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Short Communication

Comparative expression analysis of *HSP* genes in wheat and barley under heat stress

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Plants are consistently exposed to numerous biotic (herbivore and pathogen) and abiotic stresses (cold, drought, salinity, heat *etc.*). Being sessile organism, they cannot escape therefore, during the course of evolution they have evolved with sophisticated mechanism of tolerance. Among abiotic stresses, heat stress is one of the key stresss which causes great loss to the plants (Vinocur and Altman, 2005). Heat stress has a significant adverse impact on carbon assimilation and starch synthesis in these environments, which leads to reduction of grain yield and quality. In this heat acclimatization process, heat shock proteins (HSPs) play an important role in regulation of this heat-induced transcriptional reprogramming (Al-Whaibi, 2010). Heat shock proteins (HSPs) are evolutionary conserved proteins which are induced in almost all organisms by high temperature and other abiotic and biotic stresses. Most of the HSPs work as molecular chaperones in the folding and refolding of proteins (Efeoglu, 2009). Based on their molecular mass, these proteins are classified into five subfamilies viz., HSP100, HSP90, HSP70, HSP60 and small HSPs (Al-Whaibi, 2010).

Wheat is an important cereal crop and is suffered by various abiotic stress specially heat. Barley is more tolerant to heat stress compared to wheat. Heat stress causes severe loss to vegetative and reproductive performance of the wheat and barley. As a response, plants induce various heat shock proteins (small and large) to cope up with the heat stress. The objective of this study was to check the expression behaviour of two small HSPs (HSP20 and HSP26.3) and a large HSP (HSP70) in wheat genotype (WH 730) and barley genotype (RD 31) exposed to two gradient temperature regimes (35^0C and 42^0C).

The seeds of heat-tolerant genotypes of wheat WH-730 and barley RD-31 were procured from ICAR-IIWBR, Karnal. Seed of both the genotypes were sterilized in 1% sodium hypochloride for 10 min, rinsed with distilled water three times and grown in pots under controlled condition of temperature (22^0C) and humidity (50 - 60%). After fifteen days of growth, the seedlings were exposed to heat stress at 35^0C and 42^0C for 2 hours.

After 2 hours of exposure to heat stress at 35^0C and 42^0C, leaves from stressed and unstressed seedling were harvested and immediately used for RNA extraction using TRIzol® Reagent (Ambion, USA) following the manufacturer's protocol. The purity and concentration of RNAs was checked with NanoDrop spectrophotometer, ND-1000 (NanoDrop Technologies, USA). Novagen® first strand cDNA synthesis kit (Merck KGaA, Germany) was used to prepare cDNA from isolated RNA samples according to the instruction manual.

To investigate the comparative role of HSPs in wheat and barley, quantitative real-time PCR was performed with the use of HSPs-specific primers and SYBR green dye. Specific primers for HSP26.3 and HSP70 were used (Grigorova *et al.,* 2011) and designed for HSP20 using (P-BLAST Pandey *et al.,* 2015).

qRT-PCR reaction was performed in a volume of 10 µl containing 10 ng/µl of cDNA, 5 µl of 2X SYBR Green Master Mix, 1 µl of HSP gene-specific forward and reverse primers. The thermal profile for qRT-PCR reaction was as follows: 94^0C for 5 min, following by 40 cycles of 95^0C for 15 s, 55^0C for 30 sec and 72^0C for 45 sec, with a final extension of 72^0C for 10 min. The reactions were performed in three biological replicates on CFX96™ Real-Time System (Bio-Rad, USA). β-actin gene were used as mock control for expression profiling HSP genes. The threshold cycle (Ct) valve of the technical triplicates was averaged and relative expression level of all the *HSP* genes were calculated using the comparative $2^{-\Delta\Delta Ct}$ method.

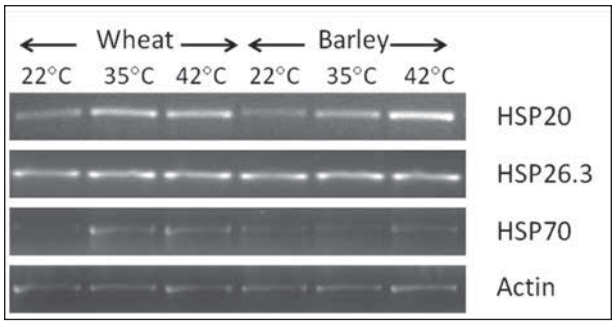

Fig. 1 RT-PCR Analysis of HSPs in relation to reference gene (β-actin) at different temperatures in wheat and barley.

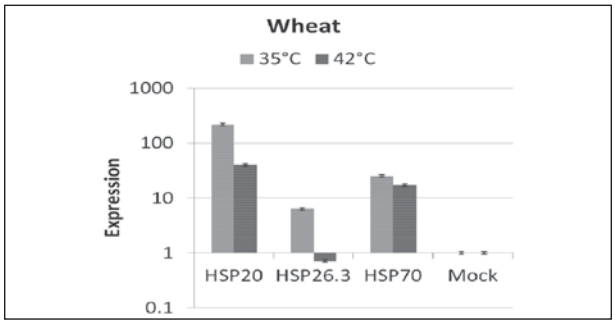

Fig. 2 Relative expression profile of HSPs at 35^{0}C and 42^{0}C using qRT-PCR in Wheat.

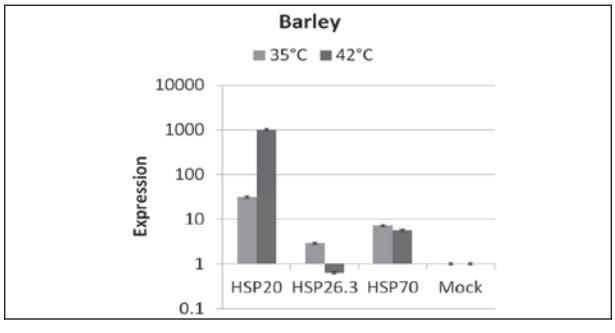

Fig. 3 Relative expression profile of HSPs at 35^{0}C and 42^{0}C using qRT-PCR in Barley.

Heat stress of 35^{0}C and 42^{0}C resulted in up regulation of HSP 20 and HSP 70 in wheat genotype WH 730 while expression of sHSP 26.3 was up regulated at 35^{0}C while down regulated at 42^{0}C compared to control (Fig 1). Among all, expression of HSP20 was more significant (>100 fold) at 35^{0}C compared to others (Fig 2). Both semi quantitative and quantitative expression analysis of all the HSPs showed similar expression pattern as of wheat (Fig 1). However, the expression of HSP20 was more significant (up to1000 fold) in barley compared to wheat (Fig 2). Expression pattern of HSP70 is in line with earlier work where they have shown up regulation (Hasan and Barthakur, 2014). Similarly, differential expression of HSP20 and HSP26.3 suggests their active involvement in modulating the heat tolerance in both wheat and barley (Pandey *et al.,* 2015; Chen *et al.,* 2014). Using semi quantitative and quantitative methods, we analysed the expression behaviour of two small HSPs (HSP20 and HSP26.3) and a large HSP (HSP70) in wheat genotype (WH 730) and barley genotype (RD 31) exposed to two gradient temperature regimes (35^{0}C and 42^{0}C).

Results indicate same differential expression pattern in both wheat and barley. However, an expression pattern of small HSP20 was higher in barley genotype as compared to wheat indicating its crucial role in barley. Further investigation on the mode of action and pathway in relation to these HSPs would open door for better understanding of heat stress tolerance in wheat and barley.

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Reference

1. Al-Whaibi MH. 2010. Plant heat shock proteins: a mini review. *Journal of King Saud University-Science-Science.* doi:10.1016/j.jksus.2010.06.022.
2. Chen X, S Lin, Q Liu, J Huang, W Zhang, J Lin, Y Wang, Y Ke, H He. 2014. Expression and interaction of small heat shock proteins (sHSPS) in rice in response to heat stress *Biochemical Biophysic Acta* **1844**(4): 818-828.
3. Efeoglu B. 2009. Heat shock protines and heat shock response. *G U Journal of Science.* **22**(2): 67-75.
4. Grigorva B, II Vaseva, K Demirevska, U Feller. 2011. Expression on of selected heat shock proteins after individually applied and combined drought and heat stress. *Acta Physiology Plantarum* **33**: 2041-2049.
5. Hasan M and S Barthakur. 2014. Hsp70 based gene expression biomarker shows growth stage specific genotypic diversity in Indian wheat (*Triticum aestivum* L.). *Cultivars Annuals of Agricultural Research News* **35**: 333-343.
6. Pandey B, A Kaur, OP Gupta, I Sharma, P Sharma. 2015. Identification of *HSP20* gene family in wheat and barley and their differential expression profiling under heat stress. *Applied Biochemistry and Biotechnology* **175**(5): 2427-2446.
7. Vinocur B and A Altman. 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Current Opinion in Biotechnology* **16**: 123-132.