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THE PROTEIN CONTENT AND HEAT SHOCK PROTEINS FROM EGGS OF RED COTTON BUG, *DYSDERCUS CINGULATUS* EXPOSED TO DIFFERENT TEMPERATURES

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ABSTRACT
The present study deals with the heat shock proteins, the protein content in control eggs exposed to heat shock to different temperature *viz*. 30ºC, 35ºC, and 40ºC showed a statistically {F (2,28) = 2.946, P < 0.05} significant difference. However, no statistically {F (2, 40) = 2.838, P < 0.05} significant difference was observed in diapause induced eggs to temperature *viz*. 30ºC, 35ºC, and 40ºC. Whereas in diapause induced eggs showed a significant difference {F(1,20) = 4.3512, P < 0.05}. Though after 9th day the eggs hatched in control, but no hatching was observed in eggs maintained at 5ºC.The analysis of electrophoresis pattern showed differences between the protein bands in normal temperature (25ºC) non-diapause eggs and diapause eggs at different temperature (5ºC, 30 ºC, 35ºC and 40ºC). In diapause and non diapause heat shock protein exposed to temperature 30ºC, 35ºC and 40ºC had same pattern of bands but one unique band of 43KDa and sHSPs of 28.0-40.0 KDa was observed in diapause and non-diapause. In cold shock a 61 KDa protein band was observed but absent in (control). Thus, protein 61 KDa and 43 KDa proteins expressed in cold shock and heat shock in diapause eggs of *Dysdercus cingulatus* might be involved in the embryonic development during stress.

INTRODUCTION

Insect diapause is centrally mediated at specific developmental stages, either in response to key stimuli fromthe environment (facultative diapause) or as a fixed component of ontogeny (obligatory diapause) as suggested by various workers (Denlinger, 2002;Kos¡ta´l, *et al*., 2008).During the initiation of diapause a physiological decreased state of metabolism, occurs that delays development and enhances stress tolerance in insects. In insects it also regulate the synthesis of heat shock proteins (HSPs). Arrigo, (2013), and Basha, *et al*. (2012) reported that, in insect sHSPs act independently of ATP and are the first line of cell defense, preventing irreversible denaturation of substrate proteins during cells in stress.

The HSPs play a significant role in the insect's responses to increased temperature and it protects cell an organism from thermal damage (Kostal and Tollarova – Borovanska, 2009; Zhao, *et al*., 2009, 2010a). In *Tribolium castaneum* when exposure at 40 ºC for 1 h HSP 83 gene is expressed (Xu, *et al*., 2010). Numerous heat shock proteins Like HSP 19.9, HSP 20.1, HSP 20.4, HSP 20.8, HSP 23.7, HSP 70, HSP 90 were identified in insects exposed to high temperature. It was observed that all proteins belonging to a small heat shock protein which protect proteins from being denatured and mainly functions as molecular chaperones during extreme conditions (Li, *et al*., 2009; Sun and MacRae, 2005; Waters *et al*., 2008). Large HSPs and small HSPs *i.e.* HSP 70, HSP 90, HSP 20.8, HSP 20.4 were highly expressed in diapause and non – diapause eggs. This shows that HSPs may play significant role in the initial embryos development in diapause and non-diapause (Fan, *et al*., 2013).

At low temperature HSPs or other cytoskeletal components are regulated to prevent the cells from damage (Michaud and Denlinger, 2004). Similarly, it was reported that HSPs can be induced by cold shocks or heat shocks (Kostal and Tollarova Borovanska, 2009; Kostal, *et al*., 2001; Rinehart, *et al*., 2006; Rinehart, *et al*., 2007). In *Pyrrhocerisapterus* adult, in non diapause showed a HSP 70 up regulated in fat bodies by both cold and heat stress. HSP 70 protein level increased by 1.6 fold at -5ºC.

Apart from HSP 70 many small HSPs are reported to be involved in the cold shock, and they have molecular masses of 16-40 KDa (Basha, *et al*., 2012; Clark and Worland, 2008). HSP 90 enhanced its expression in non-diapausing *C.suppressalis* larvae but not in diapause larvae (Sonoda, *et al*., 2006). Cold acclimated pupae of *D. antiqua* showed up-regulation of HSP 70, HSP 60 and tcpl (t-complex polypeptide-l) genes (Kayukawa, *et al*., 2005; Chen, *et al*., 2006; Kayulawa and Ishikawa, 2009).

The molecular information of diapause and protein implicated in diapause may provide information of constructing a regulatory hierarchy for diapause. In the present study the eggs of *D.cingulatus* exposed to different temperature and its effect on the protein content in diapause and non diapause eggs was observed and the electrophoretic pattern HSPs of diapause and non-diapause eggs were studied.

MATERIALS AND METHODS

The red cotton bug, *Dysdercus cingulatus*were procured from cotton field and maintained and reared under laboratory conditions as described by Kshemkalyani, *et al*. (1989). The eggs, which were laid after two generation of rearing, were only considered for the study. The freshly laid eggs were subjected to artificial induction of diapause by maintaining at room temperature and they were subjected to different temperature 30ºC, 35ºC, and 40ºC for 20 minutes. 10 eggs were subjected to cold stress at temperature 5ºC and control at 25ºC.

For studying the response of HSPs to abiotic stress, 10 eggs after laying were exposed to heat and cold shock. The induced diapause and non diapause eggs were exposed to different temperature *viz*, 30ºC, 35ºC, and 40ºC for 20 minutes. These treated eggs were homogenized with 500μl of distilled water and the soluble protein was isolated by centrifugation at 12,000 × g for 10 minutes at 4ºC. The protein content in eggs were estimated for all days of exposure. The protein content were estimated according to Lowry's method Lowry, *et al*., (1951). The SDS-PAGE was carried out according to the procedure as described by Laemelli (1970). The standard molecular weight markers used were procured from Puregene (genetix).The following standard protein marker were used: 250KDa Glycoprotein, 124KDa *â*-galactosidase, 91KDa Glycogen phosphorylase, 54KDa Glutamic dehydrogenase, 33KDa Lactate dehydrogenase, 29KDa Carbonic anhydrase, 16KDa Myoglobin.

The data were subjected to statistical analysis using ANOVA at $P < 0.05$ for significance.

RESULTS

Protein content in control eggs (25ºC) and to different temperature

The protein content of eggs maintained at room temperature *i.e* 25ºC and eggs exposed to different temperature is shown in figure 1. The protein content showed a variation on different days, highest protein content was observed on 4th day i.e. 0.59mg/ml and a least protein content was observed on 2nd day *i.e* 0.27 mg/ml. The protein content of eggs exposed to different temperature viz., 30°C, 35°C, and 40°C. It was observed that in control eggs exposed to heat shock to different temperature viz. 30°C, 35°C, and 40°C showed a statistically {$F(2,28) = 2.946$, $P < 0.05$} significant difference. However, no statistically {$F(2, 40) = 2.838$, $P < 0.05$} significant difference was observed in diapause induced eggs to temperature viz. 30°C, 35°C, and 40°C.

Protein content in cold shock eggs

The protein content for diapause induced eggs and eggs maintained at 25ºC is shown in figure 2. The diapause induced eggs showed a significant {$F(1,20) = 4.3512$, $P < 0.05$} difference in protein content compared to control. Though after 9th day the eggs hatched in control, but no hatching was observed in eggs maintained at 5ºC.

Expression pattern of protein on SDS-PAGE: The expression pattern of eggs exposed to 5ºC, at 25ºC and diapause induced eggs and control eggs exposed to different temperature are shown in figure 3. Eggs exposed to control temperature showed various protein bands *viz*. 250, 70, 54, 29 and 16 KDa. The expression of HSPs at 5ºC showed a various protein bands viz. 250, 124, 67, 61, 43, 33, 29 and 16 KDa. The cold induced eggs subjected to different temperature for 20 minutes showed different proteins bands of molecular weight, 124, 67, 61, 43 and 33 KDa.

DISCUSSION

In the present study, the eggs of *D. cingulatus* diapause induced and control eggs showed different levels of protein content throughout the study period. The protein content at various heat treatment showed a lower protein content compared with those maintained at 25ºC. The protein content at 30ºC and 25ºC showed no much difference in protein content. However

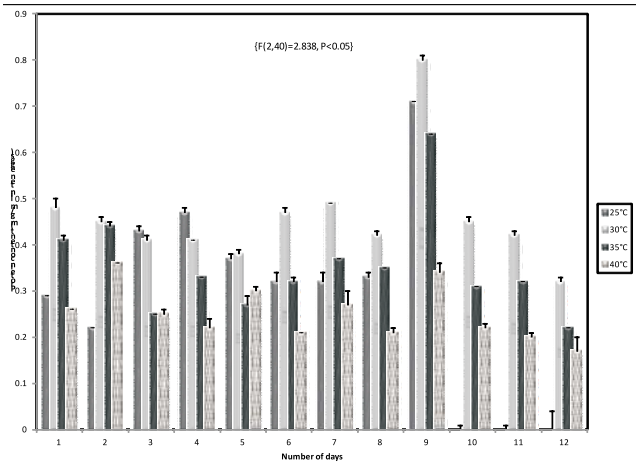

Figure 1: Heat shock protein content in non diapause eggs of *Dysdercus cingulatus*.

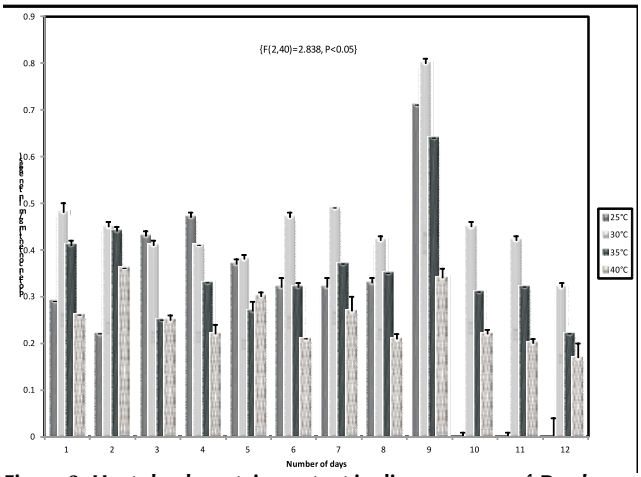

Figure 2: Heat shock protein content in diapause eggs of *Dysdercus cingulatus*.

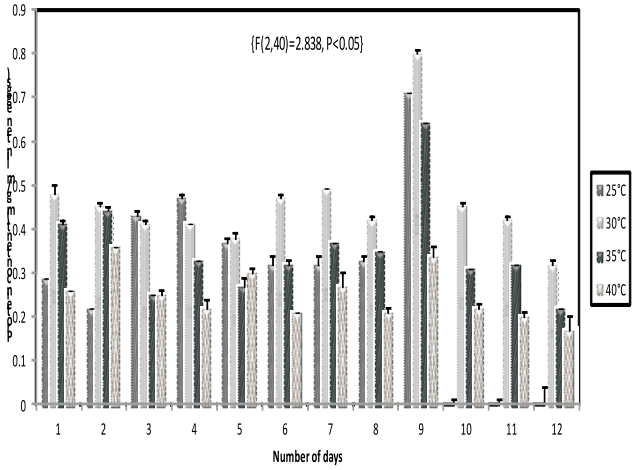

Figure 3: Cold shock protein content in diapause eggs of *Dysdercus cingulatus*.

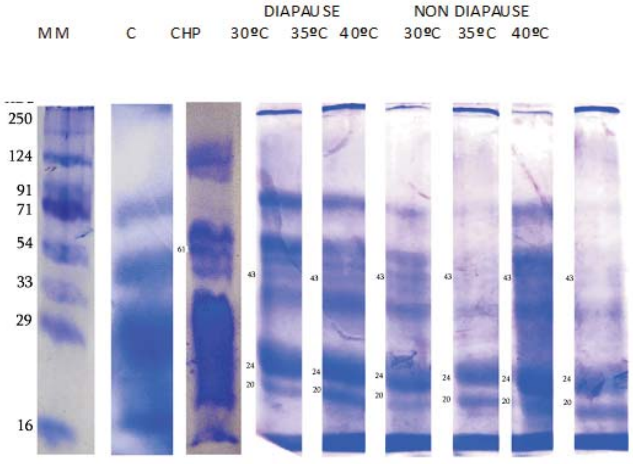

Figure 4: SDS-PAGE pattern, lane 1. Standard marker protein, lane 2. Control kept at room temperature (25ºC), lane 3. Low temperature (5ºC) cold shock protein, lane 4. Diapause heat shock protein at 30ºC, lane 5. Diapause heat shock protein at 35ºC, lane 6. Diapause heat shock protein at 40ºC, lane 7. Non diapause heat shock protein at 30ºC, lane 8. Non diapause heat shock protein at 35ºC, lane 9. Non diapause heat shock protein at 40ºC.

on exposure of eggs to temperature at 40ºC or above the protein content showed a decrease in the present study. A similar observation is reported by Yi Chuan *et al*. (2009) in protein content of non diapause and diapausing *Papiliomemnom*.

The SDS-PAGE detects quantitative differences in diapause and non-diapause in several insects (Osir, *et al*., 1989; Ichimer, *et al*., 1990; Mao and Cao. 2001). However, very few proteins play significant role in insect diapauses have been identified. In the present study HSP protein expression of normal temperature, heat shock and cold shock diapause and non diapause were identified. The SDS-PAGE of control eggs showed the protein bands of molecular weight 250, 70, 54, 29, 16 and 10 KDa proteins. The expression of HSPs in diapause eggs at 5ºC composed of protein bands of 250, 124, 90, 70, 67, 61, 54, 29, 16 and 10 KDa. In diapause eggs a special protein bands was found *i.e* 250, 124, 90, 67, 54 and 43KDa, whereas this band was not detected in the normal temperature eggs.

In heat treatment the eggs exposed to different temperature 30ºC, 35ºC and 40ºC were analysed, the expression level of non diapause and diapause was almost constant, the HSPs observed were 250 KDa, 124 KDa, 90 KDa, 70 KDa, 67 KDa, 54 KDa, 43 KDa, 29 KDa, 16 KDa and 10 KDa but significant difference between normal temperature expression level and heat shock.

The expression of HSP 90 at low temperature and at high temperature was observed and not at normal temperature. Molecular chaperone are considered as a ubiquitous feature of cells in which the HSP manage with stress-induced denaturation of other proteins (Storey and Storey, 2015). The relative expression levels of both hsp90 and hsp70 in *E. onukii* adults were upregulated as the temperature rises or falls over time, except in the -5ºC or 44ºC temperature groups. Moreover, the expression level in the temperature elevated groups was higher than that of the lower temperature groups. In addition, the hsp70 generally demonstrated a higher transcriptional level than hsp90, and both genes had a higher expression profile in female adults compared with the males. The expression profiles indicated that hsp90 and hsp70 may play important roles in *E. onukii* adult responses to ecologically relevant environmental condition studied by (Li, *et al*., 2015).

The proteins of molecular weight 43KDa observed during diapause, it may be a protein involved in embryonic development during stress. This protein is not expressed during non diapause. This observation is consistent with the fact that the metabolism of insects at low temperature and high temperature results in synthesis of lower protein and increase some chemical that are necessary for diapause survival such as glycerol and sorbitol (Denlinger, 2002).

Though SDS-PAGE analysis of different molecular proteins which are upregulated or down regulated during diapause and non-diapause. The protein in diapause may be involved in the production of phosphenolpyruvate and glyceraldehydes-3-phosphate which are precursors of the glycerol accumulation. The fact that 90 KDa molecular protein was uniquely expressed during cold tolerance and 90 KDa molecular proteins during heat shock. During diapause in the flesh fly the protein is down the cell cycle (Hayward *et al*., 2005).

The studies of molecular mechanisms of diapause have revealed several proteins associated with diapause in some insect species. (Salama and Muller, 1992) reported a 23KDa heat shock protein in the brain of diapause pupae in the flesh fly *Sarcophagacrassipalpis* (Li, *et al*.,2007).In the proteomic analysis of pre-diapause, diapause and post-diapause total 91 proteins were identified in the over summer diapause and over winter diapause in larvae of the blossom midge, *Sitodiplorismosellana* (Chen, *et al*., 2009) The molecular weight of these proteins were approximately 28.0-40.0 KDa which is also seen in the diapause eggs incubated at 5ºC of *D. cingulatus*.

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REFERENCES

Arrigo, A. P. 2013. Human small heat shock proteins: protein interactomes of homo- and hetero-oligomeric complexes: an update. Elsevier.**13:**1959-69

Basha, E. O'Neill H. and Vierling, E. 2012. Small heat shock proteins and *á*-crystallins: dynamic proteins with flexible functions. *Trends Biochem. Sci.* **37:**106-17.

Chen, Y. C., Nguyen, J., Semmens, K., Beamer, S. and Jaczynski, J. 2006. Enhancement of omega-3 fatty acid content in rainbow trout (Oncorhynchusmykiss) fillets.*J. food science*. **71(7):** C383-389.

Chen, Y.C., Tou, J. C. and Jaczynski, J. 2009. Amino acid and mineral composition of protein and other components and their recovery yields from whole Antartic krill (*Euphausiasuperba*) using isoelectric solubilization/ precipitation.*J. food science*. **74(2):** H31- H39.

Clark, M. S. and Worland, M. R. 2008. How insects survive the cold: molecular mechanisms-a review.*J.comp.Physiol*. B **178:** 917-933.

Denlinger, D. L. 2002. Regulation of diapause.Annu. Rev. Entomol. **47(1):** 93–122.

Fan, L., Lin, J., Zhong, Y., Liu, J. 2013. Shotgun proteomic analysis on the diapause and non diapause eggs of domesticated silkworm *Bombyxmori*. Plos one **8(4):** e 60386.

Hayward, S. A. L., Pavlides, S. C., Tammariello, S. P., Rinehart, J.P. and Denlinger, D. L. 2005. Temporal expression patterns of diapause associated genes in flesh fly pupae from the onset of diapause through post-diapause quiescence. *J. Insect physiol*. **51:** 631-640.

Ichimori, T., Ohtomo, R., Suzuki, K. and Kurihara, M. 1990. Specific protein related to adult diapause in the leaf beetle, *Gasteophysaatrocyanea*. *J. Insect physiol*. **36:** 85-91.

Kayukawa, T. And Ishikawa, Y. 2009. Chaperon in contributes to cold hardiness of the onion maggot *Delia antiqua*through repression of depolymerisation of actin at low temperatures. PLos One **4(12):** e8277.

Kayukawa, T., Chen, B., Miyazaki, S.,Itoyama, K.,Shinoda, T and Ishikawa, Y. 2005. Expression of the mRNA for the t-complex polypeptide-1, a subunit of chaperonin CCT, is upregulated in association with increased cold hardiness in *Delia antiqua*. Cell Stress Chaperones. **10:** 204-210.

Kos¡ta´l, V.,Tollarova´, M. and Dolez¡el, D. 2008. Dynamism in physiology and genetranscription during reproductive diapause in a heteropteran bug, Pyrrhocorisapterus. *J. Insect Physiol*. **54:** 77-88.

Kostal, V. and Tollarova-Borovanska, M. 2009. The 70 kDa heat shock protein assists during the repair of chilling injury in the insect, *Pyrrhocorisapterus*. Plos one. **4:** 4546.

Kshemkalyani, S. B., Enciy, M. R., Hakim, S. S and Prabhakar, J. D. 1989. Pathogenic fungus of *Dysdercuscingulatus.Ind. J. Entomol*. **51 (3):** 322-324.

Laemmli, E. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4; *Nature* (*London*). **227:** 680-685.

Lowry, O. H., Rosenbrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein Measurements with the Folin Phenol reagent. *JBiol.Chem*.103: 265-275.

Li, A. and Denlinger, D. L. 2009. Pupal cuticle protein is abundant during early adult diapause in the mosquito Culexpipiens. *J Med Entomol*.**46:** 1382-1386.

Li, A. Q., Popova-Butter, A., Dean, D. H. and Denlinger, D. L. 2007. Proteomics of the flesh fly brain reveals an abundance of upregulated heat shock proteins during pupal diapause. *J. Insect Physiol*. **53:** 385-391.

Li, Q., Wu, J. X., Qin, D. Z., Liu, X., C. Lu, Z. C., Li, Z., Lv.Zi, L. P. Chen, H. and Li, G. W. 2015. Gene Expression Profiles of Heat Shock Proteins 70 and 90 From*Empoascaonukii* (Hemiptera: Cicadellidae) in Response to Temperature Stress. *J. Insect Science*.**15(1):** 49.

Mao, W. F. and Cao, M. X. 2001. Isolation and purification of diapause-associated proteins from hemolymph in *Ostrinia furnacalis*.Acta Emtomol.Sin.**44:** 389-394.

Michaud, M. R. and Denlinger, D. L. 2004. Molecular modalities of insect cold survival: current understanding and future trends. International congress series.**1275:** 32-46.

Osir, E. O., Labongo, L. V. and Unntithan, G. C. 1989. A high molecular weight diapause associated protein from the stem borer, *Busseolafusca*, purification and properties. Arch. Insect Biochem. Physiol. **11:**173-187.

Rinehart, J. P., Hayward, S. A., Elnitsky, M. A.,Sandro, L. H. ,Lee, R. E. and Denlinger, D. L. 2006. Continuous up-regulation of heat shock proteins in larvae, but not adults, of a polar insect. Proc. Natl. Acad. Sci. U.S.A. **103(38):** 14223-14227.

Rinehart, J. P., Li, A., Yocum, G. D., Robich, R. M., Hayward, S. A. and Denlinger, D. L. 2007. Up-regulation of heat shock proteins is essential for cold survival during insect diapause. Proc. Natl. Acad. Sci. U.S.A. 104**(27):** 11130-11137.

Salama, M. S. and Muller T. A. 1992. A diapause associated protein of the pink bollworm *Pectinophoragossypiella*Saunder. Arch. Insect Biochem. Physiol**. 21:** 1-11.

Sonoda, S., Fukumoto, K.,Izumi, Y., Yoshida, H.,Tsumuk, I. H. 2006. Cloning of heat shock protein genes (hsp90 and hsc70) and their expression during larval diapause and cold tolerance acquisition in the rice stem borer, *Chilosuppressalis* Walker. Arch. Insect Biochem. Physiol. 63**(1):** 36-47.

Storey, K. B. and Storey, J. M. 2015. Insects in winter metabolism and regulation of cold hardiness. In: Insect Molecular Biology and Ecology (Hoffmann, K.K., ed.), CRC Press, *.J. Article*.pp. 245-270

Sun, Y. MacRae, T. H. 2005. Small heat shock proteins: molecular structure and chaperone function. Cell Mol Life Sci. **62:** 2460-2476.

Waters, E. R.,Aevermann, B. D. and Sanders-Reed, Z. 2008. Comparative analysis of the small heat shock proteins in three angiosperm genomes identifies new subfamilies and reveals diverse evolutionary patterns. *Cell Stress Chaperone*s. **13:** 127-142.

Xu, J., Shu, J. and Zhang, Q. 2010. Expression of the *Triboliumcastaneum*(Coleoptera: Tenebrionidae) hsp83 gene and its relation to oogenesis during ovarian maturation. *J. Genet. Genomics* **37:** 513-522.

Yi, Chuan- hui. 2009. Comparison of protein content among non diapause pupae, diapause pupae and Eclosion-adult from diapause pupae of *Papiliomemnon. J. Anhui agricultural sciences*.**12:**125-128.

Zhao, L., Becnel, J. J., Clark, G. G. and Linthicum, K. J. 2010a. Expression of AeaHsp26 and AeaHsp83 in *Aedesaegypti*(Diptera: Culicidae) larvae and pupae in response to heat shock stress. *J. Med. Entomol*. **47:** 367-375.

Zhao, L., Pridgeon, J., Becnel, J. J., Clark, G. G. and Linthicum, K. L. 2009. Identification of genes differentially expressed during heat shock treatment in *Aedesaegypti*. J. Med. Entomol. **46:** 490-495.

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