

High-throughput Transcriptome Sequencing for the Identification of heat-responsive Genes in wheat (*Triticum aestivum*)

Ranjeet R Kumar¹, Sushil K Sharma², Pooja Verma³, Khushboo Singh⁴,
Suneha Goswami⁵, Himanshu Pathak⁶, Raj D Rai⁷

¹Division of Biochemistry, ⁶CESCRA,
Indian Agricultural Research Institute, New Delhi, India Pin-110012

ABSTRACT

Global warming is a major threat for agriculture and food security and in many cases the negative impacts are already apparent. Wheat is one of the most important staple food crops and is highly sensitive to the heat stress; the current challenge before the researchers is to decipher the molecular mechanisms of heat stress response (HSR) and thermotolerance to protect the wheat from the heat. Transcriptomics is a powerful technique to dissect the tolerance mechanism by exploring the candidate genes. A transcript dataset of wheat (*Triticum aestivum*) was constructed containing 23,470 non-redundant transcripts with an average length of 746 bp. When compared with wheat full-length cDNAs and PlantCyc data sources, 12,992 and 8,480 were respectively aligned ($e < 1e-5$). 785 transcripts were up-regulated and 431 transcripts were down-regulated with fold 2 threshold and FDR value < 0.01 . 78 transcripts showed >10 fold high up-regulation including HSPs and metabolic-related genes. Clustering analysis of differentially expressed transcripts showed 16 expression clusters of which 6 clusters have more than 100 genes with similar expression patterns, suggesting co-expression of genes under the heat stress. 654 novel transcripts, that showed high fold expression, were further used for transcript discovery using ab initio gene prediction methods. Transcript validations in the tolerant and susceptible cultivars of wheat for 12 randomly selected heat stress-associated genes were confirmed by real-time RT-PCR. We observed that response to the heat-stress, protein-folding, oxidation-reduction process, Photosynthesis, flower development and response to the oxidative stress had higher number of up-regulated genes. Metabolic pathways including biosynthesis of secondary metabolites were highly influenced by the heat treatment. The present study culminated in greater understanding of the heat-response of tolerant genotype and has provided good candidate genes for marker development.

Keywords: *De novo* assembly, RNA-Seq, Transcriptome, *Triticum aestivum*, Differential gene expression, Next-generation sequencing, Heat stress
