



Gene expression analysis in different working groups of Indian Honey Bees, *Apis cerana indica* F.

¹Chaitra S. K., ²Badariprasad, P. R., ³Srikanth Barkeer., ¹Sujay Hurali., ³Sanganna Sajjanar., ⁴Prasad Kulkarni., and ³Sreenivas A. G.,

¹Department of Agricultural Entomology, College of Agriculture, Gangavathi, UAS, Raichur, Karnataka-584 104

²Department of Biochemistry, College of Agriculture, Gangavathi, UAS, Raichur, Karnataka-584 104

³Department of Agricultural Entomology, University of Agricultural Sciences, Raichur, Karnataka-584 104

⁴Department of Agricultural Engineering, University of Agricultural Sciences, Raichur, Karnataka

*E-mail: chaitrask1234@gmail.com

Introduction

- Honey bees (*Apis cerana indica* F.) are highly social insects exhibiting a clear division of labor among different working groups, such as foragers, nurse bees, and thermoregulatory bees.
- Each group performs specialized tasks essential for colony survival, including foraging, brood care, and maintaining hive temperature.
- These behavioral differences are underpinned by distinct gene expression profiles that regulate metabolism, stress response, and task-specific functions.

Material and Methods

- Bee samples from different working groups were collected with forceps, flash-frozen in liquid nitrogen, and stored at -80°C until RNA extraction.
- Total RNA was extracted using the Direct-zol Mini Prep Kit, and quality/quantity were assessed with NanoDrop.
- Paired-end sequencing libraries were prepared in technical replicates using the NEBNext® Ultra™ II Directional RNA Library Prep Kit (Illumina).
- Raw reads from six samples were processed with Trimmomatic v0.39 to obtain high-quality clean reads.

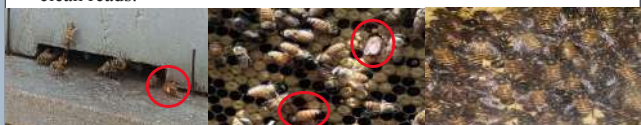
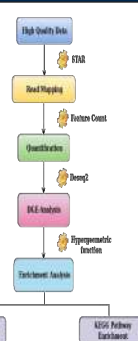


Plate 1. Different work group of bees

Objective

- To analyze differential gene expression in forager, nurse, and thermoregulatory bees

Results and Discussion

RNA from all bee groups showed high yield, purity, and integrity (RIN ≥ 8.1). Libraries were of good quality (369–387 bp, 23.4–26.1 ng/ μL) with no adapter contamination. Sequencing yielded 19.3–27.3M reads/sample with $>85\%$ HQ retention; $>90\%$ mapped to the *Apis cerana* genome. PCA explained 99% variance (PC1: 83%, PC2: 16%), clearly separating foraging, nurse, and thermoregulatory bees by transcriptional profiles.

Foragers vs. Thermoregulatory Bees (AET_GVT-1 vs. AET_GVT-3):

2,405 DEGs (1,213 $\downarrow$, 1,192 $\uparrow$); 7 unique to foragers. Thermoregulatory bees showed higher expression of PA2 Phospholipase (Gene_8430), Zinc finger protein (Gene_3999), hypothetical protein APICC_01063 (Gene_624), Zinc carboxypeptidase PBC26020.1 (Gene_3263), Lysosomal aspartic protease (Gene_6190).

Nurse vs. Thermoregulatory Bees (AET_GVT-2 vs. AET_GVT-3):

2,970 DEGs (1,497 $\downarrow$, 1,473 $\uparrow$); 4 unique to nurses, 2 to controls. Thermoregulatory bees showed higher expression of Phospholipase A1 (Gene_7204), carboxypeptidase Q-like isoform X2 (Gene_7949), retinoid-inducible serine carboxypeptidase-like (Gene_3493).

Shared DEGs (Both comparisons): 11 common genes.

Upregulated in thermoregulatory bees: cytochrome P450 9e2-like, esterase FE4, vitellogenin receptor, histone H2B-like, kinesin KIF20B, and uncharacterized proteins. Downregulated: cuticle protein 18.7-like, zinc finger 512B-like, neuronal acetylcholine receptor subunit $\alpha 4$ -like.

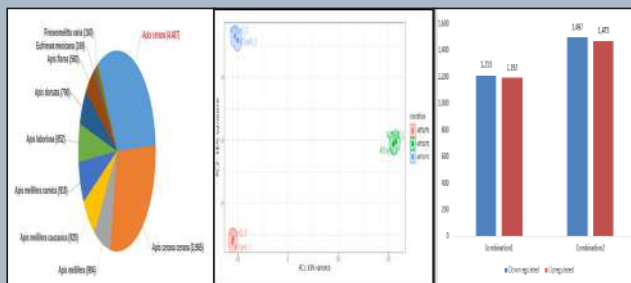


Fig. 1: Blast top hit species distribution (Top 10)

Fig. 2: PCA plot

Fig. 3: Differential Gene Expression Summary

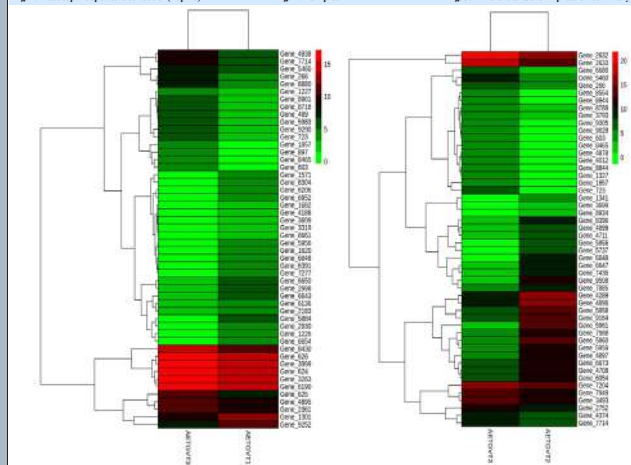


Figure 4. Heatmap of differentially expressed genes (top 50) in Combination 1 and 2

Table 1: Genes expressed in upregulated genes of thermoregulatory bees

Sl. No.	Gene	Proteins	Function
1	Gene_7714	cytochrome P450 9e2-like [Apis cerana]	Heme binding, iron ion binding, monooxygenase activity, oxidoreductase activity, acting on paired donors, with incorporation of molecular oxygen
2	Gene_5460	Esterase FE4 [Apis cerana cerana]	Calcium ion binding
3	Gene_266	Vitellogenin receptor [Apis cerana cerana]	DNA binding proteins heterodimerization activity structural constituent of chromatin
4	Gene_723	histone H2B-like (Apis cerana) PBC33380.1 Histone H2B.1/H2B.2 [Apis cerana cerana]	
5	Gene_8465	uncharacterized protein LOC107998826 isoform X2	
6	Gene_1857	kinesin-like protein KIF20B [Apis mellifera caucasicus]	ATP Binding, microtubule binding, plus end directed microtubule motor activity
7	Gene_6680	hypothetical protein APICC_09832 [Apis cerana cerana]	
8	Gene_8430	PA2 Phospholipase	PLA2 catalyzes the calcium-dependent hydrolysis of the 3-aryl groups in 3-sn-phosphoglycerides.
9	Gene_3263	Zinc carboxypeptidase PBC26020.1	Metallo-carboxypeptidase activity, zinc ion binding
10	Gene_6190	Lysosomal aspartic protease	Aspartic-type endopeptidase activity
11	Gene_7204	Phospholipase A1	Phospholipase A1 activity, triacylglycerol lipase activity
12	Gene_7949	carboxypeptidase Q-like isoform X2	
13	Gene_603	uncharacterized protein LOC108001624 [Apis cerana]	

Conclusion

- Thermoregulatory bees exhibit distinct gene expression profiles compared to foragers and nurse bees, with key metabolic, structural, and regulatory genes upregulated to support colony homeostasis.
- Shared DEGs indicate conserved molecular mechanisms underlying thermoregulation, highlighting its essential role in maintaining hive function.

References

- Fang, F., Zhou, H., Feng, X., Chen, X., Wang, Z., Zhao, S. and Li, X., 2022, Gene expression and chromatin conformation differs between worker bees performing different tasks. *Genomics*, 114(3): 110362.
- Shan, J., Cheng, R., Magaoya, T., Duan, Y. and Chen, C., 2024, Comparative Transcriptome Analysis of Cold Tolerance Mechanism in Honeybees (*Apis mellifera sinixinyuan*). *Insects*, 15(10): 790.

Chaitra S K¹, Badari Prasad P R¹, Sujay Hurali², Sanganna Sajjanar³, Prasad Kulkarni⁴, Srikanth Barkeer⁵, Sreenivas A G³

¹Department of Entomology, CoA, Gangavathi, University of Agricultural Sciences, Raichur, India

²AICRP on Rice, Agricultural Research Station, Gangavathi, University of Agricultural Sciences, Raichur, India

³Department of Agricultural Entomology, University of Agricultural Sciences, Raichur, India

⁴Department of Irrigation and Drainage, CoAE, University of Agricultural Sciences, Raichur, India

⁵Department of Agricultural Biochemistry, CoA, Gangavathi, University of Agricultural Sciences, Raichur, India

Honeybee colonies exhibit a highly organized division of labour, with distinct groups of bees performing specialized tasks essential for colony survival. Within this system, different groups of bees are assigned specific tasks based on age, physiology, and environmental demands. Thermoregulation is particularly critical, as honeybees must maintain hive temperatures within a narrow range to support the delicate balance of brood care and food storage, especially during seasonal temperature fluctuations. The interplay between these specialized roles, driven by both genetic and environmental factors, allows the colony to adapt to changing conditions across seasons. This study explores gene expression variations in three distinct groups of honey bees nurse bees, foraging bees, and thermoregulating bees across two different seasons, summer and winter. RNA sequencing was employed to investigate the molecular profiles of these groups, with a focus on understanding how seasonal changes impact the gene expression related to behaviour, metabolism, stress response and also specially to identify genes involved to perform thermoregulation across the seasons. By identifying differentially expressed genes during temperature fluctuations, this research seeks to uncover the molecular pathways that enable bees to maintain hive stability. By comparing gene activity between the summer and winter seasons, the study aims to elucidate the seasonal adaptation mechanisms at the molecular level in these bee groups, shedding light on how honeybees adjust to varying environmental conditions. This research contributes to a deeper understanding of the genetic regulation underlying the division of labour and seasonal physiological adaptations within honeybee colonies and the essential role of thermoregulation in maintaining colony homeostasis throughout the year.