CHAPTER II
REVIEW OF LITERATURE

Heat stress shows the alteration in various parameters not only in wheat but also in most of crop plants. The present review is to an attempt at bringing together some of the finding on molecular and biochemical analysis, which gives the useful information about effect of heat stress on various level of wheat plant.

The most significant factors for heat stress-related yield loss in cereals include the high-temperature-induced shortening of developmental phases, reduced light perception over the shortened life cycle and perturbation of the processes associated with carbon assimilation (transpiration, photosynthesis and respiration). Heat stress during vegetative growth causes many physiological and metabolic changes, including alterations in hormone homeostasis. Some of the heat-induced processes at the cell, organ and whole-plant levels may be hormone regulated; others may be the consequence of a new hormonal status, altered by heat stress (Hoffmann and Parsons 1991; Maestri et al., 2002).

2.1 ABIOTIC STRESS

Living organisms have evolved inherent mechanisms to cope up with the abiotic stresses. Plant’s immobility limits the range of their behavioural responses to different stresses and places a strong emphasis on cellular and physiological mechanisms of adaptation and protection. Some abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stresses, are serious threats to agriculture and abiotic stresses have become the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Rodziewicz et al., 2014).

Heat stress is one of the major problems in most cereal crops cultivated in South East Asia. Plant growth and yield are strongly affected by heat stress as it damages the function of cells, tissues, and whole plants. Wheat yield is mainly limited by heat stress and drought in arid, semi-arid, tropical, and sub-tropical areas. Difference in the tolerance level of the crop species to the elevated temperature have been found to be linked to the genetic makeup and several physiological, biochemical and molecular mechanisms respond to overcome heat stress. The optimum
temperature for wheat growth and cultivation is in the range of 18 to 24°C. Over these ranges (28 to 32°C) even 5 to 6 days short periods cause 20% or more yield losses in wheat (Stone and Nicolas, 1994). It is well documented that every 1°C increase in average temperature over 17 to 24°C during grain filling causes four percent yield reduction in grain weight (Acevedo et al., 1991).

2.2 EFFECT OF HEAT STRESS ON PHYSIOLOGICAL PARAMETERS

Wang et al. (2006) reported that wheat growth was affected more by heat stress in roots than in shoot. The cooling effect of no-till (NT) on soil may reduce the risk of root heat stress and benefit the yield compared with conventional tillage (CT). By reducing root heat stress especially during the grain growth stage and slightly increasing pre-seeding soil moisture, no-till increased above-ground biomass (33-160%) and grain yield (18-147%) every year.

Zhao et al. (2007) investigated the effects of high temperature regimes on the activities of key regulatory enzymes involved in starch and protein accumulation in grains of two winter wheat (Triticum aestivum L.) cultivars Yangmai 9 and Xuzhou 26 with different protein contents. Four day/night temperature regimes of 34°C/22°C, 32 °C/24 °C, 26 °C/14 °C and 24 °C/16 °C were established after anthesis, resulting in two daily temperature levels of 28 °C and 20 °C and two diurnal temperature differences of 12 °C and 8 °C. The activities of glutamine synthase (GS) in flag leaves and glutamate pyruvic amino transferase (GPT), sucrose synthase (SS), soluble starch synthase (SSS) and granule-bound starch synthase (GBSS) in grains were measured during the periods of grain filling. High temperature reduced both content and yield of starch in grains, while enhanced protein content and reduced protein yield in grains. High temperature significantly enhanced the activities of SS and GBSS on 14 days after anthesis (DAA). High temperature affected SSS slightly in Yangmai 9, but reduced SSS activity markedly in Xuzhou 26 on 14 DAA. However, at the middle and late stages of grain filling, high temperature reduced the activities of SS, GBSS and SSS significantly in the two wheat cultivars. High temperature reduced GPT activity in grains in the two wheat cultivars, but reduced GS activity in flag leaves of Yangmai 9 and enhanced GS activity of Xuzhou 26 on 14 DAA. In addition, under the same high temperature level, SS activity was higher at 34 °C/22 °C, whereas the activities of SSS and GBSS were higher at 32 °C/24 °C. Also, diurnal temperature differences affected GPT and GS activities differently between the two cultivars. Under optimum
temperature level, the activities of key enzymes for starch and protein synthesis were higher at 26 °C/14 °C. The activities of SS, SSS and GBSS significantly correlated with starch accumulation in grains, except for GBSS activity to starch content on 14 DAA. GPT activity was positively correlated with protein yield, and GS activity was negatively correlated with protein yield on 14 DAA, while the activities of both GPT and GS were negatively related to protein content in grains.

The yield and grain quality of two heat-stressed genotypes of bread (Triticum aestivum L.) and durum wheat (Triticum trugidum subsp. Durum) having different tolerance to high temperature after anthesis were investigated by Dais et al. (2008). Heat stress during grain filling, triggered grain shrinkage with a reduced weight and ultrastructural changes in the aleurone layer and in the endosperm cells. Heat stress also decreased the sedimentation index SDS, an effect associated with increased protein content in the grain but with decreased levels of essential amino acids. Although the responses to heat stress were similar among the Triticum genotypes, it is further suggested that during grain filling, high temperature might affect gluten strength, hence diminishing the wheat flour quality.

Farooq et al. (2011) reported that heat stress reduces plant photosynthetic capacity through metabolic limitations and oxidative damage to chloroplasts, with concomitant reductions in dry matter accumulation and grain yield. Genotypes expressing heat shock proteins are better able to withstand heat stress as they protect proteins from heat-induced damage. Heat tolerance can be improved by selecting and developing wheat genotypes with heat resistance. Nonetheless, improvement in grain yield under heat stress implies selecting genotypes for grain size and rate of grain filling. Integrating physiological and biotechnological tools with conventional breeding techniques will help to develop wheat varieties with better grain yield under heat stress during reproductive and grain-filling phases.

Wang et al. (2011) investigated the effect of pre-anthesis high-temperature acclimation on leaf physiology of winter wheat in response to post-anthesis heat stress. The results showed that both pre- and post-anthesis heat stresses significantly depressed flag leaf photosynthesis and enhanced cell membrane peroxidation, as exemplified by increased O₂ production rate and reduction in activities of antioxidative enzymes. However, under post-anthesis heat stress, plants with pre-anthesis high temperature acclimation (HH) showed much higher photosynthetic rates than those without pre-anthesis high-temperature acclimation (CH). Leaves of HH
plants exhibited a higher Chl a/b ratio and lower chlorophyll/carotenoid ratio and superoxide anion radical release rate compared with those of the CH plants. In addition, antioxidant enzyme activities in HH plants were significantly higher than in CH. Coincidently, expressions of photosynthesis-responsive gene encoding Rubisco activase B (RcaB) and antioxidant enzyme-related genes encoding mitochondrial manganese superoxide dismutase (Mn-SOD), chloroplastic Cu/Zn superoxide dismutase (Cu/Zn-SOD), catalase (CAT) and cytosolic glutathione reductase (GR) were all up-regulated under HH, whereas a gene encoding a major chlorophyll a/b-binding protein (Cab) was up-regulated by post-anthesis heat stress at 10 DAA, but was down-regulated at 13 DAA. The changes in the expression levels of the HH plants were more pronounced than those for the CH. The results indicated that pre-anthesis high-temperature acclimation could effectively alleviate the photosynthetic and oxidative damage caused by post-anthesis heat stress in wheat flag leaves, which was partially attributable to modifications in the expression of the photosynthesis-responsive and antioxidant enzymes-related genes.

Dhyani et al. (2013) reported comparative physiological changes in wheat genotypes viz., DBW-140, Raj-3765, PBW-574, K-0-307 and HS-240 and evaluated under timely and late sown conditions in rabi season. They observed that heat stress dramatically affects chlorophyll content and leaf area index (LAI) in sensitive genotypes, whereas proline and malondialdehyde (MDA) content were higher in tolerant genotypes under late sown conditions. Further, the heat susceptibility index (HIS) for 1,000-grain weight, grain weight and grain yield of wheat genotypes viz., HS 240 and K-0-307 was highest as compared with DBW 140, Raj 3765 and PBW 574 genotypes. This finding suggested that wheat genotypes were found to differ in their ability to respond to heat, thereby tolerance, which could be useful as genetic stock to develop wheat tolerant varieties in breeding programmes.

Feng et al. (2014) used two winter wheat (Triticum aestivum L.) cultivars with different sensitivities to heat stress (Jimai22 ‘JM22’, low sensitivity and Xinmai 26 ‘XM26’, high sensitivity) to study various aspects of photosynthetic characteristics during the grain filling stage under heat stress. The results showed that photosynthesis rates (Pn) in flag leaves of XM26 decreased faster than in JM22 under heat stress during the grain-filling stage. Pn decreased more rapidly under heat stress than without stress, by up to 69.9% and 59.3%, respectively, at 10 days following heat stress (10 DAS). This decline of Pn was not caused by heat-induced stomatal
limitation, but rather by a decline in Rubisco activity and a functional drop in photosystem II (PSII). After heat stress, the grain yield of JM22 decreased by 6.41%, but XM26 decreased by 11.43%, when compared with their respective controls. Heat stress also caused an alteration of mesophyll cell ultrastructure. Injury caused by heat stress to organelles in XM26 was more severe than JM22. Moreover, the JM22 cultivar showed some self-repair capacity following heat stress injury. These results indicated that declines in photosynthetic performance caused by heat stress were cultivar dependent. Compared with XM26, the JM22 cultivar had superior heat stability in terms of PSII function and carboxylation activity, both of which are susceptible to heat stress.

Ali et al. (2016) studied six genotypes for pre-anthesis growth stage (80 days after sowing), one set plants of were subjected to heat stress treatment of 35 to 40°C and 10 to 14th day and night, 50 to 70% relative humidity and illumination of 335μ mol m²s⁻² in glass house. After high temperature treatment for 3 h daily for five consecutive days, pots were moved back to normal temperature (average day/night temperature 30 ± 8 and 13 ± 5°C) conditions in open atmosphere. After heat stress treatment, flag leaf from the control and stressed plants were sampled for analysis of proline, chlorophyll a, chlorophyll b content and membrane thermos-stability. Analysis of variance for agronomic parameters revealed significant (p≤0.05) differences among wheat genotypes for days to 50% flowering, plant height, number of grains per spike, number of spikelets per spike and seed yield per pot. The proline accumulation could be used as markers in the breeding program for the development of heat tolerant wheat genotypes. Overall PSK-91, LU-26S and SARSABZ showed best performance under imposed heat stress for physiological and yield parameters.

2.3 MOLECULAR ANALYSIS

2.3.1 Transcriptome profiling

The identification of candidate genes influencing any important trait can be approached through an analysis of complementary DNA (cDNA), copies of messenger RNA (mRNA). Devoid of intronic and intergenic sequences, whose biological significance is still obscure, these mRNAs represent only a small percentage of the total genome (about 1-3% in eukaryotes). However, they do contain valuable information on gene activity since they correspond to the proteins expressed in a specific tissue and responsible for the identity of that tissue. The formal
identification of genes proceeds first by sequencing the expressed sequences tags (ESTs), partial sequences from either end of the complementary cDNA, and then by cloning the complete gene. Sequence information from ESTs can be used for deciphering the function and the organization of the genome, particularly if the strategies used have the goal of determining the order and time of gene expression, which represents another level of complexity in the analysis of biological organisms.

Qin et al. (2008) analyzed genome-wide gene expression profiles in the leaves of two wheat genotypes, namely, heat susceptible Chinese Spring (CS) and heat tolerant TAM107 (TAM). A total of 6560 (~10.7%) probe sets displayed 2-fold or more changes in expression in at least one heat treatment (false discovery rate, FDR, \( \alpha = 0.001 \)). Except for heat shock protein (HSP) and heat shock factor (HSF) genes, these putative heat responsive genes encode transcription factors and proteins involved in phyto-hormone biosynthesis/signalling, calcium and sugar signal pathways, RNA metabolism, ribosomal proteins, primary and secondary metabolisms, as well as proteins related to other stresses. A total of 313 probe sets were differentially expressed between the two genotypes, which could be responsible for the difference in heat tolerance of the two genotypes. Moreover, 1314 were differentially expressed between the heat treatments with and without pre-acclimation, and 4533 were differentially expressed between short and prolonged heat treatments. The differences in heat tolerance in different wheat genotypes may be associated with multiple processes and mechanisms involving HSPs, transcription factors, and other stress related genes. Heat acclimation has little effects on gene expression under prolonged treatments but affects gene expression in wheat under short-term heat stress.

The transcriptome of developing caryopses from hexaploid wheat (Triticum aestivum, cv. Hereward) was determined using Affymetrix wheat GeneChip® oligonucleotide arrays which have probes for 55,052 transcripts by Wan et al. (2008). Of these, 14,550 showed significant differential regulation in the period between 6 and 42 days after anthesis (daa). Large changes in transcript abundance were observed which were categorised into distinct phases of differentiation (6–10 daa), grain fill (12–21daa) and desiccation/maturation (28–42 daa) and were associated with specific tissues and processes. A similar experiment on developing caryopses grown with dry and/or hot environmental treatments was also analysed, using the profiles established in the first experiment to show that most environmental treatment effects on
transcription were due to acceleration of development, but that a few transcripts were specifically affected. Transcript abundance profiles in both experiments for nine selected known and putative wheat transcription factors were independently confirmed by real time RT-PCR. These expression profiles confirm or extend knowledge of the roles of the known transcription factors and suggest roles for the unknown ones. It has been demonstrated how it can be used to distinguish general developmental shifts from specific effects of treatments on gene expression and to diagnose the probable tissue specificity and role of transcription factors.

Aprile et al. (2010) reported that drought and heat stress were the most important factors limiting plant development and crop productivity. Two approaches were used after imposing different stress conditions (heat, drought, and combined stress): the microarray and the cDNA-AFLP analysis. Microarray transcriptome analysis was carried out using the Affymetrics 61K wheat chip on the biological replicates of mRNA extracted from flag leaf during booting stage, a phase more susceptible to drought and heat stress. Seven gene clusters were identified grouping genes with similar expression trends. This analysis defined six over-represented functional categories: metabolism, energy, and protein fate, protein with binding function or cofactor requirements, cell rescue/defence and virulence, and interaction with environment. cDNA-AFLP analysis, that is often used as a gene discovery tool, was also performed on the same samples. About 1000 cDNA fragments, ranging in size from 100 to 1900 bp, were reproducibly detected. This allowed the identification of 42 genes annotated as EST or belonging to different functional categories: response to stress, transcription regulation, transport, receptor proteins, chaperones, lipid remodelling, metabolic processes, DNA integration, unknown biological process. Moreover, 10 cDNAs that did not show significant matches in the database were identified, thus corresponding to durum wheat new sequences. In total 24cDNAs were expressed under combined stress conditions and among these some are also expressed under drought or heat stress. These analyses highlight an active modulation of gene expression in response to drought and/or heat stress.

The macroarray of approximately 2,500 maize (Zea mays L.) cDNAs was used for transcriptome profiling by Andjelkovic and Micic (2011), in response to single and simultaneous application of water and high temperature stress of maize developing kernels at 15 days after pollination. All stress treatments (water stress-WS, heat stress-HS and their combined application-CS) induced changes in expression of
106 transcripts with 54 up-regulated and 52 down-regulated. There were 11 up-regulated and 15 down-regulated transcripts in common for all three stresses. Although these common transcripts showed existence of a mutual mechanism in stress response, the 23 transcripts induced only in CS indicate that plants responded in a different manner when exposed to simultaneous effects of both stresses. A glimpse of functions regulated under WS, HS and CS is provided, and also the common and different responses between individual and simultaneous stresses.

To elucidate the effect of high temperature, Chauhan et al. (2011) gave heat shock at 37 and 42 °C for 2 h to wheat plants (Triticum aestivum cv. CPAN 1676) and responsive genes were identified through PCR-Select Subtraction technology. Four subtractive cDNA libraries, including three forward and one reverse subtraction, were constructed from three different developmental stages. A total of 5,500 ESTs were generated and 3,516 high quality ESTs submitted to Genbank. More than one-third of the ESTs generated fall in unknown/no hit category upon homology search through BLAST analysis. Differential expression was confirmed by cDNA macroarray and by northern/RTPCR analysis. Expression analysis of wheat plants subjected to high temperature stress, after 1 and 4 days of recovery, showed fast recovery in seedling tissue. However, even after 4 days, recovery was negligible in the developing seed tissue after 2 h of heat stress. Ten selected genes were analyzed in further detail including one unknown protein and a new heat shock factor, by quantitative real-time PCR in an array of 35 different wheat tissues representing major developmental stages as well as different abiotic stresses. Tissue specificity was examined long with cross talk with other abiotic stresses and putative signalling molecules. The results obtained contribute towards understanding the regulation of genes at different developmental stages in wheat crucial to withstanding and recovery from heat stress.

Jung et al. (2012) performed genome-wide transcriptome analysis of rice to identify immediate early genes strongly induced by high temperature. The effects of high temperature (37°C) treatments (for 0.5 or 1h) of seedlings relative to untreated controls (28°C) were compared using the NSF45K array. They observed that a large portion of the genes relating to the prolonged heat response were also associated with responses to other abiotic stresses such as drought, salt, cold, and submergence.

Li et al. (2013) conducted long-term heat stress treatment (38°C/30°C, day/night, for 50 days) in the switchgrass (Panicum virgatum) cultivar Alamo. Total RNA from control and heat stress samples were used for transcriptome analysis with
switchgrass Affy-metrix gene-chips. Following normalization and pre-processing, 5365 probe sets were identified as differentially expressed using a 2-fold cut-off. Of these, 2233 probe sets (2000 switchgrass unigenes) were up-regulated, and 3132 probe sets (2809 unigenes) were down-regulated. Comparative transcriptome analysis in response to heat stress among four monocots – switchgrass, rice, wheat and maize identified 16 common genes, most of which were associated with protein refolding processes. These core genes will be valuable biomarkers for identifying heat sensitive plant germplasm since they are responsive to both short duration as well as chronic heat stress treatments, and are also expressed at different plant growth stages and tissue types.

Zhang et al. (2013) studied that flag leaf is one of the key photosynthesis organs during rice reproductive stage. A time course microarray analysis of rice flag leaf was done after 40 °C treatment for 0 min, 20 min, 60 min, 2 h, 4 h, and 8 h. The identified significant heat responsive genes were mainly involved in transcriptional regulation, transport, protein binding, antioxidant, and stress response. KMC (K-means clustering) analysis discovered the time-dependent gene expression pattern under heat. MapMan analysis demonstrated that under heat treatment, Hsp genes and genes involved in glycolysis and ubiquitin-proteasome were enhanced, and genes involved in TCA, carotenoid, di-hydro flavonol and anthocyanin metabolisms and light-reaction in the photosynthesis were widely repressed. Meanwhile, some rate-limiting enzyme genes in shikimate, lignin and mevalonic acid metabolisms were up-regulated, revealing the importance of maintaining specific secondary metabolites under heat stress. This study provides more understanding regarding heat response in rice flag leaf and provided good candidate genes for crop improvement.

Alghabari et al. (2014) conducted factorial pot experiments to compare the responses of GA-sensitive and GA-insensitive reduced height (Rht) alleles in wheat for susceptibility to heat and drought stress during booting and anthesis. Grain set (grains/spikelet) of near-isogenic lines (NILs) was assessed following three day transfers to controlled environments imposing day temperatures (t) from 20 to 40 °C. Transfers were during booting and/or anthesis and pots maintained at field capacity (FC) or had water withheld. Logistic responses (y = c/1+e^(b(t-m))) described declining grain set with increasing t, and t5 was that fitted to give a 5% reduction in grain set. Averaged over NIL, t5 for anthesis at FC was 31.7 ± 0.47 °C (S.E.m., 26 d.f.). Drought at anthesis reduced t5 by <2 °C. Maintaining FC at booting conferred
considerable resistance to high temperatures \(t_5 = 33.9 \, ^\circ\text{C}\) but booting was particularly heat susceptible without water \(t_5 = 26.5 \, ^\circ\text{C}\). In one background (cv. Mercia), for NILs varying at the \textit{Rht-D1} locus, there was progressive reduction in \(t_5\) with dwarfing and reduced gibberellic acid (GA) sensitivity \(\text{Rht-D1a}\), tall, 32.7 ± 0.72; \textit{Rht-D1b}, semi-dwarf, 29.5 ± 0.85; \textit{Rht-D1c}, severe dwarf, 24.2 ± 0.72). This trend was not evident for the \textit{Rht-B1} locus or for \textit{Rht-D1b} in an alternative background (Maris Widgeon). The GA-sensitive severe dwarf \textit{Rht12} was more heat tolerant \(t_5 = 29.4 \pm 0.72\) than the similarly statured GA-insensitive \textit{Rht-D1c}. The GA-sensitive, semi-dwarfing \textit{Rht8} conferred greater drought tolerance in one experiment. Despite the effects of \textit{Rht-D1} alleles in Mercia on stress tolerance, the inconsistency of the effects over background and locus led to the conclusion that semi-dwarfing with GA-insensitivity did not necessarily increase sensitivity to stress at booting and flowering. In comparison with effects of semi-dwarfing alleles, responses to heat stress are much more dramatically affected by water availability and the precise growth stage at which the stress is experienced by the plants.

Kumar \textit{et al.} (2014) constructed a transcript dataset of wheat \textit{(Triticum aestivum)} containing 23,470 non-redundant transcripts with an average length of 746 bp. When compared with wheat full-length cDNAs and Plant Cyc data sources, 12,992 and 8,480 were respectively aligned \((e<1^{\text{e-5}})\). 785 transcripts were up-regulated and 431 transcripts were down-regulated with fold 2 threshold and FDR value < 0.01. Seventy-eight 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 \textit{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. They observed that response to the heat-stress, protein-folding, oxidation-reduction process, photosynthesis, flower development and response to 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.
Wei et al. (2014) studied that transcriptome analysis to search grain traits and elucidate their genetic regulation. Two cDNA libraries from the developing grain and leaf-stem components of bread wheat cultivar, Nongda211, were sequenced using Roche/454 technology. There were 1061274 and 1516564 clean reads generated from grain and leaf-stem, respectively. A total of 61393 high-quality unigenes were obtained with an average length of 1456 bp after de novo assembly. The analysis of the 61393 unigenes involved in the biological processes of the grain showed that there were 7355 differentially expressed genes up-regulated in the grain library. Gene ontology enrichment and the Kyoto Encyclopaedia of Genes and Genomes pathway enrichment analysis showed that many transcription products and transcription factors associated with carbohydrate and protein metabolism were abundantly expressed in the grain.

Frey et al. (2015) investigated the transcriptomic response of temperate maize to linearly increasing heat levels and to identify genes associated with heat tolerance in a set of genotypes with contrasting heat tolerance behaviour. Strong phenotypic differences with respect to heat tolerance were observed between the examined maize inbred lines on a multi-trait level. They identified 607 heat responsive genes as well as 39 heat tolerance genes.

Although many physiological studies have contributed to understand heat responses during anthesis, the most heat-sensitive stage, molecular data are still largely lacking. Gonzalez-Schain et al. (2015) studied on RNA-sequencing approach of heat- and control-treated reproductive tissues during anthesis was carried out using N22, one of the most heat-tolerant rice cultivars. This analysis revealed that expression of genes encoding a number of transcription factor families, together with signal transduction and metabolic pathway genes is repressed. On the other hand, expression of genes encoding heat shock factors and heat shock proteins was highly activated. Many of these genes are predominantly expressed at late stages of anther development. Further physiological experiments using heat-tolerant N22 (IRGC accession 19379) and two sensitive cultivars (IR64 and N22 IRGC accession 6264) suggested that reduced yield in heat-sensitive plants might be associated with poor pollen development or production in anthers prior to anthesis. In parallel, induction levels of a set of heat-responsive genes in these tissues correlated well with heat tolerance.
Liu et al. (2015) identified that heat, drought and their combination dramatically reduce wheat yield and quality, but the molecular mechanisms underlying wheat tolerance to extreme environments, especially stress combination, are largely unknown. As an allohexaploid, wheat consists of three closely related sub-genomes (A, B, and D), and was reported to show improved tolerance to stress conditions compared to tetraploid. But so far very little is known about how wheat coordinates the expression of homeologous genes to cope with various environmental constraints on the whole-genome level. To explore the transcriptional response of wheat to the individual and combined stress, they performed high-throughput transcriptome sequencing of seedlings under normal condition and subjected to drought stress (DS), heat stress (HS) and their combination (HD) for 1 h and 6 h, and presented global gene expression reprograms in response to these three stresses. Gene Ontology (GO) enrichment analysis of DS, HS and HD responsive genes revealed an overlap and complexity of functional pathways between each other. Moreover, 4,375 wheat transcription factors were identified on a whole-genome scale based on the released scaffold information by IWGSC, and 1,328 were responsive to stress treatments. The regulatory network analysis of HSFs and DREBs implicated they were both involved in the regulation of DS, HS and HD response and indicated a cross-talk between heat and drought stress. Finally, approximately 68.4 % of homeologous genes were found to exhibit expression partitioning in response to DS, HS or HD, which was further confirmed by using quantitative RT-PCR and Nullisomic-Tetrasomic lines. A large proportion of wheat homeologs exhibited expression partitioning under normal and abiotic stresses, which possibly contributes to wide adaptability and distribution of hexaploid wheat in response to various environmental constraints.

Xin et al. (2015) studied the mechanism of enhanced tolerance to post-anthesis high temperature stress induced by pre-anthesis heat priming in wheat (Triticum aestivum L.). Genome-wide gene expression profiles by Affymetrix Wheat Genome Chip were performed in the leaf after pre-anthesis heat priming and post-anthesis high temperature stress. Physiological analyses indicated that primed plants showed higher rates of photosynthesis, activities of antioxidant enzymes and lower cell membrane oxidative damage, suggesting a less high temperature damage in the primed plants. Eighty-eight gene probes spots were regulated after both pre-anthesis heat priming and post-anthesis high temperature stress, and the probes were expressed differently.
in primed plants from those in non-primed plants. Transcriptome analyses revealed up-regulation of the genes that encoded sensing and signaling, heat shock proteins, redox homeostasis, and down-regulation of the genes that encoded metabolism. The up-regulation and down-regulation might play protective roles in coping with the post-anthesis high temperature stress in the pre-anthesis heat primed plants compared with non-primed plants. It is concluded that pre-anthesis heat priming could initiate the acclimation responses at both transcriptome levels for enhancing heat tolerance at later stages in wheat plants.

Effects of heat priming applied to the first generation on tolerance of the successive generation to post-anthesis high temperature stress were investigated by Wang et al. (2016). Compared with the progeny of non-heat primed plants (NH), the progeny of heat-primed plants (PH) possessed higher grain yield, leaf photosynthesis and activities of antioxidant enzymes and lower cell membrane damage under high temperature stress. In the transcriptome profile, 1430 probes showed obvious difference in expression between PH and NH. These genes were related to signal transduction, transcription, energy, defence, and protein destination and storage, respectively. The gene encoding the lysine-specific histone demethylase 1 (LSD1) which was involved in histone demethylation related to epigenetic modification was up-regulated in the PH compared with NH.

Wheat seed development is an important physiological process of seed maturation and directly affects wheat yield and quality. Yu et al. (2016) performed dynamic transcriptome microarray analysis of an elite Chinese bread wheat cultivar (Jimai 20) during grain development using the GeneChip Wheat Genome Array. Grain morphology and scanning electron microscope observations showed that the period of 11–15 days post-anthesis (DPA) was a key stage for the synthesis and accumulation of seed starch. Genome-wide transcriptional profiling and significance analysis of microarrays revealed that the period from 11 to 15 DPA was more important than the 15–20 DPA stage for synthesis and accumulation of nutritive reserves. Series test of cluster analysis of differential genes revealed five statistically significant gene expression profiles. Gene ontology annotation and enrichment analysis gave further information about differentially expressed genes, and Map-Man analysis revealed expression changes within functional groups during seed development. They performed gene co-expression network analysis to identify genes that play vital roles in seed development and identified several key genes involved in
important metabolic pathways. The transcriptional expression of eight key genes involved in starch and protein synthesis and stress defence was further validated by qRT-PCR.

2.3.2 Expression analysis

Compared to rice, wheat exhibits characteristic growth habits and contains complex genome constituents. To assess global change in gene expression patterns in the wheat life cycle, Ogihara et al. (2002) conducted large scale analysis of expressed sequence tags (ESTs) in common wheat. Ten wheat tissues were used to construct cDNA libraries; crown and root from 14-days-old seedlings; spikelets from early and late flowering stages; spike at the booting stage, heading date and flowering date; pistil at the heading date; and seeds at 10 and 30 says post-anthesis. Several thousand colonies were randomly selected from each of these 10 cDNA libraries and sequenced from both 5’ and 3’ ends. Consequently, a total of 116 232 sequences were accumulated and classified into 25 971 contigs based on sequence homology. By computing abundantly expressed ESTs, correlated expression patterns of genes across the tissues were inferred from global gene expression patterns. Genes with similar functions were grouped with one another by clustering gene expression profiles. This technique might enable estimation of the function of anonymous genes. Multi dimensional analysis of EST data that is analogous to the microarray experiments may offer new approaches to functional genomics of plants.

Hu et al. (2010) investigated the mechanism of small heat shock proteins associated with maize tolerance to combined drought and heat stress. The expression of Hsp was analyzed through RT-PCR. The results showed that the expression levels of Hsps were more enhanced by heat and combined drought and heat stress than by control and drought.

Khurana et al. (2011) elucidated the effects of high temperatures on wheat plants (Triticum aestivum cv. CPAN 1676) which were given heat shocks at 37°C and 42°C for two hours, and responsive genes were identified through PCR-Select Subtraction technology. Four subtractive cDNA libraries, including three forward and one reverse subtraction, were constructed from three different developmental stages, viz. young seedling, pre-pollinated flower and developing grains. A total of 5500 ESTs were generated and 3516 high quality ESTs were submitted to Genbank. More than one third of the ESTs generated fall in unknown/no hit categories upon a
homology search through BLAST analysis. A large number of high temperature responsive genes have been identified and characterized. Reverse subtraction analysis in developing grains showed extensive transcriptional changes upon heat stress as revealed by comparative analysis with forward subtraction. Differential expression was confirmed by cDNA macroarray and by northern/RT-PCR analysis. Expression analysis of wheat plants subjected to high temperature stress, after one and four days of recovery, showed fast recovery in seedling tissues. However, recovery was small in the developing seed tissue after two hours of heat stress. Ten selected genes were analysed in further detail by quantitative real-time PCR in an array of 35 different wheat tissues representing major developmental stages as well as different abiotic stresses. Tissue specificity was examined along with cross talk with other abiotic stresses and putative signalling molecules. The results obtained contribute towards understanding the regulation of genes at different developmental stages in wheat crucial to withstanding and recovery from heat stress.

Jung et al. (2012) identified 710 genes exhibiting at least 2-fold up-regulation at both, for 0.5 or 1 hr, time points. The comparison of this dataset with other publicly available rice datasets under heat stress [i.e., for 10 and 30 min (early heat response), and 10 h at 42°C (late heat response)] identified 244 genes and 238 genes at least 2 fold up regulated during the early and late heat responses, respectively. Moreover, they defined 244 genes as early heat stress responsive group and 238 genes as prolonged heat stress responsive group. Gene ontology (GO) enrichment analysis revealed that a chaperone-mediated protein folding cofactor was the most significantly over-represented GO term associated with the prolonged heat response.

Kumar et al. (2011) reported cloning of HSP90 gene of 2,323 bp from C-306 cultivar of wheat having ORF from62 to 2,164 bp encoded for 700 amino acids. Quantitative real time expression analysis of HSP90 gene in C-306showed 1.5, 1.2, 2.5 fold (in root), 4.5, 4.3 and 6.5 fold increase (in flag leaf) in the transcript level at pollination, milky dough and seed hardening stages. HSP90 transcript level was observed low in root as well as shoot of susceptible cultivar (PBW343) at different stages of growth. A significant difference in the fold expression of HSP90 was observed in C-306 and PBW343 against differential heat shock. An altered expression of H2O2 and decline in proline accumulation was observed in C-306 at different stages of growth.
Rampino et al. (2012) reported effect of heat, drought and combined stress on the expression of a group of genes that are up-regulated under these conditions in durum wheat (*Triticum turgidum* subsp. durum) plants. Modulation of gene expression was studied by cDNA-AFLP performed on RNAs extracted from flag leaves. By this approach, they identified several novel durum wheat genes whose expression is modulated under different stress conditions. They focused on a group of hitherto undescribed up-regulated genes in durum wheat, among these, 7 are up-regulated by heat; 8 by drought stress; 15 by combined heat and drought stress; 4 are up-regulated by both heat and combined stress; and 3 by both drought and combined stress. The functional characterization of these genes will provide new data that could help the developing of strategies aimed at improving durum wheat tolerance to field stress.

Zhang et al. (2012a) provided a time course gene expression profile of rice panicle at anther developmental stage 8 (dough grain stage) after 40°C treatment for 0 min, 20 min, 60 min, 2 h, 4 h, and 8 h. The identified differentially expressed genes were mainly involved in transcriptional regulation, transport, cellular homeostasis, and stress response. The predominant transcription factor gene families responsive to heat stress were Hsf, NAC, AP2/ERF, WRKY, MYB, and C2H2.KMC(K-means clustering) analysis discovered the time-dependent gene expression pattern under heat stress. The motif co-occurrence analysis on the promoters of genes from an early up-regulated cluster showed the important roles of GCC box, HSE, ABRE, and CE3 in response to heat stress. The regulation model central to ROS combined with transcriptome and ROS quantification data in rice panicle indicated the great importance to maintain ROS balance and the existence of wide cross-talk in heat response. The present study increased understanding of the heat response in rice panicle and provided good candidate genes for crop improvement.

Zhang et al. (2012b) reported expression profile of young rice panicle under heat stress of 40°C on the whole genome level using rice 44k oligo-array. The results demonstrated that carbohydrate metabolism and stress-related genes consist of major group that were affected during the time course. Hsps, Hsfs, enzymes responsible for carbon partitioning, secondary metabolism, cell wall morphogenesis-related genes, and ETC were all sensitive to heat.

Li et al. (2013) worked on differential expression of 42 randomly selected genes and validated using RT-PCR. Rice orthologs were retrieved for 78.7% of the
heat stress responsive switchgrass probesets. Gene ontology (GOs) enrichment analysis using AgriGO program showed that genes related to ATPase regulator, chaperone binding, and protein folding was significantly up-regulated. GOs associated with protein modification, transcription, phosphorus and nitrogen metabolic processes were significantly down-regulated by heat stress. Plausible connections were identified between the identified GOs, physiological responses and heat response phenotype observed in switchgrass plants.

Rerksiri et al. (2013) experimented on six highly heat-responsive genes identified in rice by microarray data analysis. The qRT-PCR analysis confirmed that the expression of these six genes were highly heat inducible promoters of the three highly heat inducible genes (OsHsfB2cp, PM19p, and Hsp90p) which were used to drive GUS gene expression in rice. The three promoters exhibited similar high activity level in rice leaf under heat, but OsHsfB2cp and PM19p showed much higher activities in panicles under heat stress. They confirmed that the OsHsfB2c and PM19 promoters were highly heat inducible.

Hasan and Barthakur (2014) examined terminal stress in wheat during grain filling period which negatively affects growth, yield and grain quality, causing tremendous economic losses in many wheat growing regions of the world. Abiotic stress tolerance is a complex phenomenon and controlled by multiple defense genes including heat shock proteins and heat shock factors. The 70 Kd heat shock protein (Hsp70), is a molecular chaperone expressed during an intriguing array of developmental processes in plants and involved in preserving cellular functions under adverse environmental conditions. They carried out an extensive growth stage specific expression profiling of a wheat Hsp70 gene under rainfed field conditions in generative stages and under high temperature stress in seedling stage in ten diverse cultivars. The differential induction indicated an important role for Hsp70 and potential application as gene expression biomarker in identifying phenophasic and developmental stage specific vulnerability in contrasting genotypes. The Hsp70 gene expression biomarker will also be useful tool for identifying and validating wheat cultivars and germplasm collections for early and terminal stress tolerance traits.

Hu et al. (2014) analyzed genome-wide gene expression profiles in the leaves of two tall fescue genotypes, heat tolerant ‘PI578718’ and heat sensitive ‘PI234881’ using high-throughput RNA sequencing. A total of 262 million high-quality paired-end reads were generated and assembled into 31,803 unigenes with an average length
of 1,840 bp. Of these, 12,974 unigenes showed different expression patterns in response to heat stress and were categorized into 49 Gene Ontology functional subcategories. In addition, the variance of enrichment degree in each functional subcategory between PI578718 and PI234881 increased with increasing treatment time. Cell division and cell cycle genes showed a massive increase in transcript abundance in heat-stressed plants and more activated genes were detected in PI 578718 by Kyoto Encyclopedia of Genes and Genomes pathways analysis. The assembled transcriptome of tall fescue could serve as a global description of expressed genes and provide more molecular resources for future functional characterization analysis of genomics in cool-season turfgrass in response to high-temperature. Increased cell division, LMW/HMW-HSP, dissimilation and antioxidant transcript amounts in tall fescue were correlated with successful resistance to high temperature stress.

To elucidate the molecular basis of high temperature response in pearl millet, 12 days old seedlings of *P. glaucum* cv. 841A were subjected to heat stress at 46°C for different time duration (30 min, 2, 4, 8, 12 and 24 h) and a forward subtractive cDNA library was constructed from pooled RNA of heat stressed seedlings by James *et al.* (2014). A total of 331 high quality Expressed Sequence Tags (ESTs) were obtained from randomly selected 1050 clones. Sequences were assembled into 103 unique sequences consisting of 37 contigs and 66 singletons. Of these, 92 unique sequences were submitted to NCBI dbEST database. Gene ontology through RGAP data base and BLASTx analysis revealed that about 18% of the ESTs showed homology to genes for “response to abiotic and biotic stimulus”. About 2% of the ESTs showed no homology with genes in dbEST, indicating the presence of uncharacterized candidate genes involved in heat response in *P. glaucum*. Differential expression of selected genes (hsp101 and CRT) from the SSH library were validated by qRT-PCR analysis. The ESTs thus generated are a rich source of heat stress responsive genes, which can be utilized in improving thermo tolerance of other food crops.

Xue *et al.* (2014a) studied heat shock factors (Hsfs) that play a central regulatory role in acquired thermo tolerance. To understand the role of the major molecular players in wheat adaptation to heat stress, the Hsf family was investigated in *Triticum aestivum*. Bioinformatic and phylogenetic analyses identified 56 TaHsf members, which were classified into A, B, and C classes. Many TaHsfs were
constitutively expressed. Subclass A₆ members were predominantly expressed in the endosperm under non-stress conditions. Upon heat stress, the transcript levels of A₂ and A₆ members became the dominant Hsfs, suggesting an important regulatory role during heat stress. Many TaHsfA members as well as B₁, C₁, and C₂ members were also up-regulated during drought and salt stresses. The heat-induced expression profiles of many heat shock protein (Hsp) genes were paralleled by those of A₂ and A₆ members. Trans-activation analysis revealed that in addition to TaHsfA members (A₂b and A₄e), over expression of TaHsfC₂a activated expression of TaHsp promoter-driven reporter genes under non-stress conditions, while TaHsfB₁b and TaHsfC₁b did not. Functional heat shock elements (HSEs) interacting with TaHsfA₂b were identified in four TaHsp promoters. Promoter mutagenesis analysis demonstrated that an atypical HSE (GAACATTTTGGAA) in the TaHsp₁₇ promoter is functional for heat-inducible expression and trans-activation by Hsf proteins. The trans-activation of Hsp promoter-driven reporter genes by TaHsfC₂a also relied on the presence of HSE. An activation motif in the C-terminal domain of TaHsfC₂a was identified by amino residue substitution analysis. These data demonstrate the role of HsfA and HsfC₂ in regulation of Hsp genes in wheat.

Xue et al. (2014b) investigated the role of TaHsfA₆f, a member of the A₆ subclass of heat shock transcription factors, in the regulation of heat stress protection genes in *Triticum aestivum* (bread wheat). Expression analysis showed that TaHsfA₆f was expressed constitutively in green organs but was markedly up-regulated during heat stress. Over expression of TaHsfA₆f in transgenic wheat using a drought-inducible promoter resulted in up-regulation of heat shock proteins (HSPs) and a number of other heat stress protection genes that included some previously unknown Hsf target genes such as Golgi anti-apoptotic protein (GAAP) and the large isoform of *Rubisco activase*. Transgenic wheat plants over expressing TaHsfA₆f showed improved thermo-tolerance. Trans-activation assays showed that TaHsfA₆f activated the expression of reporter genes driven by the promoters of several HSP genes (*TaHSP₁₆.₈, TaHSP₁₇, TaHSP₁₇.₃*, and *TaHSP₉₀.₁-A₁*) as well as *TaGAAP* and *TaRof₁* (a co-chaperone) under non-stress conditions. DNA binding analysis revealed the presence of high-affinity TaHsfA₆f-binding heat shock element-like motifs in the promoters of these six genes. Promoter truncation and mutagenesis analyses identified TaHsfA₆f-binding elements that were responsible for trans activation of *TaHSP₉₀.₁-A₁* and *TaGAAP* by TaHsfA₆f. These data suggested that TaHsfA₆f was a
transcriptional activator that directly regulated TaHSP, TaGAAP, and TaRof1 genes in wheat and its gene regulatory network had a positive impact on thermo-tolerance.

Yang et al. (2014) studied comprehensive bioinformatics analysis in wheat A and D genome donors, Triticum urartu and Aegilops tauschii, the genomic sequences of which were published recently. The results showed that 13 Hsf proteins were identified in both T. urartu and Ae. tauschii and they could be classified into three groups according to structure; seven Hsfs belonged to group A; two to group B; and three to group C. Expression analyses of these Hsf genes in different tissues of T. urartu and in the response to heat stress were conducted using quantitative RT-PCR. Several Hsf genes in group A (Tuhsf03, Tuhsf05, Tuhsf06, Tuhsf10) had 19–292-fold increases in transcript levels versus the control in different tissues of T. urartu and could be induced by heat stress, while the transcripts of group B and group C Hsf genes could hardly be detected. These results provide important information for cloning, expression, and functional studies of Hsfs in wheat.

To identify heat stress response (HSR) genes, Dong et al. (2015) profiled gene expression in two Chinese cabbage inbred lines with different thermo tolerances, Chiifu and Kenshin. Many genes exhibited >2-fold changes in expression upon exposure to 0.5–4 h at 45°C (high temperature, HT): 5.2% (2,142 genes) in Chiifu and 3.7% (1,535 genes) in Kenshin. The most enriched GO (Gene Ontology) items included ‘response to heat’, ‘response to reactive oxygen species (ROS)’, ‘response to temperature stimulus’, ‘response to abiotic stimulus’, and ‘MAPKKK cascade’. In both lines, the genes most highly induced by HT encoded small heat shock proteins (Hsps) and heat shock factor (Hsf)-like proteins such as HsfB2A (Bra029292), whereas high-molecular weight Hsps were constitutively expressed. Other upstream HSR components were also up-regulated: ROS-scavenging genes like glutathione peroxidase 2 (BrGPX2, Bra022853), protein kinases, and phosphatases. Among heat stress (HS) marker genes in Arabidopsis, only export in 1A (XPO1A) (Bra008580, Bra006382) can be applied to B. rapa for basal thermo tolerance (BT) and short-term acquired thermo tolerance (SAT) gene. CYP707A3 (Bra025083, Bra021965), which was involved in the dehydration response in Arabidopsis, was associated with membrane leak again both lines following HS. Although many transcription factors (TF) genes, including DREB2A (Bra005852), were involved in HS tolerance in both lines, Bra024224 (MYB41) and Bra021735 (ab ZIP/AIR1 [Anthocyanin-Impaired-Response-1]) were specific to Kenshin. Several candidate TFs involved in thermo-
tolerance were confirmed as HSR genes by real time PCR, and these assignments were further supported by promoter analysis. Although some of these findings were similar to those obtained using other plant species, clear differences in *Brassica rapa* reveal a distinct HSR in this species. These data could also provide a spring board for developing molecular markers of HS and for engineering HS tolerant *B. rapa*.

Khomdram and Barthakur (2015) designed an experiment by constructing a portable heat trap chamber and grew two wheat ideotypes WR544 and HD2967 in pots under normal and exposed to heat stress (4hr) at generative stages inside the heat chamber. The cultivar WR544 was also exposed to drought by water withholding for 15 days and exposed to high temperature within the chamber. Along with this, heat acclimatized seeds of both the varieties were also utilized for heat tolerant analyses. The portable heat trap chamber maintained ± 5°C differences in temperature from the ambient temperatures during the heat stress period. Gene expression profiling were carried out with two heat stress responsive genes, S-phase kinase associated protein 1-like protein (*TaSKP1*) and WRKY transcription factor (*TaWRKY10*) in flag leaf and post anthesis stages. Differential gene expression was seen for both the genes. Highest induction level of *TaSKP1* was observed in acclimatized WR544 both in flag leaf and post anthesis. Whereas *TaWRKY10* expression was maximum in combination of drought and heat stress exposed WR544 in both the stages. Overall the results point out to the effectiveness of this heat trap chamber for evaluation of high temperature stress mechanism of wheat in field.

Pandey *et al.* (2015) reported genome-wide identification and characterization of 27 newly *TaHSP20* candidate genes in wheat and 13 *HvHSP20* in barley, describing structures, phylogenetic relationships, conserved protein motifs, and expression patterns using Hidden Markov Model and BLAST algorithm. The structural analysis highlights that this gene family possesses a conserved ACD region at the C-terminal. Detailed pattern analysis of HSP20 revealed presence of P-G doublet and I/V/L-X-I/V/L motif that helps in oligomerization. Identification of conserved motif sequences of wheat and barleyHSP20 strongly supported their identity as sHSP families. This study illustrates for the first time 3D model prediction of full-length wheat HSP20 (*TaHSP20*) protein and ACD region. Digital expression analysis was also carried out in order to reveal a widespread distribution of the sHSP family genes at various developmental stages of wheat and barley. In addition, five selected transcripts of both wheat and barley were validated for their expression
profile under 35 °C and 42 °C heat stress conditions. Results indicated up-regulation of all the transcripts under heat stress condition except TaCBM38894 candidate, which showed down-regulation in wheat.

2.4 BIOCHEMICAL ANALYSIS (PROTEIN PROFILING)

Perrotta et al. (1998) observed high temperatures during grain filling that can modify dough properties and quality in wheat. They analysed four Italian wheat cultivars grown under different temperature conditions to study the influence of high temperature on storage-protein-gene expression. Plants were grown both in the field and in growth cabinets and were subjected to different thermal regimes. PolyA+ mRNAs were extracted from control and stressed plants at different stages of kernel development. Northern blot hybridisations were performed using probes for storage and heat shock proteins to monitor the expression of the relative genes under different temperature conditions. Northern analyses, performed using storage protein probes, indicated that temperature variation does not influence the synthesis of any of the storage protein mRNAs. On the contrary, the hybridisation signals obtained using heat shock probes were more intense in the stressed samples, indicating that the expression of heat shock genes is modulated by the temperature variation.

Treglia et al. (1999) studied Poly(A)+RNA isolated from durum and common wheat seeds exposed to different thermal regimes during ripening and translated in vitro using a rabbit reticulocyte system. The modification of protein synthesis was studied with particular regard to heat shock proteins produced under high temperature conditions. One-dimensional poly acryl amide gel electrophoresis analysis showed products ranging in size from 14 to 100 kDa, some of which were present only when mRNA samples from high temperature-treated plants were translated. The mRNAs were also analysed by northern hybridization with specific probes for heat shock proteins. The results clearly show that wheat plants respond to thermal stress by triggering the typical mechanisms of the heat shock response including activation of the heat shock genes, in developing grains as well as other plant parts.

A proteomic approach was used for analysis and characterisation of wheat-grain endosperm proteins at a developmental stage (17 days post-anthesis) of the wheat cultivar “Wyuna” by Skylas et al. (2000). This involved the extraction, solubilisation and subsequent two-dimensional separation of total wheat-grain endosperm proteins. About 1300 polypeptides were resolved. Separation in the first
dimension was performed using isoelectric focusing across two pH ranges: pH 4.0–7.0 and pH 6.0–11.0. Proteins were blotted to PVDF, excised and characterised using conventional N-terminal Edman degradation micro-sequencing. Sequences were submitted to SWISS-PROT and TrEMBL databases via FASTA algorithm. In total, 321 proteins were submitted for post-separation characterisation; 177 (55%) proteins were identified from database matches, 55 (17%) proteins were not matched and 89 (28%) proteins did not yield any N-terminal sequence data. Protein expression within the endosperm at both the developmental (17 days post-anthesis) and mature (45 days post-anthesis) stages of growth were then compared using Melanie II 2-D PAGE image analysis software. This approach has provided an insight into the complex nature of endosperm protein heterogeneity and provides a basis for the future examination of the effects of environmental variation on protein composition.

Skylas et al. (2002) studied two complementary approaches to determine the effects of heat shock on wheat-grain quality. Heat-susceptible (cv. Wyuna) and heat-tolerant (cv. Fang) cultivars of wheat were compared after heat shock, utilising both dough-quality testing and proteome analysis. Plants were grown at day/night temperatures of 24/18 °C during development, with stressed plants being subjected to heat shock at day/night temperatures of 40/25 °C on 15, 16 and 17 days post-anthesis. Grain samples were taken during development (17 days post-anthesis) and at maturity (45 days post-anthesis). Dough-quality testing of flour indicated that the Wyuna cultivar exhibited a decrease in dough strength after heat shock, whilst the Fang cultivar exhibited an increase. Proteome studies conducted on endosperm (at 17 days post-anthesis) showed that the heat-tolerant Fang cultivar exhibited a stronger and more diverse ‘heat shock response’ than Wyuna. In total, 48 protein spots exhibiting differential expression between control and heat shock treatments were excised from gels and analysed by mass spectrometry. The resultant tryptic-peptide mass fingerprint data was submitted to SWISSPROT and TrEMBL databases for protein identification. The majority of heat-shock associated proteins had low molecular mass and showed database similarity to previously characterised small heat shock proteins. Several discrete isoforms of the low molecular weight heat shock proteins were observed as differentially expressed between the two cultivars. Furthermore, seven protein spots were expressed in heat shocked Fang but not in heat shocked Wyuna and were further characterised utilising tandem mass spectrometry. These possible marker
proteins for heat-tolerance may assist the breeders in the selection of heat-tolerant cultivars that would not be expected to lose dough strength in such an environment.

Sule et al. (2004) analyzed stress-responsiveness in plants to discovery genes conferring stress tolerance and their use in breeding programs. High temperature is one of the environmental stress factors that can affect the growth and quality characteristics of barley (Hordeum vulgare). In this study a proteomic analysis (2D-PAGE and MS) was used to detect the effects of heat shock on the protein pattern of an abiotic stress-tolerant (Mandolina) and an abiotic stress-susceptible (Jubilant) barley cultivar. Evaluation of two-dimensional gels revealed several proteins to be differentially expressed as a result of heat stress in both cultivars. The protein spots of interest were, after an in-gel tryptic digestion, further investigated by mass spectrometry. For the analysis of the peptide mixture, we both used a matrix-assisted laser desorption/ionization (MALDI) tandem time of flight mass spectrometer (TOF/TOF) and an automated nano-HPLC system coupled to an electrospray ionization-quadrupole linear ion trap (Q-TRAP) instrument. The hyphenation of the latter techniques proved to be a powerful technique as shown by the identification of six isoforms of a 16.9 kDa HSP in one single spot. They observed that S-adenosyl methionine synthetase (SAM-S) was differentially expressed between the two cultivars. Recent results refer to the role of SAM-S as being involved in abiotic stress tolerance. Furthermore, comparison of the heat shock treated samples also revealed several small heat shock proteins (sHSP), of which distinct isoforms could be characterised.

Hurkman and Tanaka (2007) studied total protein extracts of wheat endosperm used for analysis of the highly abundant gliadins and glutenins. The most popular total endosperm extraction methods were compared for their effectiveness in proteome coverage. A drawback of total endosperm extracts is that the enormous dynamic range of protein abundance limits the detection, quantification, and identification of low abundance proteins. Protein fractionation is invaluable for improving proteome coverage, because it reduces sample complexity while enriching for specific classes of less abundant proteins. A wide array of techniques is available for isolating protein subpopulations. Sequential extraction is a method particularly suited for sub-fractionation of wheat endosperm proteins, because it takes advantage of the specific solubility properties of the different classes of endosperm proteins. This method effectively separates the highly abundant gliadins and glutenins from the much less
abundant albumins and globulins. Subcellular fractionation of tissue homogenates is a classical technique for isolating membranes and organelles for functional analysis. This approach is suitable for defining the biochemical processes associated with amyloplasts, specialized organelles in the endosperm that function in the synthesis and storage of starch. Sub-proteome fractionation, when combined with 2-DE and protein identification, provides a powerful approach for defining endosperm protein composition and providing new insights into cellular functions.

Lee et al. (2007) investigated rice leaf proteome in response to heat stress. Rice seedlings were subjected to a temperature of 42°C and samples were collected 12 and 24 h after treatment. Increased relative ion leakage and lipid peroxidation suggested that oxidative stress frequently was generated in rice leaves exposed to high temperature. 2-DE, coupled with MS, was used to investigate and identify heat-responsive proteins in rice leaves. In order to identify the low-abundant proteins in leaves, samples were pre-fractionated by 15% PEG. The PEG supernatant and the pellet fraction samples were separated by 2-DE, and visualized by silver or CBB staining. Approximately 1000 protein spots were reproducibly detected on each gel, wherein 73 protein spots were differentially expressed at least at one time point. Of these differentially expressed proteins, a total of 34 and 39 protein spots were found in the PEG supernatant and pellet fractions, respectively.

Irmak et al. (2008) conducted an experiment on two near-isogenic lines of the wheat variety Lance having Glu-D1a (HMW-GS 2 ¼ 12) and Glu-D1d (HMW-GS 5 ¼ 10) which were subjected to several regimes of heat stress. In 2001, the temperature regimes were (i) 20/16 (day/night, ºC) from planting to maturity, (ii) 20/16 except for a 3 day heat treatment of 35/20, 25 days after anthesis and (iii) 20/16 until 25 DAA, after which plants were subjected to 40/25 until maturity. In 2002, treatments (i) and (iii) were the same, while treatment (ii) used a temperature of 40/25 ºC for 3 days at 25 DAA. Seed was collected at 3 days interval starting from 16 days after anthesis and analyzed for protein composition by SE-HPLC. The line with the Glu-D1d allele showed an earlier polymerization of glutenin than its allelic counterpart and a higher molecular weight of glutenin at maturity, this being deduced from measurements of the percentage of un-extractable polymeric protein.

Yildiz and Terzi (2008) studied effect of heat stress on soluble proteins extracted from leaf tissues of bread (Triticum aestivum cv. G¨onen-98, tolerant; cv. Cumhuriyet-75, susceptible; genome ABD) and durum (Triticum durum cv. Ege-88,
tolerant; cv. Ankara-98, susceptible; genome AB) wheat cultivars differing in sensitivity to high temperature was examined by two-dimensional gel electrophoresis. At acclimation (37°C) and acclimation → high temperature (37°C→50°C) treatments compared to control (25°C), evaluation of gels revealed 31 proteins to be differentially expressed in first leaves as a result of heat stress in heat-susceptible and heat-tolerant cultivars of bread and durum wheat. All of the increased or decreased proteins in amount, newly synthesized and/or disappeared were in low-molecular-weight (LMW, 16.1–24.0 kDa) and generally acidic character (pI 4.8–6.9). The responses of the four cultivars were compared: Twenty-two of 31 proteins were detected as newly synthesized LMW heat shock proteins (LMW HSPs = small HSPs). The number of these sHSPs was different in cultivars which have the same genome. In addition, the number of the sHSPs in heat-tolerant cultivars was higher than in heat-susceptible cultivars. Some of the sHSPs were specific to cultivar. Most of the sHSPs synthesized at 37°C were also detected at 37°C→50°C treatment. It is suggested that sHSPs have special importance in two points: Firstly, sHSPs in cultivars showed abundance and diversity. Secondly, these proteins may play an important role in the acquiring of thermal tolerance.

Efeoglu and Terzioglu (2009) investigated effects of heat stress at 37°C and 45°C for 8 h on the seedlings of Karacadag and Firat wheat cultivars differing in sensitivity by 2-D SDS polyacrylamide gel electrophoresis. Examination of the 2-D SDS polyacrylamide gel electrophoresis analysis of the thylakoid membrane proteins from the two wheat cultivars showed that heat treatment at 37°C and 45°C did not induce or enhance the synthesis of any protein. While the synthesis of some proteins were repressed when compared to the control temperature cultivars, the photosynthetic responses of Karacadag were less altered than Firat to the effect that; Karacadag showed lower reduction in the chlorophyll content, FV and FV/FM parameters where the F₀(initial chlorophyll fluorescence) parameter only increased in the Firat cultivar at 45°C. Therefore, Karacadag was determined to be a heat tolerant cultivar that can be used for cultivation in warmer regions.

Han et al. (2009) performed proteomic analyses in leaf tissue, exposed to high temperatures at 35 °C, 40 °C and 45 °C. The proteomics analysis showed that proteins such as lignifications related proteins were regulated by high temperature and distinct proteins related to protection were up-regulated at different high temperatures. All the results indicated that different strategies were adopted at different levels of high
temperature: the higher the temperature, the more protection machineries were involved. At 35 °C, some protective mechanisms were activated to maintain the photosynthetic capability. At 40 °C, antioxidative pathways were also active. When rice seedlings encountered high-temperature stress at 45 °C, in addition to those induced at 35 °C and 40 °C, heat shock proteins were effectively induced.

Hurkman et al. (2009) studied accumulation of KCl-soluble/methanol-insoluble albumins and globulins in the endosperm of developing wheat (Triticum aestivum, L. cv. Butte 86) grain produced under a moderate (24ºC/17ºC, day/night) or a high temperature regimen (37ºC/28ºC) imposed from 10 or 20 days post-anthesis (dpa) until maturity. Proteins were separated by 2-DE and developmental profiles for nearly 200 proteins were analyzed by hierarchical clustering. Accumulation of proteins shifted from those active in biosynthesis and metabolism to those with roles in storage and protection against biotic and abiotic stresses. Few proteins responded transiently when plants were transferred to the high temperature regimens, but levels of a number of proteins were altered during late stages of grain development. Specific protein responses depended on whether the high temperature regimens were initiated early or mid development. Some of the heat responsive proteins have been implicated in gas bubble stabilization in bread dough and others are suspected food allergens.

Hu et al. (2010) characterized small heat shock proteins associated with maize tolerance to combined drought and heat stress. Two-dimensional electrophoresis was used to identify combined drought- and heat-responsive protein spots in maize leaves. After Coomassie brilliant blue staining, approximately 450 protein spots were reproducibly detected on each gel, wherein 7 protein spots were expressed only under heat and combined drought and heat stress but were almost undetected under control and drought. Using MALDI-TOF mass spectrometry, a total of seven proteins were identified, including cytochrome b6-f complex iron sulfur subunit, shSP17.4, shSP17.2, shSP26, guanine nucleotide-binding protein b-subunit-like protein, putative uncharacterized protein, and granule-bound starch synthase IIa. Moreover, the gene expression of three shSPs was analyzed at the transcriptional level and indicated that all three shSPs were expressed under several treatments although their expression levels were obviously more enhanced by heat and combined drought and heat stress than by control and drought. The effect of abscisic acid (ABA) on the three shSPs, pretreatment with 100 µM ABA substantially enhanced the expression of the three shSPs at the protein level, but only slightly at the mRNA level.
To identify proteins accumulating in mature embryos which can be used as potential markers for dehydration tolerance, Irar et al. (2010) compared the embryo proteome from two durum wheat genotypes (Triticum durum Desf.), Mahmoudi (sensitive, S) and Om Rabia3 (tolerant, T). Total protein extracts from wheat embryos were analyzed by using conventional 2-DE and Proteome Lab PF-2D. Among all, 803 protein spots in the (S) variety and 894 spots in the (T) variety had detected. The percentage of similar protein for the two varieties was 75%, demonstrating extensive homology between tolerant and sensitive genotypes.

Two hundred and seventeen protein spots of interest were (after an in-gel tryptic digestion) identified using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry studied by Kamal et al. (2010). Ten percent of abiotic stress responsive proteins were identified in cv. Keumgang followed by 7% in cv. Jinpum and cv. China-108, 12% in cv. Yeonnon-78, 31% in cv. Norin-61 and 33% in cv. Kantou-107 in our experiment. Of the total number of 575 identified proteins, 345 proteins were recognized as abiotic stress responses unique proteins with isoforms, of which 34% are induced by heat, 27% by drought, 15% by salt, 13% by cold and 11% by other environmental stress. Furthermore, elucidating the function of proteins expressed by genes in stress tolerant and susceptible plants will not only advance our understanding of plant adaptation and tolerance to environmental stresses, but also may provide important information for designing new strategies for crop improvement.

Laino et al. (2010) observed that in Central and Southern Italy, where durum wheat represents one of the most widely cultivated crops, grain filling occurs during Spring, a period characterized by sudden increases in temperature. Wheat grain proteins are classified into albumins, globulins, and prolamins. The non-prolamin fractions include proteins with metabolic activity or structural function. In order to investigate the consequences of heat stress on the accumulation of non-prolamin proteins in mature durum wheat kernels, the Italian cultivar Svevo was subjected to two thermal regimes (heat stress versus control). The 2-D patterns of non-prolamin proteins were monitored to identify polypeptides affected by heat stress during grain fill. This study shows that heat stress alters significantly the durum wheat seed proteome, although the changes range is only between 1.2- and 2.2-fold. This analysis revealed 132 differentially expressed polypeptides, 47 of which were identified by MALDI-TOF and MALDI-TOF-TOF MS and included HSPs, proteins involved in
the glycolysis and carbohydrate metabolism, as well as stress-related proteins. Many of the heat-induced polypeptides are considered to be allergenic for sensitive individuals.

In order to investigate the consequences of heat stress on the accumulation of non-prolamin proteins in mature durum wheat kernels, Paolo et al. (2010) studied the Italian cultivar Svevo which was subjected to two thermal regimes (heat stress vs. control) during grain filling. The 2D patterns of non-prolamin proteins were monitored to identify polypeptides affected by heat stress. This study showed that heat stress alters significantly the durum wheat seed proteome, although the fold changes range only between 1.2 and 2.2. This analysis revealed 132 differentially expressed polypeptides, 47 of which were identified by MALDI TOF and MALDI-TOF-TOF MS and included heat shock proteins, proteins involved in the glycolysis and carbohydrate metabolism, as well as stress related proteins. Many of the heat induced polypeptides are considered to be allergenic for sensitive individuals. The differences observed with previously reported data regarding bread wheat may be explained by the absence of the D genome in durum wheat.

Chen et al. (2011) displayed phosphor-protein in the leaves of rice under heat stress using two-dimensional electrophoresis (2-DE) and Pro-Q Diamond dye. Differentially expressed phosphor-proteins were identified by MALDI-TOF-TOF-MS/MS and confirmed by Western blotting. Ten heat-phospho-proteins were identified from twelve protein spots, including ribulose bisphosphate carboxylase large chain, 2-Cys peroxiredoxin BAS1, putative mRNA binding protein, Os01g0791600 protein, OSJNBa0076N16.12 protein, putative H(+)-transporting ATP synthase, ATP synthase subunit beta and three putative uncharacterized proteins. The identification of ATP synthase subunit beta was further validated by Western blotting. Four phosphorylation site predictors were also used to predict the phosphorylation sites and the specific kinases for these 10 phosphoproteins. Heat stress induced the de-phosphorylation of RuBisCo and the phosphorylation of ATP-β, which decreased the activities of RuBisCo and ATP synthase. The observed de-phosphorylation of the mRNA binding protein and 2-Cys peroxiredoxin may be involved in the transduction of heat-stress signalling, but the functional importance of other phosphoproteins, such as H+-ATPase, remains unknown.

Mahla et al. (2011) screened thirty-six genotypes for thermo-tolerance based on wilting of primary leaf and values of chlorophyll fluorescence. Four wheat
genotypes, i.e., two tolerant (HW-2045 and WH-1021) and two susceptible (HS-277 and WH-147) were selected. SDS-PAGE of seedlings under stress conditions revealed the appearance of polypeptide bands of different molecular weight in tolerant and susceptible genotypes, and these polypeptides bands disappeared on revival of 2nd and 4th day. Based on genotypes screening and on polypeptide pattern, out of four genotypes, HW-2045 was found to be the most tolerant and WH-147 as the most susceptible genotype.

Guo et al. (2012) displayed variable expression patterns of grain proteins in grains from two wheat cultivars Jimai 20 and Zhoumai 16 with different gluten quality properties at five development stages. Proteome characterization during grain development in Chinese bread wheat cultivars Jimai 20 and Zhoumai 16 with different quality properties was investigated by 2-DE and tandem MALDI-TOF/TOF-MS. Identification of 117 differentially accumulated protein spots representing 82 unique proteins and five main expression patterns enabled a chronological description of wheat grain formation. Significant proteome expression differences between the two cultivars were found; these included 14 protein spots that accumulated in both cultivars but with different patterns and 27 cultivar-different spots. Among the cultivar-different protein spots, 14 accumulated in higher abundance in Jimai 20 than in Zhoumai 16, and included NAD-dependent isocitrate dehydrogenase, triticin precursor, LMW-s glutenin subunit and replication factor C-like protein. These proteins are likely to be associated with superior gluten quality. In addition, some proteins such as class II chitinase and peroxidase 1 with isoforms in developing grains were shown to be phosphorylated by Pro-Q Diamond staining and phosphorprotein site prediction. Phosphorylation could have important roles in wheat grain development. Differences in seed storage proteins were considered to be related to different quality performance of the flour from these wheat cultivars. Some proteins with isoforms were phosphorylated, and this may reflect their importance in grain development. These results provide new insights into proteome characterization during grain development in different wheat genotypes.

Hurkman et al. (2013) determined flour quality by the gluten proteins, a complex mixture of proteins consisting of high molecular weight-glutenin subunits (HMW-GS), low molecular weight-glutenin subunits (LMW-GS), and α-, γ- and ω-gliadins. Detailed proteomic analyses of the effects of fertilizer and high temperature on individual gliadin and glutenin protein levels are needed to determine how these
environmental factors influence flour quality. Wheat plants (*Triticum aestivum* L. cv. Butte 86) were grown in greenhouses under moderate and high temperature regimens with and without post-anthesis fertilizer. Quantitative two-dimensional gel electrophoresis was used to construct accumulation profiles in developing endosperm for the entire complement of gluten proteins identified previously by tandem mass spectrometry. Amounts of individual gliadins and glutenins were also determined in flour produced under each of the regimens. Under all environmental regimens, most HMW-GS, LMW-GS, γ- and ω-gliadins accumulated rapidly during early stages of grain development and levelled off during middle stages of development. A subset of LMW-GS showed a second distinct profile, accumulating throughout development, while α-gliadins showed a variety of accumulation profiles. In flour, fourteen distinct gluten proteins responded similarly to fertilizer, high temperature, and high temperature plus fertilizer. The majority of HMW-GS and ω-gliadins and some α-gliadins increased while two LMW-GS and a minor γ-gliadin decreased. Fertilizer did not influence gluten protein accumulation under high temperature conditions. Additionally, the effects of fertilizer and high temperature were not additive; very few changes were observed when plants that received fertilizer were subjected to high temperature. Although post-anthesis temperature and fertilizer have very different effects on grain development and yield, the two treatments elicit surprisingly similar effects on the accumulation of gluten proteins. The similarity of the responses to the different treatments is likely due to source-sink activities of nitrogen reserves in the wheat plant. Because each protein that showed a response in this study is linked to a gene sequence, the work sets the stage for transgenic studies that will better elucidate the roles of specific proteins in flour quality and in the response to the environment.

Kumar *et al.* (2013a) screened 22 wheat genotypes for their cell membrane stability (CMS), out of which C-306 showed the maximum CMS value of 74%. Protein profiling revealed the expression of many new and existing proteins in C-306 cultivar compared to PBW343 under differential heat shock (HS). An altered protein expression was also observed in tolerant and susceptible cultivars at different stages of growth. Likewise, Kumar *et al.* (2013b) also reported western blot analysis which revealed the presence of 5, 6 and 5 multi protein chaperone complexes of HSP90 in the range of 95 kDa to 70 KDa at pollination, milky dough and seed hardening stages.

Rollins *et al.* (2013) studied leaf proteome alterations in the context of physiological and morphological responses to drought and heat stress in barley
The objective of this study was to identify barley leaf proteins differentially regulated in response to drought and heat and the combined stresses in context of the morphological and physiological changes. The Syrian landrace Arta and the Australian cultivar Keel were subjected to drought, high temperature, or a combination of both treatments starting at heading. Changes in the leaf proteome were identified using differential gel electrophoresis and mass spectrometry. The drought treatment caused strong reduction of biomass and yield, while photosynthetic performance and the proteome were not significantly changed. In contrast, the heat treatment and the combination of heat and drought reduced photosynthetic performance and caused changes of the leaf proteome. The proteomic analysis identified 99 protein spots differentially regulated in response to heat treatment, 14 of which were regulated in a genotype-specific manner. Differentially regulated proteins predominantly had functions in photosynthesis, but also in detoxification, energy metabolism, and protein biosynthesis. The analysis indicated that de novo protein biosynthesis, protein quality control mediated by chaperones and proteases, and the use of alternative energy resources, i.e. glycolysis, play an important roles in adaptation to heat stress. In addition, genetic variation identified in the proteome, in plant growth and photosynthetic performance in response to drought and heat represent stress adaption mechanisms to be exploited in future crop breeding efforts.

A comparative proteomic analysis of paired, genetically similar heat-tolerant and heat-sensitive rice lines was conducted by Liao et al. (2014). Two-dimensional electrophoresis (2-DE) revealed a total of 27 differentially expressed proteins in rice grains, predominantly from the heat-tolerant lines. The protein profiles clearly indicated variations in protein expression between the heat-tolerant and heat-sensitive rice lines. Matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry (MALDI-TOF/TOF MS) analysis revealed that 25 of the 27 differentially displayed proteins were homologous to known functional proteins. These homologous proteins were involved in biosynthesis, energy metabolism, oxidation, heat shock metabolism, and the regulation of transcription. Seventeen of the 25 genes encoding the differentially displayed proteins were mapped to rice chromosomes according to the co-segregating conditions between the simple sequence repeat (SSR) markers and the target genes in recombinant inbred lines (RILs).
Hu et al. (2014) worked protein profiles in the leaves of two tall fescue genotypes, heat tolerant ‘PI578718’ and heat sensitive ‘PI234881’ by 2DE. Low molecular weight heat shock protein (LMW-HSP, HSP20) showed activated in two stressed genotypes and high molecular weight HSP (HMW-HSP,HSP90) just in PI578718.

Moradpour et al. (2014) studied ten wheat cultivars for proteome analysis. After applying stress and extraction of leaf proteins, two-dimensional gels were prepared and scanned. Analysis of gel images was performed using Same Spot Progenesis. About 657 protein spots were identified by the software. After alignment of the spots and their correspondence, 148 spots were identified visually and by using the software and statistical analysis was carried out. Five spots with Fold ≥ 1/5 at P≤ 0.05 were identified, of which 4 spots were significant at P ≤ 0.05 and 1 spot was significant at P ≤0.01.

Xin et al. (2015) performed proteome analysis by2D electrophoresis and MALDI TOF/TOF in the leaf after pre-anthesis heat priming and post-anthesis high temperature stress. Proteome analyses revealed up-regulation of the genes that encoded sensing and signalling, heat shock proteins, redox homeostasis, and down-regulation of the genes that encoded metabolism. The up-regulation and down-regulation might play protective roles in coping with the post-anthesis high temperature stress in the pre-anthesis heat primed plants compared with non-primed plants. Eight protein spots were regulated after both pre-anthesis heat priming and post-anthesis high temperature stress; proteins were expressed differently in primed plants from those in non-primed plants. These results are of primary importance for understanding the effects of multi-heat stress on production of wheat crops in future climate change scenarios.

Kaneko et al. (2016) studied proteomic analysis of rice chalky grains and revealed that deregulations in the expression of multiple proteins implicated in diverse metabolic and physiological functions, such as protein synthesis, redox homeostasis, lipid metabolism, and starch biosynthesis and degradation. The extracted proteins were trypsin-digested and labeled by iTRAQ (isobarictag for relative and absolute quantitation), followed by tandem mass spectrometry (MS/MS) analysis. Analysis of protein extracts from the different samples resulted in the detection 938 of proteins.
Among them 6.5% (around 61 expressed proteins) were deregulated. HSP 81–1, HSP 81–3, and 70 kDa HSP involved strongly up-regulated.