiwari et al., 1995), and 50°C in *Locusta migrotoria* (Qin et al., 2003).

However, comparison of the resultant robust bivoltine hybrids (CSR18, CSR19, HT1 etc.,) subjected to thermal treatment revealed more tolerance to high temperature treatments than productive breeds, affecting not only the survivability but also other cocoon traits of the insect (Suresh et al., 1999). Unfortunately, the performance of the thermotolerant bivoltine breeds under fluctuated environment was very poor in the field. Thus, the question regarding the role of stress responses in thermal adaptation in nature still remains unanswered in *B. mori* as well as other organisms with different geographical origins. Additionally, some related questions, which were asked 10 years ago are still valid and remain unresolved. Perhaps, cross-disciplinary approaches integrating proteomic, genomic, evolutionary, biological, and physiological methods might help to address these questions.
1.2 Proteomic approach

1.2.1 Molecular analysis

The cataloging of proteins from single-celled microorganism to multi-celled organism has progressed over the past 15 years at rapid pace in parallel with advances in protein and peptide separation, detection and identification. In recent years, gathering information about a proteome from any biological component or system employed both experimental observation ('wet lab' procedure) and data analysis ('dry lab' procedure). Integration of various electrophoresis techniques with high-resolution two dimensional electrophoresis (2-DE), liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Aebersold and Mann, 2003) followed by mass spectrometry (MS) resulted in exponential generation of data. As a consequence copious amount of data generated and stored in various types of databases that are accessible for comparative analysis at http://us.expasy.org/ch2d/. The proteomic data are stored in public repositories for re-use by other scientists and/or combined with other data sets to inform system biologists. Moreover, while 2-DE is one of the most important technologies for separating complex protein mixture, peptide mass fingerprints (PMF) of metrix assisted laser disorption and ionization time of flight mass spectrometry (MALDI-TOF-MS) has typically been employed for the identification of relatively abundant proteins in 2D gel spots and success depends on obtaining good sequence coverage from a reasonable number of peptides matched to each protein are a number of bioinformatics tools available for the analysis of MALDI-TOF-MS data (Palagi et al., 2006). Further, peptide mass spectrometry has become a powerful tool to discover and validate protein-coding genes, while
annotation of these genes is a key goal of genome sequencing projects. The advances in proteomic tools and methods facilitated identification and analysis of HSPs and other proteins not only in \textit{B. mori} but also in other organisms.

The expression, regulation, localization, and functions of HSPs have been studied extensively in different organisms. The kinetics of HSP synthesis revealed distinct and reproducible differences between cell cultures of \textit{B. mori} and the gypsy moth \textit{Lymantria dispar}. While mulberry silkworm cells synthesize all HSP classes at temperature reaching 48°C, the gypsy moth cells synthesize no proteins at a 40°C and above, and die under extreme conditions (Evgen'ev \textit{et al.}, 1987). In view of this, Evgen'ev \textit{et al.}, (1987) proposed to investigate whether high thermo-resistance was inherent only in the cultured cells or if cells also behave in a similar way \textit{in vivo}.

The differential expression of heat shock proteins in newly evolved bivoltine strains, NP2, KSO1, CSR2, and CSR4, was compared with that of the NB4D2 strain, which exhibited acclimation in the field over three decades (Vasudha \textit{et al.}, 2006). Interestingly, expression of only one set of HSPs with a molecular mass of 90 kDa in first, second and third instars, and an 84 kDa HSP in the fourth instar was confirmed by Vasudha \textit{et al.}, (2006). Surprisingly, five different sets of 84, 62, 60, 47, and 33 kDa HSPs were also observed in the fifth instar larvae of NB4D2, KSO1, and CSR2 strains. Where as in other two bivoltine strains, expression of three HSPs (84, 47, and 33 kDa) in the NP2 and only two HSPs (84 and 47 kDa) in the CSR4 strains was reported (at 35 and 40°C for two hours, Vasudha \textit{et al.}, 2006). In a multivoltine silkworm strain, pure mysore, 84, 70, 31, 30, and 29 kDa HSPs at 42°C (one hour, Lohmann and Riddiford, 1992) and 83, 80, 74, 70, 68, 65, and 23 kDa at
48°C were found expressed in cells and organs (for one hour, Evgen'ev et al., 1987). Between two multivoltines, 93, 46, and 28 kDa HSPs from pure mysore and 93, 70, 46, and 28 kDa HSPs from C. nichi were reported (Joy and Gopinathan, 1995). This clearly indicated that different sets of HSPs were being expressed at various heat shock temperatures, in different breeds of B. mori of which 90 and 84 kDa HSPs were ubiquitous. In addition, expression of HSPs in different tissues varied depending on the stage of development or the temperature, and also at which stage exposure was performed (Joy and Gopinathan, 1995). Notably, concentration of HSPs and their distribution to specific sub-cellular sites is an important factor in acquisition of thermotolerance (Kampinga, 1993).

Most of these studies were carried out following single dimensional electrophoresis (1-DE) and blotting techniques that made it possible to interrogate underlying mechanisms with greater certainty, albeit higher resolution of the proteins could not be achieved. To resolve these constraints in B. mori, advanced proteomic tools and techniques were employed, which paved the way for understanding differentiation and identification of different HSPs in the whole body of B. mori. A small number of protein spots were excised from the sample and separated by 2-DE. After analysis of the resultant mass peptide fingerprints with search engine Protein prospector, they were identified as the protein HSP70 (Rajesh et al., 2008). In addition, a comparative analysis of silk gland proteins in 2-DE gels of heat shock induced and normal silkworm larvae of NB4D2 revealed discrete differences with new and over expressed protein spots (Rajesh et al., 2009). Thus, application of advanced proteome techniques proved to be a promising
approach in identification of different HSPs and opened new avenues to uncover more HSPs in *B. mori*.

### 1.2.2 Purification of proteins

All living organisms confronted with environmental insults elicit a common and seemingly highly conserved stress response. The stress response appears in the form of expression of either particular or similar set of proteins with definite or cumulative functions. This represents an emerging paradigm for the coordinated multi-step regulations of apoptotic signaling events to provide protection from and to facilitate cellular recovery after exposure to damaging stimuli (Beere and Green, 2001; Beere, 2004). Several HSPs including major and smaller HSPs are known to be expressed in the biological system; the up regulation of HSP90 (2-10 fold) in malignant cells (Elah *et al.*, 2007) and neuronal cells (Paul, 2000) and its down regulation during insect diapause have been well documented (Joseph *et al.*, 2007). Despite, HSPs have number of important natural functions in all the living organisms including human are involved as regulator in protein synthesis and protein folding. In addition, an important function lies in the regulation of gene function during cell growth and death. Hence, in early 1990s, it attracted the scientific community across the world to use as a potential drug target.

Continued investigations on HSPs and their applications as promising anti-tumor vaccine highlighted the need of simple and effective method for their purification. Basically, protein purification involves series of processes intended to isolate a single type of protein from a complex mixture and considered as fundamental tool for characterization, structure and function determination of a protein of
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interest. Towards this, methods followed for purification of HSPs (HSP70 and HSP96) from cells and tissues are based on affinity chromatography using ADP (ATP) - sepharose and Concavalin A (Con A) - sepharose (Welch and Feramisco, 1985; Meng et al., 2002). The affinity matrix purification considerably complicates purification of HSP90 that includes multistage processes with several chromatographic procedures. Immuno-affinity purification was also used (Denis, 1988; Isii et al., 1999; Menoret and Bell, 2000). Recently, thiophilic interaction chromatography was used for the first time to purify 8 major HSPs from cells and tissues of mammals (Yuri et al., 2009).

HSP preparations derived from any cell type contain the peptides repertoire of that particular cell type. This has immunological significance, as the vaccination of mice and rats with HSP peptide complexes leads to powerful antigenic specific, CD8+ cellular response against the peptides chaperoned by the HSPs, but not against the HSP itself (Udono and Srivastava, 1993; Udono et al., 1994). HSP based vaccines have been shown to immunize against cancer and infectious diseases in both prophylactic and therapeutic sense. So far gp96, HSP90, and HSP70 have been used successfully and a separate purification methods are now readily available. However, the gross availability of stress proteins has been posing a problem as they are often isolated from porcine brain (Chu et al., 1997) and tumor samples (Antoine and Gillian, 2000). The large impediment for the biomedical use of these proteins due to possible presence of BSE in brain is low and high cost for its isolation. Thus, HSPs are being produced from microorganisms and cancer cells but their production methods are too expensive and low yield with poor activity. This limitation offers an alternative model
organism for isolation and purification of pharmaceutically important HSPs for example HSP90.

Keeping this gap and importance of HSPs in biomedical research in view any attempt that could facilitate purification of HSP90 from either B. mori or insects has scientific relevance.

1.2.3 Interaction of proteins and proteases - Zymographic analysis

The structural and functional properties of proteins, which are significant in cell function and protection, are governed by different classes of proteases and analyzed employing a simple and sensitive technique "Zymography". Normally, many proteins are synthesized as inactive precursors known as zymogens and are subsequently converted to physiologically active forms by selective enzymatic cleavage of peptide bonds called zymogen activation (Neurath and Walsh, 1976). As SDS activates the zymogen into active protease, various proteases like matrix metalo proteases (MMPs) expressed during cancer development were identified and characterized (Tanriverdi et al., 2009; Lokeshwar et al., 1993). Plasminogen activators constitute a group of specific serine proteases that convert the plasma zymogen and plasminogen into proteolytic enzyme and plasmin respectively. These activators are normally present in blood, tissue, urine and other body fluids (Astrup, 1978) and acts as important regulators of local proteolytic activity. Plasminogen activators have been implicated in the process of malignant transformation, trophoblast implantation, tumor invasion, mammalian ovulation and mammary gland involution. Mammalian plasminogen activators are principally characterized and distinguished according to their size and immunological cross-reactivity. The molecular weight of plasminogen
activators has been determined by gel filtration and SDS-PAGE (Patrick et al., 1983). Thus, electrophoretic technique employed for analysis of plasminogen activators has become potential tool for detection of multiple bands of enzymatic activity in plasminogen-containing and plasminogen-free gels.

Interestingly, insect (lepidoptera) larval mid gut hosts a complex proteolytic environment of various proteases like trypsin, chymotrypsin, elastases, cathepsin-B like proteases, aminopeptidases and carboxypeptidases, which are responsible for protein digestion, killing bacteria and viruses (Nakazawa et al., 2004). In B. mori, serine proteases are known to dominate the larval midgut environment and contribute about 95% of the total digestive activity. In addition, two types of membrane bound alkaline proteases (a caseinolytic, chymotrypsin-like protease and a BAPNA-lytic, trypsin-like protease) were detected, which are synthesized in the epithelial cells of the midgut and transported into the digestive fluid into the gut lumen. The molecular weights of these proteases are 32K (Ca-p) and 42K (Ba-p) upon synthesis and then changed to 27K and 26K in the digestive fluid (Suzuki et al., 1991). Interestingly, silkworm egg also posses some proteases that are involved in vitelline and yolk degradation during embryo development (Ikeda et al., 1990; Maki & Yamashita, 2000). Since most of these studies confined to tissue specific, interaction between protein and proteases in the whole organism that complete its larval life cycle in natural environmental conditions remain enigmatic.

1.3 Genomic approach

The HSP family consists of ubiquitous proteins, which are phylogenetically conserved from bacteria to mammals including plants
(Craig, 1985). Although, expression of HSPs has been reported from different silkworm strains only a few have been characterized in B. mori. Recently, Landais et al., (2001) characterized a cDNA encoding a 90 kDa HSP in B. mori and compared with Spodoptera frugiperda (both lepidopteran insects). These two cDNAs encode 716 aa (amino acid) and 717 aa proteins in B. mori and S. frugiperda, respective, with calculated molecular mass of 83 kDa, which is similar to Drosophila. Unlike in vertebrates, hsp90 does not contain introns and is a unique gene both in B. mori and S. frugiperda genomes. Comparison of aa sequences of B. mori and S. frugiperda with that of D. melanogaster, Homo sapiens, and S. cerevisiae revealed a high percentage of similarity and phylogenetic relationships (for details see Landais et al., 2001). Apparently, extensive study is required to determine their expression at different developmental stages of different silkworm strains as the HSP90 expression is found rather in early instars than late instars (Vasudha et al., 2006) and expression of some hsp genes changes during development (Craig, 1985). In D. melanogaster, hsc70-4 (constitutive hsp gene family) was expressed at a high level in embryos, larvae, and adults, whereas, the hsc70-1 and hsc70-2 expression was highest in adults but not detected in larvae. The hsc70-1 was expressed at a low level while no expression of hsc70-2 was observed in the embryo. In Chironomus tentans, hsc70 expression was evident at all developmental stages but slightly lower in the embryo than older stages (Karouna-Renier et al., 2003).

Small heat shock proteins (SmHSPs or sHSPs) belong to a family of genes that are seemingly less conserved compared with those of major hsp gene families, but occur ubiquitously in a variety of organisms. These proteins are involved in apoptosis as well as
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Protection against heat stress (Arrigo, 2005; Feder and Hofmann, 1999). In B. mori (strain p50), six genes encoding sHSP19.9, sHSP20.1, sHSP20.4, sHSP20.8, sHSP21.4, and sHSP23.7 were reported (Sakano et al., 2006) although their biological and commercial roles remain unknown. The deduced amino acid residues of these sHSPs are quite similar to each other. CLUSTALW multiple alignments indicated 82, 80, and 80% identity between Pia25 and sHSP20.8, sHSP20.8 and sHSP20.4, sHSP20.4 and sHSP19.9, respectively. Besides the α-crystallin domain, the N-terminal XXLXDQXFG motifs are commonly conserved in the sequences of these HSPs (Sakano et al., 2006). Further, reverse transcriptase-polymerase chain reaction (RT-PCR) analysis showed no difference in expression levels of SmHSP genes in different organs (Sakano et al., 2006), but indicated an increased amount of transcripts following heat shock in B. mori strains p50 (Sakano et al., 2006), Nistari and NB4D2 (Velu et al., 2008), which was found to be strain specific.

1.4 Role of HSPs in acquired thermotolerance

In most insects, the integument consisting of cuticle and underlying epidermis is the initial defence organ and serves as an important barrier between itself and the environment. It protects silkworm from physical injury, evaporation of water from the body and the invasion of pathogens. Response of the integument to heat shock will therefore be an integral part of survival for multivoltine (tropical), univoltine, and bivoltine (temperate) silkworms in the field and in other wild insect species, which are geographically distinct. Hence, a new strategy was adopted wherein the whole egg (Manjunatha et al., 2005) and larvae (Vasudha et al., 2006) of B. mori were subjected to heat shock at various temperatures during different developmental stages, to determine the importance of HSPs in acquired thermotolerance. These
studies revealed that different sets of HSPs expressed in different developmental stages have a profound influence not only on the performance of larvae (rated in terms of mortality) but also to complete life cycle in the natural environmental condition. The well-defined role of HSPs (expressed either individually or collectively) in acquired thermotolerance in the silkworm and other insects is not known. In order to derive more accurate and novel hypotheses, the expression of heat shock proteins should be correlated with currently available information on the tolerance of silkworm strains reared in tropical environments.

1.5 Role of HSPs in relation to commercial traits

To date, the greatest emphasis has been given to HSP70 and HSP90 as molecular chaperons that help organisms to cope with stresses of internal and external nature. Recent approaches not only revealed the importance of HSP90 in normal growth and development of eukaryotes, and parasite (*Plasmodium falciparum*) growth in human erythrocytes (Banumathy *et al.*, 2003), but also elucidated the relationship between HSPs and life history traits focusing on the ecological and evolutionary relevance (Sorensen *et al.*, 2003; Sorensen and Loeschcke, 2007). Concomitantly, the relationship between heat shock, HSPs expression, and commercial traits was studied in great detail in the case of *B. mori* (Vasudha *et al.*, 2006). Notably, an increased cocoon weight of 17.52 vs. 13.48%, and increase in shell weight of 19.44 vs. 13.45% in NB4D2 over its control was observed following heat shock at 35 and 40°C respectively. Concurrently, CSR2 also exhibited a 13.11 vs. 6.44% increase in cocoon weight and 16.26 vs. 5.03% increase in shell weight at 35 and 40°C heat shock over their respective controls. The increased cocoon and shell weight observed in heat shock induced
bivoltine silkworm strains against control would be due to expression of HSPs at larval stage. While Joy and Gopinathan (1995) did not observe any heat shock effects on commercial traits, Lohmann and Riddiford (1992) reported that of the nine animals heat shocked at 44°C for 1 hr, only five resumed feeding, while three spun cocoons. Commercial traits of these insects were not evaluated and compared with that of controls. Consequently, as an effective strategy, heat shocked larvae (whole organism) were allowed to grow under natural environmental conditions and they spun better quality cocoons than the non heat shocked larvae reared in natural environmental conditions (Vasudha et al., 2006).

These investigations highlighted the fact that knowledge obtained from model organisms under “comfortable” laboratory conditions does not always reflect what happens out in the field, where conditions are continuously changing and unpredictably hostile. Interestingly, the increased cocoon weight and shell weight over control, reflects the positive correlation between heat shock responses and silk protein content in the cocoon. Abramova et al., (1991) reported suppression of fibroin synthesis in the silk gland following heat shock, but recently, Zhang et al., (2006) identified HSP90, HSP70, and HSP60 in the silk glands of B. mori, offering the opportunity for further systematic investigation in different breeds of silkworm. None of the larvae recovered from heat shock at 45°C (Vasudha et al., 2006) and 46°C (Lohmann and Riddiford, 1992), were able to spin cocoons. However, the observed differences between cocoon weight, shell weight and shell ratio among various silkworm strains, would require further investigations to determine species-specific responses to heat shock. Altogether, these observations clearly indicate that mild heat shock
between 35 and 40°C for two hours facilitates bivoltine silkworm larvae to respond and overcome the fluctuating natural environmental conditions in succeeding instars. The practical application of this phenomenon will need to be explored positively and systematically (using multivoltine and bivoltine silkworm strains) in laboratory and field conditions in order to achieve stabilized sericulture farming in tropical countries like India.

An increasing body of experimental evidences from *B. mori* would be useful for comparative genomic and proteomic research among lepidopterans and other organisms. The genome wide analysis of *hsp* genes (Hahn *et al.*, 2004) and their regulatory factors provide novel insights into the complex metabolic reprogramming that occurs within cells in response to stress, particularly among lepidopterans. The domesticated silkworm *B. mori*, together with its wild progenitor, *Bombyx mandarina*, and non-mulberry silkworms (Tasar - *Antheraea mylitta*; Muga - *A. assamensis*; Eri - *Samia cynthia ricini*), which are reared in nature, open ample scope to investigate the ecological and evolutionary modification of HSPs and pinpoint the candidate gene(s). The individual or collective role of HSPs in relation to biological, commercial, physiological, and immunological features among different silkworm races/breeds/strains (including non-mulberry silkworms) is important for understanding the factors that govern thermotolerance and acclimation in insects. Knowledge of HSPs and their use as molecular markers would facilitate conventional breeders to select better parents, with a reduction in laborious crosses for development of suitable silkworm strains, important for tropical countries under silkworm race improvement programmes.
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Even to date, although some information’s are available, but it is very scarce and not substantiate to envisage the biological importance of HSPs in silkworm. Hence, the present investigation was undertaken with the following objectives in order to explore the possibility of using heat shock proteins as molecular markers in conventional-molecular breeding for improvement of silkworm strains for tropics.

Objectives

1. To screen the heat shock proteins expressed during different instars in polyvoltine and bivoltine silkworm strains of *B. mori*,
2. Identification, confirmation and characterization of HSPs expressed following proteomic tools and techniques,
3. Isolation and purification of major heat shock protein by chromatographic techniques and