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Analysis of total protein to identify thermo tolerant strains- as a biochemical tool under heat stress condition in silkworm, *Bombyx mori* L

Savarapu Sugnana Kumari[1*], Veeragoni Dileep Kumar[2], Sunil Misra[3]
[1-3] Applied Biology Division, Indian Institute of Chemical Technology, Tarnaka, Hyderabad, Telangana, India

Abstract
The study reveals effect of high temperature on the total protein content level in the haemolymph, fat body and cuticle of fifth instar silkworm larvae and its effect on commercial traits among few silkworm races. 7 bivoltine and 12 multivoltine silkworm races were exposed to high temperature of 40^0 C - 45 ^{0}C and quantitative analysis of total protein profile carried out. Variation in the temperature significantly influenced the protein content in the heamolymph, fat body and cuticle of fifth instar larvae. Total protein content of heamolymph increased with increase in the temperature. The impact of heat shock on commercial traits of cocoons analyzed for acquired thermo tolerance level over the control. The data obtained was recorded for each trail in a one way ANOVA and was subjected with the Graph pad prism software analysis for its significance. Results revealed that, significantly high protein content recorded in bivoltine compared to multivoltine. Further, KNT, AP-White, GFP-C, GLPF of multivoltine strains and BO_2, BD_2S, BO1S and ISK of bivoltine strains exhibited better survivability over the other strains. Finally, all the obtained data reveals the varying response of a tolerance and non-tolerance among the silkworm strains in total protein analysis in correlation with economical traits.

Keywords: tolerance, silkworm, high temperature, bivoltine, multivoltine, haemolymph, fat body, cuticle, survivability

1. Introduction
Abiotic stress response has become an indispensable field of research in recent years due to ongoing climate change in order to understand its effect on the health and behaviour of animals (King and MacRae, 2015) [13]. Insects in particular are known to have a pervasive effect of temperature, which varies with the different life stages because they inhabit different micro habitats and may differ in tolerance to high temperature. Among beneficial insects, the mulberry silkworm, *Bombyx mori* L. has actually deprived an opportunity to acquire tolerance to hot environmental conditions due to intensive and careful domestication over more than 4000 years (Punyavathi *et al.*, 2017) [20]. Long term care and selective breeding targeting only at enhancement of quantitative traits (with reference to silk production) has deprived the animal of many of its attributes for self-defense against predators, pathogens and extreme climatic conditions. The success of any new silkworm strain/races in the field depends on its inbuilt adaptability to varied environmental conditions. Undoubtedly, the better survivability of a silkworm strain in the field is governed by the molecular mechanism of the cell. The mechanism of cellular protection involves expression of a polypeptide family known as heat shock proteins (hsps).They act as molecular chaperones to ensure better survival under stressful conditions, including thermostress (Ellis and Van Der Vies, 1991 [4]; Kregel, 2002 [14]; Stromer *et al*., 2003 [30] ; Parcellier *et al*., 2003 [17]) Further, the versatility of insect species to temperature tolerance is illustrated by the examples of larvae of a Dipteran insect Polypedilum vanderplanki inhabiting Nigeria, which can tolerate temperature as low as -270 and +102°C (Hinton, 1960) [8] and the larvae of Coleopteran insects *Rhyzopertha dominica* and *Jtophylus oryzae* as high as 80°C (Evans, 1981) [5] but, *B. mori* is one of most thermo sensitive organism (Sorensen and Loeschcke, 2007) [26].

Earlier most of the heat shock studies in silkworm have been confined to cell culture or organisms from temperate climates (Evgenev *et al.*, 1987 [6]; Broude *et al.*, 1988[2]; Abramova *et al.*, 1991 [1]; Lohmann and Riddiford, 1992 [15]; Hsieh *et al*., 1995 [10]). But not much work has been correlated with the commercial traits of *B. mori* with respect to heat tolerance. With the objective to determine the sensitivity at different larval stages and to analyze the hsps expressed at different developmental stages in different silkworm strains of *B. mori* to determine the impact of heat shock on biological and commercial traits (Vasudha *et al*., 2006 [33]). Study on expose of fifth instar silkworm larvae from day three at 36°C and 40°C for 6 h every day until spinning and which forms as part of acclimation/hardening of the silkworm strains/breeds (Suresh Kumar *et al.*, 2005 [28]; Suresh Kumar *et al.*, 2011 [29]). While others induce HS for 2-3 h during I-V instars that enhance the ability of the silkworm strains to acquire tolerance to critical environmental conditions (Vasudha *et al*., 2006 [33]; Howrelia *et al.*, 2011 [9]). The effect of heat shock on various quantitative and qualitative traits of bivoltine hybrid FC2 and screening for thermo tolerant races among bivoltine silkworm germplasm revealed (Prasanth *et al.*, 2013 [19]; Sugnana kumari *et al.*, 2011[27]); Shivkumar and Subramanya, 2015 [24]).

The current study deals with the screening for thermo tolerance level in silkworm larvae by the role of temperature on total protein profile of different strains silkworm in the haemolymph, fat body and cuticle. Furthermore, also focused on overall silkworm rearing performance with respect to economical traits to obtain valuable information that will help to identify thermo tolerant silkworm strains exposed to high temperature based on the total protein profile as a molecular marker.

2. Material and Methods

1. These experiments were performed in the Indian Institute of Chemical Technology (IICT), Hyderabad, Telangana State, India during 2017-18.
2. **Heat shock treatment in silkworm strains:** Twelve multivoltine (KNT, CFP, GCM, CLPF, GLPF, PAF, GFP-C, AP-White, ISK, CDFP, IIA, GDFP) and seven bivoltine silkworm strains (BD2S, BO2, SOF-Br, BO1S, BO1N, SOC-B, BO3BL) were subjected to heat shock during 5th instar 4th day. 100 larvae of each strain were placed in a thin walled beaker in three replicates for heat shock at 40ºC and 45ºC in water bath for 1 hour. The treated larvae were then transferred to room temperature for recovery for 2 hours and mulberry leaf was fed to resume immediately after the heat shock recovery period. Thereafter, heat shocked and control larvae were reared until spinning under natural environmental conditions with 28ºC - 30ºC and humidity of 60% - 80% in a day. All the experiments were done in triplicate.
3. **Estimation of Protein Content in treated silkworms:** The total protein content in the haemolymph, fat body and cuticle of larvae was estimated according to Lowry *et al*, (1951)[16] modified by Singh and Agarwal, 1989 [25] using Bovine Serum Albumin (BSA) as standard (Tables 1-3). For analysis of proteins from fat body and the cuticle they were dissected from the heat shocked as well from control larvae, and washed in Ringer (Irvine, 1969) [11]. The tissues (1 g/ml of Ringer) were homogenized using a Teflon glass homogenizer. NP40 (0·5% v/v) was also included in the buffer while homogenizing the cuticle (Kiely and Riddiford, 1985 [12]). The homogenates were centrifuged at 5000*g* for 10 min at 4°C, and the supernatant after removal of the floating lipid layer was used for protein analysis.
4. **Influence of high temperature on Biological and commercial traits:** Thermo tolerance of heat shock at varied temperature was assessed based on percent mortality (inability to enter succeeding instars or to spin cocoon), and effective rate of rearing (ERR, which refers to number of cocoons harvested from number of larvae brushed). Commercial characteristics like effective rearing rate (ERR), Pupation % and shell ratio were also recorded and statistically analyzed using the *ANOVA* program (Fig 1- 3).

3. Results

Changes in protein content with increasing temperature: The results on the total protein content in haemolymph, fat body and cuticle after heat shock presented in the Tables 1-3.

Heamolymph: Based on the results, it is clearly indicates that the quantity of total protein content varied in all the nineteen strain. In multivoltine, the total protein content of heamolymph subjected to 40ºC and 45ºC is high compared to control and it range between 35.37 - 48.18 µg/ml and 40.74 - 54.31 µg/ml respectively when compared to control (31.89 - 41.38 µg/ml). Similarly, among bivoltine strain subjected at 40ºC and 45ºC comparatively high protein content range between 45.67- 58.83 µg/ml and 47.04 - 66.34 µg/ml was observed in all the silkworm strains respectively when compared to control (44.78- 49.7 µg/ml) (Table 1).

Fat body: In multivoltine, the total protein content in the fat body exposed to 40ºC is in the range of 8.14- 12.36 µg/ml and significant variation was observed in all the silkworm strains. Among all the silkworm strains exposed to 45ºC, slightly low protein content (8.12- 11.15 µg/ml) was observed compared to strains exposed at 40ºC. The total protein content of fat body in both the treatments (40ºC and 45ºC) was low when compared to control (9.26 - 13.63 µg/ml). In bivoltine, total protein content of 9.2 -12.06 µg/ml at 40ºC and 7.77 - 9.78 µg/ml at 45ºC when compare to control 10.94-13.92 µg/ml (Table 2).

Cuticle: The total protein content in the cuticle among the multivoltine strain exposed to 40ºC in the rage of 8.27-11.26 µg/ml and in strains exposed at 45ºC comparatively low protein content of 7.14 -9.93 µg/ml observed. Protein content in the Control batch is in the range of 10.17 -13.70 µg/ml. With reference to bivoltine strains, it varied from 8.30 -14.51 µg/ml of total protein content in the strains exposed to 40ºC, 7.18 - 10.92 µg/ml was observed in the strains exposed to 45ºC and in control 10.43-16.96 µg/ml was observed (Table 3).

Table 1: Total protein content in silkworm haemolymph

Multivoltine silkworm strains				
S.No	**Silkworm strain**	**Control**	**40ºC**	**45ºC**
1	KNT	39.65±0.27	46.15±0.51	54.31±0.31
2	CFP	41.38±0.25	45.15±0.31	49.26±0.16
3	GYM	36.79±0.31	39.24±0.15	42.18±0.44
4	CLPF	40.67±0.48	44.6±0.25	50.6±0.20
5	GLPF	33.28±0.41	40.92±0.29	43.63±0.27
6	PAF	31.89±0.29	35.37±0.27	40.74±0.37
7	GDFP	39.15±0.34	43.19±0.51	45.18±0.51
8	Ap-white	38.65±0.41	46.34±0.36	47.72±0.48
9	CCM	39.83±0.31	42.18±0.19	45.61±0.78
10	CDFP	42.87±0.15	45.78±0.87	50.12±0.45
11	IIA	40.79±0.24	48.18±0.47	52.81±0.89
12	GFP-C	39.26±0.26	45.71±0.4	48.18±0.19
Bivoltine silkworm strains				
1	BD2S	45.81±0.27	52.14±0.41	54.56±0.31
2	SOC-B	49.11±0.32	50.86±0.45	54.11±0.45
3	SOF-BR	44.91±0.25	47.04±0.44	50.14±0.67
4	BO1S	47.93±0.56	49.77±0.56	52.39±0.98
5	ISK	44.78±0.53	49.67±0.23	53.04±0.38
6	BO2	49.7±0.35	58.83±0.29	66.34±0.59
7	BO3BL	45.83±0.67	47.89±0.67	53.22±0.31

Values represent mean ± SD

Table 2: Total protein content in silkworm fat body

S. No	Silkworm strain	Control	40ºC	45ºC
Multivoltine silkworm strains				
1	KNT	9.26±0.16	8.14±0.12	7.79±0.29
2	CFP	11.26±0.27	10.23±0.12	8.12±0.16
3	GYM	12.23±0.15	11.78±0.25	9.23±0.25
4	CLPF	9.97±0.24	9.48±0.29	8.42±0.38
5	GLPF	13.63±0.19	12.26±0.38	10.91±0.34
6	PAF	11.12±0.25	9.55±0.23	8.33± 0.31
7	GDFP	13.56±0.15	12.36±0.23	11.15±0.13
8	Ap-white	13.18±0.25	10.22±0.38	9.65±0.21
9	CCM	10.18±0.28	9.56±0.35	8.34±0.27
10	CDFP	13.56±0.31	11.18±0.53	10.45±0.38
11	IIA	12.12±0.17	10.43±0.67	9.18±0.33
12	GFP-C	11.76±0.26	10.38±0.39	9.89±0.12
Bivoltine silkworm strains				
1	BD2S	10.94±0.36	9.2±0.36	7.77±0.35
2	SOC-B	11.4±0.34	9.5±0.18	8.3±0.23
3	SOF-BR	11.24±0.16	10.93±0.52	8.13±0.11
4	BO1S	12.34±0.21	10.15±0.25	9.18±0.36
5	ISK	13.39±0.28	12.06±0.38	9.78±0.31
6	BO2	13.92±0.11	10.16±0.16	8.34±0.26
7	BO3BL	12.98±0.27	9.26±0.34	8.13±0.23

Values represent mean ± SD

Table 3: Total protein content in silkworm cuticle

S.No	Silkworm strain	Control	40ºC	45ºC
Multivoltine silkworm strains				
1	KNT	13.7±0.32	9.55±0.3	8.46±0.42
2	CFP	12.36±0.23	8.35±0.79	7.18±0.99
3	GYM	12.95±0.76	10.16±0.22	8.23±0.34
4	CLPF	12.27±0.47	11.26±0.38	9.68±0.28
5	GLPF	13.52±0.27	10.47±0.3	8.57±0.17
6	PAF	11.93±0.38	10.46±0.29	9.93±0.41
7	GDFP	13.54±0.68	10.62±0.53	9.53±0.12
8	Ap-white	10.48±0.23	8.68±022	7.45±0.41
9	CCM	11.96±0.96	8.58±0.13	7.15±0.28
10	CDFP	12.48±0.87	9.15±0.45	8.23±0.96
11	IIA	13.29±0.34	11.23±0.65	9.16±0.57
12	GFP-C	10.17±0.46	8.27± 0.18	7.14±0.49
Bivoltine silkworm strains				
1	BD2S	10.43±0.26	9.29±0.32	7.45±0.32
2	SOC-B	11.56±0.15	8.3±0.56	7.18±0.11
3	SOF-BR	16.96±0.36	14.51±0.28	10.3±0.35
4	BO1S	14.18±0.14	11.26±0.16	9.32±0.18
5	ISK	15.36±0.36	13.24±0.38	10.49±0.23
6	BO2	14.63±0.23	13.89±0.11	10.92±0.21
7	BO3BL	13.21±0.24	12.93±0.26	9.83±0.22

Values represent mean ± SD

Biological and commercial traits:

1. **Heat shock effect on biological and commercial traits of different silkworm strains**

Thermal sensitivity and heat shock response of different silkworm strains are measured by observing the survival rate of larvae till cocoon stage and cocoon parameters.

Effective rearing rate (ERR): Among the silkworm strains exposed to 45ºC heat shock, maximum ERR of 18% was observed in KNT followed by GLPF (15%), CCM (14%) and GFP-C (13%) among multivoltine. ERR ranging from 3 to 11% was observed among the bivoltine. Whereas strains exposed to 40ºC heat shock, maximum ERR % was observed in KNT (77.53%) followed by GFP-C (76.89%), AP-White (74.16%), GLPF (73.34%) among multivoltine. In bivoltine, maximum ERR was observed in BO_2 (70.12%) followed by BD2-S (69.05%), BO1-S (68.13%), ISK (65.36%). In control batch maximum ERR was observed in KNT (96.18%) and GFP-C (95.96%) in multivoltine. Among bivoltine, maximum ERR was observed in BO3BL (93.49%) and ISK (93.18%) (Fig: 1).

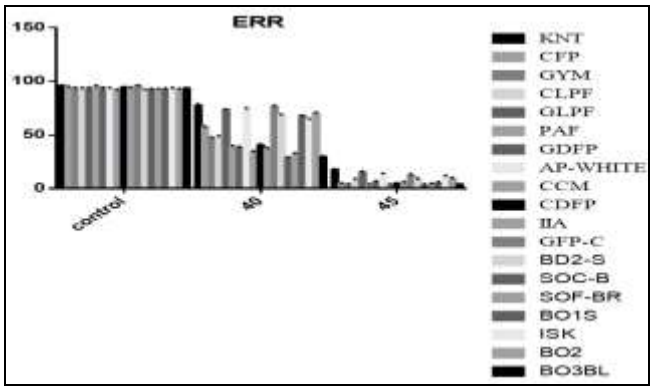

Fig 1: Effect of heat shock (40ºC) on the ERR among all the silkworm strains.

Pupation Rate: Silkworm strains exposed to 45ºC heat shock, zero pupation rates was observed in all the silkworm strains. Among 12 multivoltine strains > 70% of pupation rate was observed in four silkworm strains exposed to 40ºC (Table 1). Maximum pupation was observed in KNT (75%) followed by GFP-C (74.33%), AP White (72.33%) and GLPF (71.33%). Among 7 bivoltine strains > 60% of pupation was observed in four strains. Maximum pupation was observed in BO2 (67.66%), BD2-S (67%) followed by BO1-S (66%), and ISK (63.66%). With regard to control batch, maximum pupation was observed in KNT (93%) and GFP-C (92.66%) among multivoltine. In bivoltine, highest pupation rate was observed in ISK (91.33%) and BO2 (90.66%) (Fig 2).

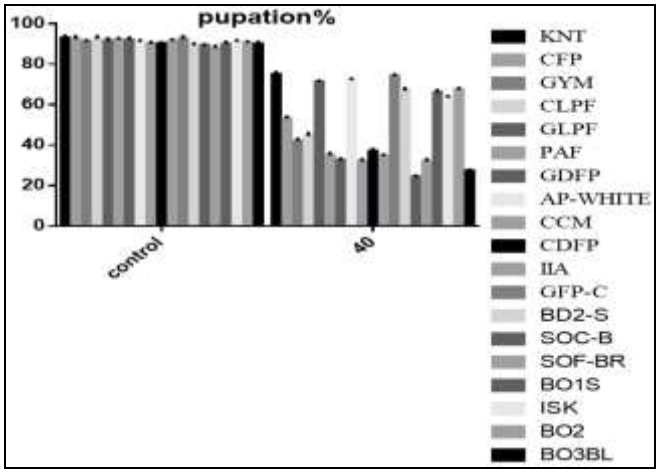

Fig 2: Effect of heat shock (40ºC) on the pupation among silkworm strains.

Shell Ratio (SR %): Among the silkworm strains exposed to 40ºC, maximum SR% was observed in CLPF (16.72%) CDFP (16.04%) and GDFP (16.02%) among multvoltine. In bivoltine, maximum SR% was observed in BD2S (20.35%) BO1S (20.35%) and BO2 (20.27 %). In control batch maximum SR% of 16.37 % was observed in CDFP followed by 16.29% in CLPF among multivoltine and among bivoltine highest SR% was observed in BO3BL (20.62 %) (Fig 3).

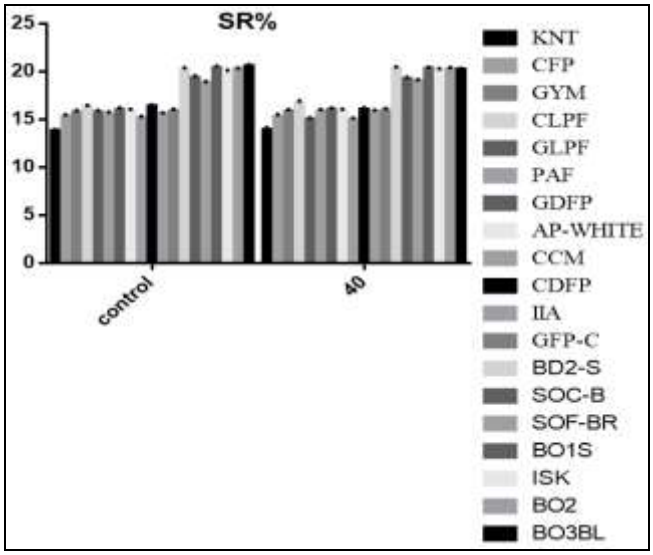

Fig 3: Effect of heat shock (40ºC) on the shell ratio % among all silkworm strains.

4. Discussion

The current study revealed the effect of heat shock at 40ºC

and 45ºC on twelve strains of multivoltine and seven strains of bivoltine silkworm strains for their tolerance to temperature higher than their normal growth temperature. Four strains *viz.*, KNT, GFP-C, AP-White, GLPF of multivoltine, and four strains of bivoltine *viz.*, BO2, BD2S, BO1S, ISK were found to be tolerant at 40ºc as judged by their survival and capacity to spin cocoons after 1 hour heat shock. Temperature above 45°C proved to be a lethal for all of all selected silkworm strains. A differential in thermal tolerance among silkworm strains subjected to 40°C, the effect of heat shock on the protein concentration of individual tissues of haemolymph, cuticle and fat body were analysed on a comparative basis. Results revealed that in multivoltine and bivoltine the total protein content of heamolymph subjected to 40ºC and 45ºC is high compared to control and these results relatively comparable to Ramani *et al.* (2017) [21]. Total protein content of fat body and cuticle in both the treatments (40ºC and 45ºC) were low when compared to control. The increase in protein content is may be due to presence of a set of highly conserved proteins, the HSPs, ubiquitous to all organisms examined so far and HSPs considered conferring protection against the adverse effect of heat shock (Hightower, 1991)[7].

Apart from *Bombyx mori*, biochemical and physiological constituents and their correlation in wheat (*Triticum aestivum* L.) genotypes under high temperature at different development stages was carried out by Ramani *et al.* (2017) [21]. Protein level changes under magnetic exposure of silkworm larvae has also revealed by Santosh kumar Tripati, (2012) [22].

The haemolymph being an open circulation system, most of the tissues including the fat bodies and silk glands lay bathed in haemolymph within the silkworm larvae. Consequently several proteins synthesized in the fat body find their way into the haemolymph. The fat body is the reservoir and active synthetic tissue for most of the haemolymph and cuticular proteins (Shigematsu, 1968 [23]; Dean *et al.*, 1985 [3]; Wyatt and Pan, 1978 [34]; Tojo *et al.*, 1981 [32]; Plantevin *et al.*, 1987 [18]. Integuments consisting of the cuticle and the underlying epidermis are the external tissues which act as initial defence organ in most insects. Response of the integument to heat shock will therefore be an integral part of the survival of the silkworm.

Proteins, the key factors within the cells which are governed by the genes and evident from the biochemical changes reflected in the larva (Irvine, 1969) [11] which are important biological macromolecules that are required for growth and development of the silkworm and for the synthesis of silk (Kiely and Riddiford, 1985[12]; Talukdar *et al.*, 2015[31]). Silk worm synthesizes silk threads in the form of cocoons which are structural protein fibre. At the end of the fifth instar larval stage, the silkworm larvae by to and fro movement of their head produce fibres which finally covers the larva and the larva becomes pupa. Therefore silk fibre synthesis is completely dependent upon the protein content of the larval body.

From current study, it was observed that haemolymph protein was found significantly highest in heat tolerant strains and this may be used as bench mark to screen silkworm germplasm for heat resistance/tolerance.

5. Conclusion

The following studies concludes that at 40°C, four multivoltine and four bivoltine silkworm strains were proved to be tolerant at high temperature with effective rearing rate (yield) ranging from 65.36% (ISK) to 77.53% (KNT). In this context, the present study proved that after heat shock, if these larvae are reared under natural environmental conditions where frequent fluctuations occur, they will perform better and spin better quality cocoons than the non-heat shocked individuals. Hence, these strains may be used as parental strains in developing high yielding heat resistant silkworm breeds. Therefore, sscreening of silkworm genetic resources using total protein analysis can be more useful for the silkworm selection in breeding programmes as well as its exploitation for commercial purpose.

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7. References

1. Abramova IYU, Ulmasov KHA, Akopov SB, Karaev KK, Babaeva AKH, Evgenev MB, *et al*. Dynamics of the synthesis of fibroin and heat shock proteins in different organs and at different stages of silkworm, *Bombyx mori* ontogenesis. Prikladnya Biokhimiya Mikrobiologiya, 1991; 27:147-156.
2. Broude ZTY, Titarenko E, Denisenko ON, Karaev KK, Ulmasov KA, Levin AV, *et al*. Molecular mechanisms of adaptation to hyperthermia in eukaryotic organisms. (III). Heat shock response in *Bombyx mori* cell infected by nuclear polyhedrosis virus. Molecular Biology, 1988; 22:1128-1131.
3. Dean RL, Locke Mand Collins JV. Structure of the fat body; in Comprehensive insect physiology biochemistry and pharmacology (eds) GA Kerkut, LI Gilbert (New York: Pergemon Press), 1985, 198-210.
4. Ellis RJ, Van Der Vies SM. Molecular chaperones. Annual Review of Biochemistry, 1991; 60:321-347.
5. Evans DE. The influence of some biological and physical factors on the heat tolerance relationships for *Rhyzopertha dominica* (F.) and S*itophilus oryzae* (L.) (Coleoptera: *Bostrychidae and Curculionidae*). J. Stored Products Res. 1981; 17:65-72.
6. Evgenev MB, Sheinker VS, Levin AV, Braude ZTY, Titarenko EA, Shuppe NG, *et al*. Zolotareva. Molecular mechanisms of adaptation to hyperthermia in higher organisms. (I). Synthesis of heat- shock proteins in cell cultures of different species of silkworms and in caterpillars. Molecular Biology, 1987; 21:410-419.
7. Hightower LE. Heat shock stress protein, chaperones and proteotoxicity. Cell, 1991; 66:191-197.
8. Hinton HE. A fly larva that tolerates dehydration and temperatures of -270ºC to +102ºC. Nature, 1960; 188:336-337.
9. Howrelia HJ, Patnaik BB, Selvanayagam M, Rajakumar. Impact of temperature on heat shock protein expression of *Bombyx mori* cross-breed and effect on commercial traits. J Environ. Biol, 2011; 32:99-103.
10. Hsieh FK, Yu SJ, Su SY, Peng SJ. Studies on the

thermotolerance of the silkworm, *Bombyx mori*. Chinese Journal of Entomology, 1995; 15:91-101.
11. Irvine HB. Sodium and potassium secretion byisolated insect malpigian tubules. Appl. J Physiol, 1969; 217:1520-1527.
12. Kiely ML, Riddiford LM. Temporal programming of epidermal cell protein synthesis during the larval pupal transformation of Manduca sexta; Rous Archs. Dev. Biol, 1985; 194:325-335.
13. King AM, MacRae TH. Insect heat shock proteins during stress and diapause. Annu. Rev. Entomol, 2015; 60:59-75.
14. Kregel KC. Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. Journal of Applied Physiology, 2002; 92:2177-2186.
15. Lohmann CMF, Riddiford LM. The heat shock response and heat sensitivity of *Bombyx mori*. Sericologia, 1992; 32:533-537.
16. Lowry OH, Rosenbrought NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J. Biol. Chem, 1951; 193:265-275.
17. Parcellier A, Gurbuxani S, Schmitt E, Garrido C. Heat shock proteins, cellular chaperones that modulate mitochondrial cell death pathways. Biochemical Biophysical Research Communications, 2003; 304:505-512.
18. Plantevin G, Bosquet G, Calvez B, Nardon C. Relationship between juvenile hormone levels and synthesis of major haemolymph proteins in *Bombyx mori* larvae; Comp. Biochem. Physiol, 1987; 86:501-507.
19. Prashanth MA, Bhat, Punyavathi HB, Manjunatha. Heat shock response of FC2 - a bivoltine hybrid of the mulberry silkworm, *Bombyx mori*, International Journal of Biotechnology and Bioengineering Research, 2013; 1238:73-88. © Research India Publications http://www.ripublication.com/ijbbr.htm
20. Punyavathi Muzafer A. Bhat, Manjunatha H. Comparative proteome analysis and thermal stress induced changes in the embryo of poly- and bi-voltine strains of *Bombyx mori*. Journal of Applied Biology & Biotechnology, 2017; 5:59-67.
21. Ramani HR, Ramani R, Mandavia MK, Dave RA, Bambharolia RP, Silungwe H, *et al*. Biochemical and physiological constituents and their correlation in wheat (*Triticum aestivum* L.) genotypes under high temperature at different development stages. International Journal of Plant Physiology and Biochemistry, 2017; 9:1-8, DOI: 10.5897/IJPPB2015. 0240 Article Number: D7C1CE064193 ISSN 2141-2162 Copyright ©2017
22. Santosh Kumar Tripati. Protein level changes under magnetic exposure of larvae in *Bombyx mori*: A Multivoltine mulberry silkworm. Academic Journal of Entomology, 2012; 5:73-80.
23. Shigematsu H. Synthesis of blood protein by the fat body in the silkworm, *Bombyx mori* L. Nature, 1968; 182:880-882.
24. Shivkumar Subramanya G. Quanititative estimation of haemolymph protein during different days of 5th instar larvae in bivoltiness, multivoltines and mutants of the *Bombyx mori*. Global J Biosci. & Biotech, 2015; 4:239-241.
25. Singh DK, Agarwal RA. Toxicity of piperanyl butaxide carbonyl synergism on the snail Lymnaea accuminata, International Review Dergesamtem Hydrobiologic, 1989: 74:689-699.
26. Sorensen JG, Loeschcke V. Studying stress responses in the post genomic era: its ecological and evolutionary role. Journal of Bioscience, 2007; 32:447-456.
27. Sugnana Kumari S, Subbarao SV, Sunil Misra, Suryanarayana Murty U. Screening for the thermo tolerance in identification of bivoltine germplasm resources of mulberry silkworm, *Bombyx mori*. Journal of Insect Science, 2011; 11:116. available online: insectscience.org/11.116.
28. Suresh Kumar N, Harjeet Singh, Kalpana GV, Basavaraja HK, Nanje Gowda B, Mal Reddy N, *et al*. Evaluation of temperature tolerant and temperature sensitive breeds of bivoltine silkworm, *Bombyx mori* L. Indian J Seri, 2005; 44:186-194.
29. Suresh Kumar N, Harjeet Singh. Expression of heterosis in silkworm hybrids, *Bombyx mori* (Lepidoptera: *Bombycidae*) tolerant to high temperature and high and low humidity conditions of the tropics. IJP AES, 2011; 1:188-204.
30. Stromer T, Ehrnsperger M, Gaestel M, Buchner J. Analysis of the interaction of small heat shock proteins with unfolding proteins. Journal of Biological Chemistry, 2003; 278:18015-18021.
31. Talukdar K, Rajkhowa RC, Sarma S, Kalita JC, Rahman A. Quantification and electrophoretic profile of haemolymph proteins of muga silkworm (*Antheraea assamnsis*) larvae reared on two major host plants(*Litsea monopetala* and *Persea Bombycina*) for two different crops (season). J. Entom. & Zoo. Studies, 2015; 3:473-475.
32. Tojo S, Kiguchi K, Kimura S. Hormonal control of storage protein synthesis and uptake by the fat body in the silkworm *Bombyx mori*; J Insect Physiol, 1981; 27:491-497.
33. Vasudha BC, Aparna HS, Manjunatha HB. Impact of heat shock on heat shock proteins expression, biological and commercial traits of *Bombyx mori*. Insect Science, 2006; 13:243-250.
34. Wyatt GR, Pan ML. Insect Plasma Protein. Annu. Rev. Biochem, 1978; 47:779-817.