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An Expression of Heat Shock Protein in Diapause Egg of Dysdercus Cingulatus

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Abstract: ***An expression of heat shock protein is a critical mechanism in diapause egg of Dysdercus cingulatus. As all organisms possess a stress response relating up regulation of heat shock proteins (Hsps) which increases survival rate at altering temperature. The results of present study revealed that the protein content was significantly different on different days in non-diapause egg and protein content in heat shock diapause eggs was decreasing as the diapause eggs exposed to elevated temperature. The room temperature (control) non diapause eggs showed five Protein bands in SDS PAGE of molecular weight viz. 250 KDa, 54 KDa, 33 KDa, 20 KDa and 16 KDa. Whereas Heat shock protein electrophoresis pattern of protein of diapause eggs were analysed at 24 hrs intervals for different days it is observed that, 8 similar protein bands of molecular weight viz., 250 KDa, 70 KDa, 54 KDa, 43 KDa, 33 KDa, 29 KDa, 23 KDa and 20 KDa were found at different temperature viz., 30 °C, 35 °C, and 40 °C. On comparison of protein profile of non-diapause control and diapause heat shock protein of molecular weight 70 KDa, 43 KDa and 23 KDa bands were not observed in the control non diapause eggs.***
Keywords: Heat shock protein, non-diapause egg and diapause egg, Dysdercus cingulatus.

I. INTRODUCTION

Insects have evolved a variety of molecular adaptation to cope with stressful changing temperature. [2] Response of heat shock proteins (Hsps) considered as significant for thermal tolerance and adaptation in vinegar fly Drosophila melanogaste. [8] At increased temperature and chemical or other stress in insect synthesis heat shock protein (Hsps). These Hsp play significant role in the insect's responses to abiotic stressors such as increased temperature [4], [5], [14], [15] at elevated temperature Hsps can protect cells and organisms from thermal damage. It was observed in Tribolium castaneum exposure at 40 % C for 1 h Hsp 83 gene was expressed .[13] Hsp 83 in Aedes aegypti larvae and pupae are important markers of stress and may function as important proteins to protect and enhance the survival rate.[14] Different heat shock protein expression observed when insects were pre-treated with a mild heat stressor. This is known as rapid heat hardening and is apparently cause the denatured of protein during heat shock. [2] Number of heat shock proteins such as 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 and unfavourable conditions. All the Hsp belong to a small heat shock protein which protect proteins from being denatured and mainly functions as molecular chaperones during extreme conditions. [6], [12] Large Hsp and small Hsp i.e. Hsp 70, Hsp 90, Hsp 20.8, Hsp 20.4 were highly expressed in diapause eggs. This shows that Hsps may play significant role in the initial embryos development in diapause.[3] cDNAs encoding sHsp: sHsp 19.9, sHsp 20.1, sHsp 20.4, sHsp 20.8, sHsp 21.4 and sHsp 23.7were isolated and sequenced from Bombyx mori and sHsp 21.4 showed substantial increase from other sHsp.[11] In this study we tested the expression of heat shock protein in diapause egg of Dysdercus cingulatus at elevated temperature.

II. MATERIAL AND METHODS

A. Experimental strain

Dysdercus cingulatus commonly known as red cotton bug was selected for the study. Strains were procure from the nearby field of cotton and reared in the laboratory and acclimated to laboratory condition. Adults were reared on cotton soaked seeds. After two generation rearing, eggs were taken for experiment.

B. Sample Preparation

Eggs of red cotton bug were collected and made two batches one treated as control and one experimental group. Control eggs remained within a room temperature at 25 ± 2°C for the 9 days which were treated as non-diapause eggs. At an interval of 24 hours batches of 10 eggs at room temperature were homogenized with 500 µl of distilled water and a soluble protein was isolated by centrifugation at 12,000 rpm for 10 min at 4°C and treated as non-diapause protein.

Experimental egg collected and placed within a 5°C in refrigerator for 24 hours so that they enter in diapause condition. After 24 hours egg were exposed to different temperature viz., 30 °C, 35 °C, and 40 °C in batches of 10 eggs for 20 minutes at a interval of 24 hours (i.e.1 day interval). These treated eggs were homogenized with 500 µl of distilled water and a soluble protein was isolated by centrifugation at 12,000 rpm for 10 min at 4°C.

C. Determination of Protein Concentration by [7] Method

The soluble protein obtained after heat shock treatment from diapause eggs and protein of non-diapause eggs at 25 ± 2°C were estimated by using Lowry's method. BSA as a standard protein solution was prepared and a graph was plotted between µg of protein and optical density. The extract of 500 µl protein from the sample was pipette and mixed with 3ml of reagent 'c' (2% Na2CO3 in 0.1N NaOH, 1% CuSO4, 2% C4H4KNaO6.4H2O) kept for 20 minutes in dark. After 20 minute 0.5 ml of Folin Ciocalteau reagent was added. The blue colour developed was read at optical density 660 nm on spectrophotometer against the reagent blank. The value obtained was calculated from the standard graph of BSA.

D. Statistical Analysis

Values of protein concentration were expressed as mean ± SE (standard error) and ANOVA was applied to know the difference in heat shock protein at varied temperatures. Each test difference at P <0.05 was considered significant.

E. SDS- PAGE

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using microkin unit, Techno source (Mumbai). Protein samples were boiled for 4-5 min to denature the protein. 40 µl of protein sample was loaded on 10 % SDS polyacrylamide gel (4 % stacking gel, 10 % resolving gel). Electrophoresis was carried out for 3 hours at a constant voltage of 150 V until the tracking dye reached the bottom of the gel. 0.1 % coomassie brilliant blue R-250 in 40 % methanol and 10 % acetic acid was used to stain the gel for 1 hr and destained overnight with the destaining solution containing 40 % methanol and 10 % acetic acid . The standard molecular marker ranging 10 KDa -250 KDa was used.

III. RESULT

Effects of temperature on the protein content of the non-diapause eggs kept at 25 ± 2°C and diapause eggs exposed at different temperature viz., 30°C, 35°C and 40°C**.**

The diapause and non- diapause eggs showed varying levels of heat tolerance on different days. The protein content at various heat treatments was lowering in comparison with those incubated at room temperature non diapause protein (25 ± 2°C). The diapause protein content at 30°C showed very little decrease when compared with non-diapause protein content at 25°C. However diapause eggs on exposure to temperature of 40°C or above the protein content decreased due to which rate of survival also reduced.

To determine the difference between the heat tolerance at varied temperature, the data was subjected to one way ANOVA test it was observed that the F (3, 43) = 5.3323 which was larger than the value at P< 0.05 compared from table which showed that the null hypotheses, that there is no significant difference in the protein content in the non-diapause eggs and heat shock diapause protein of Dysdercus cingulatus was rejected.

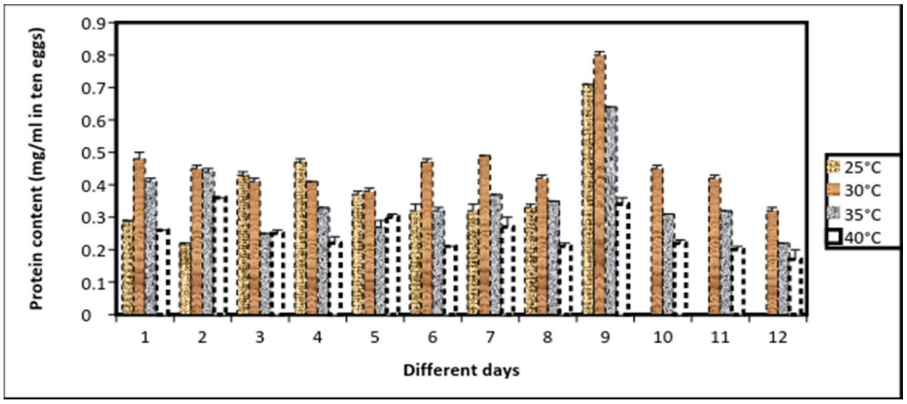

Fig 1. Protein content of non-diapause eggs kept at room temperature 25 ± 2°C and Heat shock Protein of diapause eggs exposed to different temperature viz., 30°C, 35°C and 40°C.

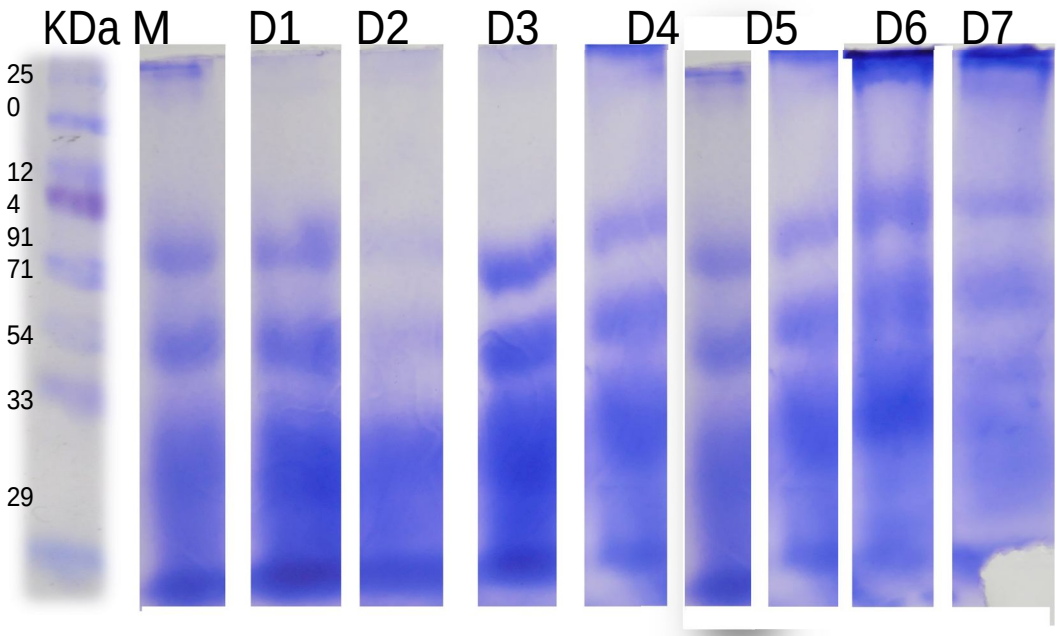

Fig 2. SDS-PAGE patterns of the non-diapause eggs (control) kept at room temperature 25 ± 2°C.The sample were separated by 10% SDS-PAGE. The lane M is standard marker protein, lane D1- D9 are different days control protein.

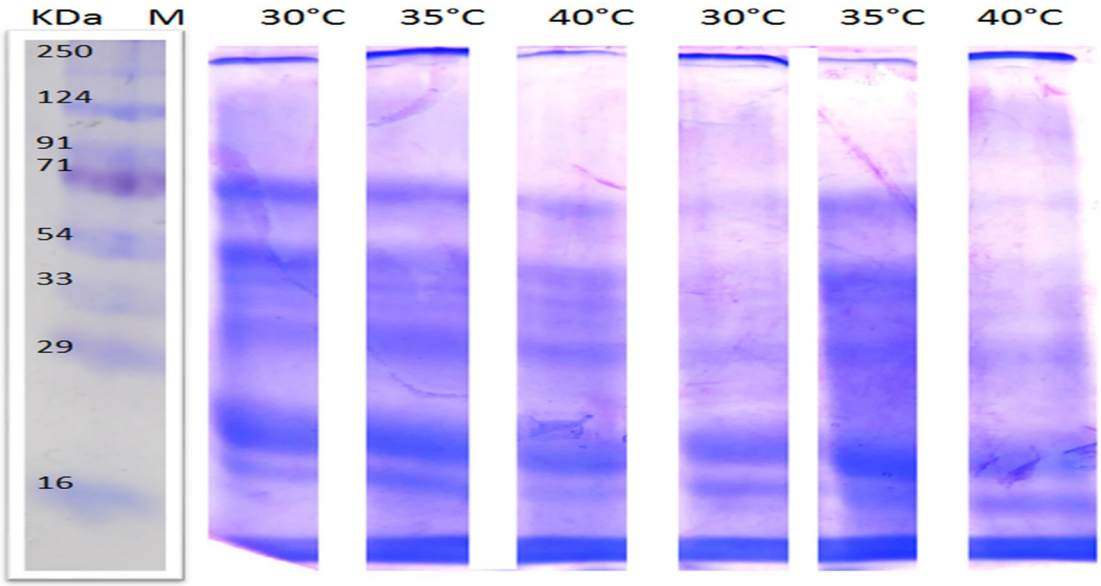

Fig 3. SDS PAGE patterns of the diapause eggs were exposed to different temperature 30 °C, 35 °C, and 40 °C. The samples were separated by 10% SDS PAGE. The lane M is standard marker protein, lane are diapause protein exposed to different temperature viz. 30 °C, 35 °C, and 40 °C.

The protein profile of the non- diapause eggs (control) kept at room temperature 25 ± 2°C. The electrophoresis pattern of protein of non-diapause eggs were analyzed at 24 hours intervals unto 9 days. Similar protein migration pattern was observed in non – diapause eggs between 24 hours intervals until 9 days. The room temperature (control) non diapause eggs showed five Protein bands in SDS PAGE of molecular weight viz. 250KDa, 54KDa, 33KDa, 20KDa and 16KDa. Heat shock protein electrophoresis pattern of protein of diapause eggs were analyzed at 24 hours intervals for different days it is observed that, 8 similar protein bands of molecular weight viz., 250 KDa, 70KDa, 54 KDa, 43 KDa, 33 KDa, 29 KDa, 23 KDa and 20 KDa were found at different

temperature viz., 30 °C, D2 -35 °C, and 40 °C. On comparison of protein profile of non-diapause control and diapause heat shock protein of molecular weight 70KDa, 43KDa, and 23KDa bands were not observed in the control non diapause eggs.

IV. DISCUSSION

The present study provide an initial analysis of protein content and protein bands of different molecular weight in the non-diapause and diapause eggs of Dysdercus Cingulatus when exposed to different temperature. The protein content in non-diapause eggs showed variation on different days where as in diapause eggs it was decreasing due to heat shock. As protein play an important role in the cell influencing growth and development in diapause larvae. Diapause larvae accumulate more protein content than non-diapause larvae.[9] is has been reported that multiple HSPs occur in living cells. Hsps are involved to cope up heat stress out of which some Hsp are known to be upregulated during recovery from cold stress and heat stress in insects.[1] In the present study protein profile of heat shock diapause eggs when compared with the protein profile of room temperature non diapause eggs, protein band of 70KDa, 43KDa, 23KDa and 20KDa was observe in heat shock diapause protein exposed to temperature viz., 30°C, 35°C and 40°C and not in room temperature non diapause eggs. In earlier studies report reveals that small Hsp23 and Hsp70 are highly up-regulated during diapause in pupa of S.crassipalpis. It is just due to pupa enter diapause and not due to temperature stress. Contrast result to the stress response where there is a nearly global shut down in the expression of other genes when the Hsps are turned on the Hsps is expressed simultaneously with the other genes during diapause.[10] The present study result of Hsp70 and Hsp23 observed in the heat shock protein diapause eggs possible due to eggs kept at 5°C as they enter diapause and then heat shock treatment was given. Whereas Hsp70, Hsp43, Hsp23, Hsp20 band were observed in heat shock diapause eggs. It is also reported that large Hsps and small Hsps i.e. Hsp70, Hsp 90, Hsp20.8, Hsp20.4 were highly expressed in diapause eggs. This shows that Hsp may play significant role in embryos development in diapause.[3]

V. CONCLUSION

This study shows that proteins involved in stress response play key function in survival of eggs. Expression of Hsp 70, Hsp 43, and Hsp 23 shows that it is important for the Dysdercus cingulatus diapause eggs to survive heat shock and adapt to the changing environments.

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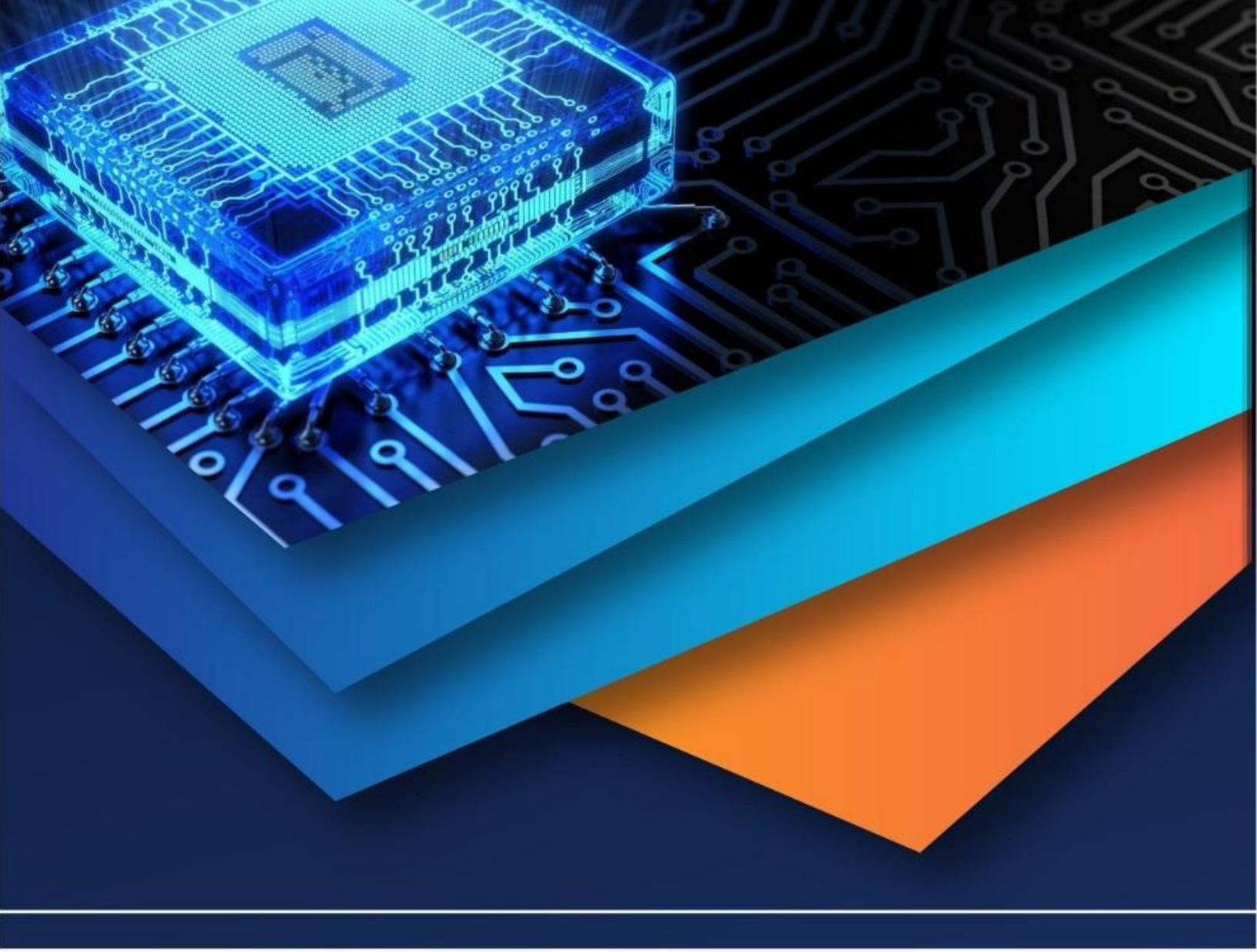

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