

# Tackling the Heat-Stress Tolerance in Crop Plants: A Bioinformatics Approach

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## Abstract

Plants are exposed to different types of environmental factors including heat stress that affect negatively various regular activities of the plant. Plants, as sessile organisms, must have developed efficient strategies of response to cope with and adapt to different types of abiotic stresses imposed by the adverse environment. Plant responses to environmental stress are complex and appear to be a difficult task to study in the classical plant-breeding program due to several technical limitations. The current knowledge of the regulatory network governing environmental stress responses is fragmentary, and an understanding of the damage caused by these environmental stresses or the plant's tolerance mechanisms to deal with stress-induced damages is far from complete. The emergence of the novel "omics" technologies from the last few years, such as genomics, proteomics, and metabolomics, is now allowing researchers to enable active analyses of regulatory networks that control abiotic stress

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22 responses. Recent advances in different omics approaches have been  
23 found greatly useful in understanding plant responses to abiotic stress  
24 conditions. Such analyses increase our knowledge on plant responses and  
25 adaptation to stress conditions and allow improving crop improvement  
26 programs including plant breeding. In this chapter, recent progresses on  
27 systematic analyses of plant responses to heat stress including genomics,  
28 proteomics, metabolomics, and phenomics and transgenic-based  
29 approaches to overcome heat stress are summarized.

#### Keywords

30 Omics • Phenomics • Molecular regulatory networks • NGS-based  
31 transcriptome analysis • Heat-shock response • Heat-shock proteins •  
32 Heat-shock element  
33

#### 34 Abbreviations

35	HSFs	Heat-Shock Transcription Factors
36	ROS	Reactive Oxygen Species
37	GEO	Gene Expression Omnibus
38	TAIR	The Arabidopsis Information Resource
39	NGS	Next-Generation Sequencing
40	GC-MS	Gas Chromatography Coupled to Mass Spectrometry
41		
42	LC-MS	Liquid Chromatography Coupled to Mass Spectrometry
43		
44	SGN	Sol Genomics Network

#### 45 1 Introduction

46 Since plants are sessile in nature, they develop  
47 many physiological and molecular mechanisms  
48 to cope with different abiotic stresses. Plants  
49 started to emerge 1.5 billion years ago  
50 (Lehninger et al. 1993), and the evolutionary  
51 pressure has shaped plant responses to environ-  
52 mental fluctuations that minimize damage and  
53 ensure protection of cellular homeostasis. Heat  
54 stress is one of the main abiotic stresses that can  
55 limit the crop productivity drastically in the com-  
56 ing years due to global warming. High  
57 temperatures can be detrimental to all phases of  
58 plant development. Heat stress causes irrevers-  
59 ible damage to plant function and development  
60 (Hall 2001). Heat stress affects a broad spectrum

of cellular components and metabolism. The 61  
timing, duration, and severity of heat stress influ- 62  
ence pollen-pistil interactions in crop plants 63  
(Snider and Oosterhuis 2011). To counter the 64  
effects of heat stress on cellular metabolism, 65  
plants and other organisms respond to changes 66  
in their ambient temperature by reprogramming 67  
the composition of certain transcripts, proteins, 68  
and metabolites. Heat stress leads to a series of 69  
phenotypical and genetical changes, creates 70  
osmotic imbalances, and produces ubiquitous 71  
and evolutionarily conserved proteins known as 72  
heat-shock proteins (Hsps) (Gupta et al. 2010). 73  
Stress responses involving extreme temperature 74  
result in excess production of reactive oxygen 75  
species (ROS), leading to oxidative damage and 76  
thus limiting the growth and productivity of agri- 77  
cultural crops. Genome-wide transcriptional 78  
profiles during temperature and oxidative stress 79  
revealed coordinated expression patterns and 80  
overlapping regulons in crop plants (Mittal 81  
et al. 2012). Therefore, understanding plant 82  
responses to heat stress is now thought to be 83  
one of the hottest topics in agricultural science. 84  
Major progress in this research field has come 85  
from the application of different bioinformatics/ 86  
systems biology approaches. These high- 87  
throughput techniques have made it possible to 88  
analyze thousands of genes in one shot (Smita 89  
et al. 2013). With the introduction of bioinfor- 90  
matics tools, many heat-stress-inducible genes 91  
were identified from the huge genome databases, 92

93 their promoter sequences were identified, and the  
 94 putative functions of the genes were functionally  
 95 characterized through transgenic approaches.  
 96 This provides the information to understand the  
 97 molecular mechanisms for improving heat toler-  
 98 ance in crops. Availability of these data sets  
 99 publicly has broadened and deepened the view  
 100 of heat-stress responses and tolerance not only in  
 101 model plants but also in agricultural crops.

## 102 **2 Bioinformatics Approaches**

103 Recent advances in functional genomics have  
 104 allowed us the use of different bioinformatics  
 105 approaches such as transcriptomics (global gene  
 106 expression), proteomics (protein profiling/modi-  
 107 fication), metabolomics (metabolite profiling),  
 108 and phenomics to understand the complex  
 109 molecular regulatory networks associated with  
 110 stress adaptation and tolerance (Cramer et al.  
 111 2011). These technologies generate enormous  
 112 amounts of information which has boosted up  
 113 the field of bioinformatics, with thousands of  
 114 new algorithms and software published every  
 115 year. System-based approaches with a combina-  
 116 tion of multiple omics analyses have been an  
 117 efficient tool to determine the global picture of  
 118 cellular events which would increase our under-  
 119 standing of the complex molecular regulatory  
 120 networks and find out the interacting partners  
 121 associated with heat-stress adaptation and toler-  
 122 ance. The data collected from transcriptomics,  
 123 proteomics, and metabolomics needs to be com-  
 124 bined to achieve a better understanding of the  
 125 plant as a system. In this context, different  
 126 omics data should contribute greatly to the iden-  
 127 tification of key regulatory steps to characterize  
 128 the pathway interactions. The integration of a  
 129 wide spectrum of omics data sets from various  
 130 plant species facilitates to promote translational  
 131 research for future biotechnological applications  
 132 in crop plants and also in fruit trees. These  
 133 approaches demonstrate the power of systems  
 134 biology for understanding the key cellular  
 135 components underlying plant functions during

temperature stress. Thus, cooperation between 136  
 and beyond disciplines has a role to play in 137  
 unraveling the intricacies associated with heat- 138  
 stress adaptation in plants. 139

### 2.1 Transcriptomics 140

Transcriptomics is a powerful approach for 141  
 studying the responses of plants in relation to 142  
 their environment. The transcriptome consists of 143  
 the entire set of transcripts that are expressed 144  
 within a cell or organism at a particular develop- 145  
 mental stage or under various environmental 146  
 conditions. Recent transcriptomic studies have 147  
 helped to provide a better understanding of plant 148  
 response to different abiotic stresses like cold, high 149  
 salinity, drought, high light intensity, hyper- 150  
 osmolarity, and oxidative stresses (Deyholos 2010; 151  
 Wang et al. 2012). The overlap of large number of 152  
 genes induced by various stress conditions reveals 153  
 the molecular cross talk of gene regulatory net- 154  
 work responses to various abiotic stress conditions 155  
 (Weston et al. 2011; Friedel et al. 2012). This 156  
 contribution has enabled the discovery of novel 157  
 stress-responsive genes on the basis of expression 158  
 profiles in different developmental stages of the 159  
 plant under stress conditions (Sreenivasulu et al. 160  
 2008; Smita et al. 2013). The availability of com- 161  
 plete genome sequences of *Arabidopsis* and *Oryza* 162  
*sativa* model plants and other important crops has 163  
 provided sufficient genomic information to per- 164  
 form high-throughput genome-wide functional 165  
 analysis. Compared to other stresses, heat-stress 166  
 responses in plants have received increasing atten- 167  
 tion in recent years, and accordingly global trans- 168  
 criptome expression in response to heat stress has 169  
 been reported in different plant species (Mang- 170  
 elsen et al. 2011; Liu et al. 2012). Lim et al. 171  
 (2006) found that *Arabidopsis* suspension cells at 172  
 a moderate heat enhanced the expression profiling 173  
 of 165 genes, with high quantity of heat-shock 174  
 proteins (Hsps). Frank et al. (2009) found from 175  
 the cDNA microarrays and qPCR analysis that 176  
 Hsp70, Hsp90, and heat-shock transcription 177  
 factors (HSF) were important to tomato 178

179 microspore resistance to heat stress. Transcrip- 227  
180 ptomic data from *Triticum aestivum* and other 228  
181 plants show that 5 % of the genes are significantly 229  
182 affected in response to heat stress (Finka et al. 230  
183 2011). But *Arabidopsis* transcriptomic data show 231  
184 that 11 % of the genes expressed in response to 232  
185 heat stress are encoded for heat-induced 233  
186 chaperones (Qin et al. 2008). The rest of the 234  
187 transcripts encode products involved in calcium 235  
188 signaling, phytohormone signaling, sugar and 236  
189 lipid signaling, and metabolism. Additionally, 237  
190 some studies have identified various transcripts 238  
191 increased during heat treatment, including 239  
192 members of the *DREB2* family of transcription 240  
193 factors, *AsEXPI* encoding an expand in protein, 241  
194 genes encoding for galactinol synthase and 242  
195 enzymes in the raffinose oligosaccharide pathway, 243  
196 and antioxidant enzymes (Xu et al. 2007). Reports 244  
197 exist which show decrease in transcript levels 245  
198 related to programmed cell death, basic metabo- 246  
199 lism, and biotic stress responses under heat-stress 247  
200 conditions (Larkindale and Vierling 2008).

AU4 193 factors, *AsEXPI* encoding an expand in protein, 241  
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196 and antioxidant enzymes (Xu et al. 2007). Reports 244  
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199 lism, and biotic stress responses under heat-stress 247  
200 conditions (Larkindale and Vierling 2008).

AU5 201 Affymetrix Grape Genome Array and qRT- 249  
202 PCR techniques were used to identify the heat- 250  
203 stress- and recovery-regulated genes in the grape 251  
204 and found that about 8 % of total probe sets were 252  
205 responsive to heat stress and subsequent recovery 253  
206 in grape leaves. The responsive genes identified 254  
207 in this study belong to a large number of impor- 255  
208 tant factors and biological pathways, including 256  
209 those for cell rescue (i.e., antioxidant enzymes), 257  
210 protein fate (i.e., Hsps), primary and secondary 258  
211 metabolism, transcription factors, and signal 259  
212 transduction and development (Liu et al. 2012). 260  
213 Wheat Genome Array was applied to measure 261  
214 the transcriptome changes in response to heat 262  
215 stress in the contrasting genotypes and identified 263  
216 a total of 6,560 probe sets that responded to heat 264  
217 stress (Qin et al. 2008). A combination of heat 265  
218 and drought stresses had a significantly higher 266  
219 detrimental effect on growth and productivity of 267  
220 maize, barley, sorghum, and different grasses 268  
221 than each of the stresses applied individually 269  
222 (Abraham 2008). Nonetheless, apart from a nota- 270  
223 ble study on the effects of simultaneous drought 271  
224 and heat stress (Rizhsky et al. 2004), the effects 272  
225 of stress combinations have been little studied 273  
226 (Atkinson and Urwin 2012). Transcriptome 274

analysis has been used to investigate the expres- 227  
sion in response to heat stress as well as com- 228  
bined stresses in several plant species (Oshino 229  
et al. 2007; Rasmussen et al. 2013). 230  
Transcriptome profiling of *Arabidopsis* plants 231  
during a combination of drought and heat stress 232  
influences the changes in the expression pattern 233  
of more than 400 transcripts (Rizhsky et al. 234  
2004). Distinct responses were also observed in 235  
plants exposed to a combination of heat and high 236  
light intensity (Hewezi et al. 2008), heat, and 237  
salinity (Keles and Oncel 2002). Their work 238  
suggests that some pathways/mechanisms are 239  
dependent on genotype, duration, intensity, and 240  
type of abiotic stress. 241

Until now, most of the transcriptome 242  
responses have focused on an improved stress 243  
tolerance during the vegetative phase of plant 244  
growth. Heat stress decreases the duration of 245  
developmental phases leading to fewer organs, 246  
smaller organs, reduced light perception over the 247  
shortened life cycle, and perturbation of the pro- 248  
cesses related to carbon assimilation. These 249  
parameters ultimately contribute to losses in the 250  
final yield of plants (Hussain and Mudasser 251  
2007). But the most sensitive developmental 252  
stages to heat stress are flowering and grain 253  
filling (Wei et al. 2010). High temperature 254  
drastically reduced both yield and quality of 255  
wheat (Sharma et al. 2012). In this context, 256  
recently few attempts were made to reveal the 257  
transcriptome alterations in developing seeds to 258  
understand the yield stability under heat stress 259  
in rice (Yamakawa and Hakata 2010) and 260  
barley (Mangelsen et al. 2011). Altogether, 261  
transcriptome analyses provide novel insight 262  
into the plant response to heat stress and have 263  
great implications for further studies on gene 264  
function annotation and molecular breeding. In 265  
the era of post-genomics, large-scale gene 266  
expression data are generated by whole-genome 267  
transcriptome platforms. There are a few soft- 268  
ware applications that have been developed to 269  
query high-throughput microarray gene expres- 270  
sion databases at the genome-wide gene content 271  
level for various stress responses. The available 272  
expression data are mostly deposited in online 273  
repositories such as GEO (Barrett et al. 2007), 274

275 NASC Arrays (Craigon et al. 2004), PLEXdb  
276 (Dash et al. 2012), and ArrayExpress  
277 (Kapushesky et al. 2012). In parallel, various  
278 online query-oriented tools have been developed  
279 such as Genevestigator (Zimmermann et al.  
280 2004), *Arabidopsis* eFP browser (Winter et al.  
281 2007), RiceArrayNet (Lee et al. 2009), or  
282 *Arabidopsis* and rice co-expression data mining  
283 tools (Ficklin et al. 2010; Movahedi et al. 2011)  
284 Gramene (Youens-Clark et al. 2011), TAIR  
285 (Swarbreck et al. 2008), and MaizeGDB  
286 (Schaeffer et al. 2011) to extract development-  
287 and stress-specific regulons by implementing  
288 global normalization and clustering algorithms  
289 (Sreenivasulu et al. 2010).

### 290 2.1.1 NGS-Based Transcriptome 291 Analysis

292 Next-generation sequencing (NGS)-based  
293 transcriptome analysis is superior to other avail-  
294 able techniques since sequencing-based method  
295 is digital, high throughput, highly accurate, and  
296 easy to perform and is capable of identifying  
297 allele-specific expression. The principal advan-  
298 tage of NGS is that their throughputs are much  
299 higher than that of classical sequencing. In recent  
300 years, researchers have developed various  
301 platforms such as the Illumina Genome Ana-  
302 lyzer, the Roche/454 Genome Sequencer FLX  
303 Instrument, and the ABI SOLiD System that  
304 have proven to be powerful and cost-effective  
305 tools for advanced research in many areas,  
306 including genome sequencing, resequencing of  
307 the genome, miRNA expression profiling, DNA  
308 methylation analysis, and especially the de novo  
309 transcriptome sequencing of non-model  
310 organisms (Morozova and Marra 2008). NGS  
311 transcriptome analysis is fast and simple because  
312 it does not require any cloning of cDNAs and  
313 generates an extraordinary depth of short reads. It  
314 is a more comprehensive and efficient way to  
315 measure transcriptome composition, obtain  
316 transcriptome sequencing using NGS techno-  
317 logies provides better alternative for the gene  
318 expression studies. Application of NGS technol-  
319 ogy in the plant transcriptome analysis has been  
320 very limited, and only a few proof-of-concept  
321 studies have been performed to reveal the

transcriptional complexity in plants. Here, we 322  
provide some examples of the RNA-seq-based 323  
gene expression studies performed in plants, 324  
which provide novel insights into the various 325  
biological aspects. The Illumina sequencing 326  
analysis in maize revealed the differential 327  
expression of a very high fraction of genes 328  
(64.4 %) and provided the evidence for dynamic 329  
reprogramming of transcriptome with transcripts 330  
for basic cellular metabolism like photosynthesis 331  
(Li et al. 2010). An integrated transcriptome atlas 332  
of the soybean has been generated, which 333  
resulted in the identification of tissue-specific 334  
genes (Libault et al. 2010). Further, this expres- 335  
sion data has been utilized for comparative 336  
analyses of gene expression from other legumes, 337  
*Medicago truncatula* and *Lotus japonicus*. In 338  
another independent study, Severin et al. (2010) 339  
identified more than 177 genes involved in the 340  
agronomically important trait, like seed filling 341  
process using RNA-seq in soybean. Garg et al. 342  
(2011) identified the differentially expressed 343  
genes in a tissue-by-tissue comparison and 344  
tissue-specific transcripts in the chickpea, using 345  
massively parallel pyrosequencing. The tran- 346  
scriptional complexity in rice has also been 347  
unraveled via sequencing of mRNA from various 348  
tissues in two subspecies and identified more 349  
than 15,000 novel transcriptionally active 350  
regions and 3,464 differentially expressed genes 351  
(Lu et al. 2010). A novel sequence-based 352  
approach using Roche 454 technology focused 353  
on sequencing unique 3'-UTRs of genes to dis- 354  
tinguish highly conserved, related transcripts 355  
such as members of same gene family and quan- 356  
tify their expression (Eveland et al. 2008). 357

RNA-seq is a popular approach in NGS 358  
technologies to collect and quantify the large- 359  
scale sequences of coding and noncoding RNAs 360  
rapidly (Garber et al. 2011). NGS-based RNA- 361  
seq has been used for the rapid development of 362  
genomic resources in many plants (Gowik et al. 363  
2011). NGS was employed to create transcrip- 364  
tome databases of species without a sequenced 365  
genome such as mangroves (Dassanayake et al. 366  
2009), eucalyptus (Novaes et al. 2008), olive 367  
(Alagna et al. 2009), and chestnut (Barakat 368  
et al. 2009). For this RNA-seq approach, either 369

**AU7** 370 fragmented mRNA or fragmented cDNA (Wang  
371 et al. 2009a, b) can be used as input, and read  
372 lengths ranging from 100 to 250 nts and 500 nts  
373 model length can be received depending on the  
374 sequencer and sequencing kit employed. A major  
375 challenge in the near future for those who like to  
376 begin the work with NGS data is retooling for  
377 methods to store data. This is due to the short  
378 history of the technology and its continuous  
379 development, and there are as yet no standard  
380 methods available to detect and analyze differ-  
381 entially expressed genes based on NGS data. Such  
382 deep sequencing data from crop plants can help  
383 to identify the candidate genes associated with  
384 final yield, grain quality, disease resistance, and  
385 abiotic including heat-stress tolerance. These  
386 data are also useful to identify and isolate new  
387 genes and promoters involved in agronomical  
388 traits of economically important crops. Genera-  
389 tion of such bioinformatics data would be useful  
390 in crop improvement programs. NGS-based  
391 sequencing applications have rapidly expanded  
392 in plant genomics by browsing the Sequence  
393 Read Archive (SRA) in NCBI (<http://www.ncbi.nlm.nih.gov/sra>), European Nucleotide Archive  
394 (<http://www.ebi.ac.uk/ena/home>), and DDBJ  
395 Sequence Read Archive ([http://trace.ddbj.nig.ac.jp/dra/index\\_e.shtml](http://trace.ddbj.nig.ac.jp/dra/index_e.shtml)), all of which store raw  
396 sequencing data from NGS platforms; users can  
397 determine how thoroughly a given species has  
398 been sequenced and retrieve the publicly avail-  
399 able sequencing data for further use.  
400  
401

## 402 **2.2 Proteomics**

403 Proteomics is not only a powerful molecular tool  
404 used in describing complete proteomes at the  
405 organelle, cell, organ, or tissue levels, but it can  
406 also compare the status of protein profiling under  
407 different physiological conditions, such as those  
408 resulting from the exposure to stressful condi-  
409 tions (Cushman and Bohnert 2000). The prote-  
410 ome reflects the actual state of the cell or the  
411 organism and is an essential bridge between the  
412 transcriptome and the metabolome (Zhu et al.  
413 2003) and also acts directly on biochemical pro-  
414 cesses and thus must be closer to the phenotype.

In the last decade, proteomics has been shown to 415  
be a powerful tool in exploring many biological 416  
mechanisms which brought much deeper insight 417  
in the abiotic stress-responsive mechanisms in 418  
the crop plants (Rinalducci et al. 2011; Yin 419  
et al. 2012). However, the proteomic studies of 420  
crop plants under heat stress are not well under- 421  
stood (Neilson et al. 2010; Rinalducci et al. 422  
2011). To understand the modulation mecha- 423  
nisms of heat tolerance in plants, a detailed 424  
study of the response to high temperature at the 425  
proteomics level is essential. Thus far, there have 426  
been only a few proteomic studies regarding heat 427  
stress in plants (Koussevitzky et al. 2008; 428  
Neilson et al. 2010). Recent developments in 429  
protein analysis methods have made possible 430  
the evaluation and identification of many 431  
proteins and to exploit proteomic data in the 432  
context of stress response particularly heat stress 433  
(Nanjo et al. 2010). Proteome approach has been 434  
successfully used to study the effect of heat 435  
shock on wheat grain quality and to identify 436  
protein markers that enable breeders to produce 437  
cultivars with desired characters especially 438  
cultivars that tolerate heat-stress conditions 439  
(Skylas et al. 2002). The effect of heat stress in 440  
the wheat endosperm by MALDI-TOF coupled 441  
with 2-DE analysis identified a total of 48 differ- 442  
entially expressed proteins (Majoul et al. 2003). 443  
Of these, more than 37 % of the proteins have 444  
been identified as Hsps that are involved in pro- 445  
tein stability and folding, which suggests that 446  
high temperature has severe effects on protein 447  
denaturation and regulation. Rice leaf proteomic 448  
analysis by 2-DE-MS method in response to heat 449  
stress identified 1,000 protein spots, wherein 73 450  
protein spots were differentially expressed at 451  
least at one time point. These proteins were fur- 452  
ther categorized into different classes related to 453  
heat-shock proteins, energy and metabolism, 454  
redox homeostasis, and regulatory proteins. 455

Proteomic analysis in barley cultivars under 456  
heat stress identified several isoforms of sHsps 457  
and S-adenosylmethionine synthetase (SAM-S) 458  
and found to be upregulated (Sule et al. 2004). 459  
Several studies that analyzed the proteomic 460  
response to heat stress have been carried out in 461  
*Arabidopsis* and identified 45 spots which were 462

463 unique to the combined heat and drought  
464 stresses. Proteins uniquely regulated by heat in  
465 *A. scabra* included sucrose synthase, superoxide  
466 dismutase, glutathione S-transferase, and stress-  
467 inducible Hsps. This suggests that these proteins  
468 may contribute to increased survival of *A. scabra*  
469 under high-temperature conditions. Using differ-  
470 ential metabolic labeling, Palmblad et al. (2008)  
471 identified a number of known Hsps as well as  
472 other proteins previously not associated with heat  
473 shock in *Arabidopsis*. Polenta et al. (2007)  
474 identified the Hsps from tomato pericarp by ther-  
475 mal treatment. They highlighted the importance  
476 of class I sHsps that are involved in the process  
477 and further characterized them by using mono-  
478 specific polyclonal antiserum and MS/MS analy-  
479 sis. Thus, the results of this study suggest that  
480 plants cope with heat stress in a complex manner,  
481 where Hsps play a pivotal role in a complex  
482 cellular network. The identification of some  
483 novel proteins in the heat-stress response  
484 provides new insights that can lead to a better  
485 understanding of the molecular basis of heat sen-  
486 sitivity in plants (Lee et al. 2007).

487 Protein profiling of two ecotypes (low and high  
488 elevations) of Norway spruce was investigated in  
489 response to high-temperature stress using 2-DE  
490 and LC-MS/MS. This analysis showed an accu-  
491 mulation of sHsps during the recovery from heat  
492 stress, specifically in the low-elevation ecotype  
493 (higher level of thermotolerance) (Valcu et al.  
494 2008). Root protein profiling under heat stress  
495 identified 70 protein spots which showed differ-  
496 ential accumulation in at least one species. More  
497 proteins were downregulated as a result of heat  
498 stress, but *A. scabra* exhibited many upregulated  
499 protein spots under heat-stress regimes. The two  
500 grasses displayed different proteomic profiles.  
501 Some of the uniquely regulated genes by heat  
502 stress in *A. scabra* included sucrose synthase,  
503 superoxide dismutase, glutathione S-transferase,  
504 and stress-inducible heat-shock proteins. This  
505 suggests that these proteins may contribute to  
506 increased survival of *A. scabra* under high-  
507 temperature conditions (Xu et al. 2008). Heat-  
508 stress treatment in combination with drought  
509 resulted in the expression of approximately 650  
510 protein spots in *C. spinarum*. Forty-nine spots

511 changed their expression levels upon heat and  
512 drought treatment, and 30 proteins were identified  
513 by MS and 2-D Western blot. These proteins were  
514 classified into Hsps, photosynthesis-related  
515 proteins, RNA-processing proteins, and proteins  
516 involved in metabolism and energy production  
517 (Zhang et al. 2010). Proteomic profiling of radish  
518 leaves in response to high-temperature stress  
519 resulted in the identification of 11 differentially  
520 expressed protein spots, and they were divided  
521 into four categories: Hsps, redox homeostasis-  
522 related proteins, energy- and metabolism-related  
523 proteins, and signal transduction-associated  
524 proteins (Zhang et al. 2012). Such studies provide  
525 a good starting point in understanding the overall  
526 thermal responses of plants; however, further heat  
527 treatments and comparative analyses should be  
528 conducted in order to gain a better understanding  
529 of the overall thermal responses of plants.

530 Availability of proteomics data is important to  
531 support published results and conclusions. Several  
532 proteomics resources and repositories available  
533 for plant species were updated (Schneider et al.  
534 2012), e.g., Plant Proteome Database ([http://ppdb.  
536 tc.cornell.edu/](http://ppdb.<br/>535 tc.cornell.edu/)) which provides information on  
537 maize and *Arabidopsis* proteomes, RIKEN Plant  
538 Phosphoproteome Database (RIPP-DB, [http://  
540 phosphoproteome.psc.  
541 database.riken.jp](http://<br/>539 phosphoproteome.psc.database.riken.jp)) updated  
542 with a data set of large-scale identification of rice  
543 phosphorylated proteins (Nakagami et al. 2012),  
544 and OryzaPG-DB launched as a rice proteome  
545 database based on shotgun proteomics (Helmy  
546 et al. 2011). Besides those repositories, numerous  
547 very valuable resources, each focused on a spe-  
548 cific aspect like tandem mass spectra evidences,  
549 quantitative information, and localization of phos-  
550 phosphorylation sites, are available for plant proteo-  
551 mics such as ProMEX (Wienkoop et al. 2012);  
552 PhosPhAt, a plant phosphorylation site database  
553 (Arsova and Schulze 2012); PaxDb (Wang et al.  
554 2012), a meta-resource integrating information on  
555 absolute protein abundance levels across different  
556 organisms, including *A. thaliana*; MASCP Gator  
557 (Joshi et al. 2011), an aggregation portal for the  
558 visualization of *Arabidopsis* proteomics data; or  
559 PPDB, the Plant Proteome Database (Sun et al.  
560 2009) to cite only a few. UniProtKB is cross-  
561 linked to several of those proteomics resources,  
562

559 including PRIDE, IntAct, ProMEX, PeptideAtlas,  
560 and PhosphoSite. A complete list of the cross-  
561 references, with bibliographic references, is avail-  
562 able at <http://www.uniprot.org/docs/dbxref>. These  
563 databases help us in identifying and understanding  
564 the complex protein networks associated with the  
565 heat-stress tolerance and the functions of these  
566 proteins during heat stress.

### 567 2.3 Metabolomics

568 Metabolomics is the qualitative and quantitative  
569 collection of all low-molecular-weight meta-  
570 bolites present in a cell that participate in general  
571 metabolic reactions and are required for the main-  
572 tenance, growth, and normal function of a cell  
573 (Arbona et al. 2009; Jordan et al. 2009).  
574 Metabolome directly influences the phenotype  
575 when compared to transcriptome or proteome  
576 and bridges the gap between genotype and pheno-  
577 type. The study of the metabolome represents the  
578 integration of the genetic background and the  
579 influence of the environmental conditions, thus  
580 describing more accurately the phenotype of a  
581 given plant species. Metabolic regulation during  
582 stressful events has been facilitated much in the  
583 last decade, and the identification of metabolites  
584 has been improved through mass spectrometric  
585 studies (Sawada et al. 2009). More comprehensive  
586 coverage can only be achieved by using several  
587 extraction and detection technologies in parallel  
588 and subjecting them to chemical analysis using  
589 different analytical methods like gas chromatog-  
590 raphy coupled to mass spectrometry (GC-MS) and  
591 liquid chromatography coupled to mass spectrom-  
592 etry (LC-MS) (De Vos et al. 2007). Other analyti-  
593 cal techniques include liquid chromatography  
594 (photodiode array detection) coupled to mass  
595 spectrometry (LC-PDA/MS) (Huhman and  
596 Sumner 2002), capillary electrophoresis coupled  
597 to mass spectrometry (CE-MS) (Harada et al.  
598 2009, Takahashi et al. 2009), Fourier transform  
599 ion cyclotron resonance mass spectrometry  
600 (FTICR/MS) (Oikawa et al. 2006), and NMR  
601 spectroscopy (Krishnan et al. 2005). Among all  
602 analyzers that can be used with the separation  
603 techniques mentioned above, the most popular in

metabolomics are MS and, particularly, those 604  
providing accurate mass measures (Arbona et al. 605  
2013). Therefore, the future objective is the 606  
standardization and annotation of data from mul- 607  
tiple metabolomics technologies in public 608  
databases (Castellana and Bafna 2010). The data 609  
obtained can then be investigated by multivariate 610  
and correlation analyses for functional genomics 611  
in order to study the systems biology of plant 612  
metabolism and make use of the data for crop 613  
improvement (Arbona et al. 2013). From the 614  
genome sequence information of the *A. thaliana* 615  
and other model plants, it is evident that plants 616  
reorganize their metabolic network in order to 617  
adapt to such adverse conditions (Kaplan et al. 618  
2004). Many plants respond to different stresses 619  
by a progressive adjustment of their metabolism 620  
with early and late responsive gene networks. 621  
Some metabolic changes are common to salt, 622  
drought, and temperature stresses, whereas others 623  
are specific (Urano et al. 2009; Lugan et al. 2010). 624  
Using metabolic changes as a “map” or “marker,” 625

AU9

factors regulating metabolic movements were 626  
investigated by Saito et al. (2008) in combination 627  
with other “omics” approaches. It appears, there- 628  
fore, metabolomics plays a key role in understand- 629  
ing cellular functions and decoding the functions 630  
of genes (Hagel and Facchini 2008). 631

In plant systems, metabolomics approach has 632  
already been used to study metabolomic changes 633  
during a variety of stresses, for example, water and 634  
salinity (Cramer et al. 2007), sulfur (Nikiforova 635  
et al. 2005), phosphorus (Hernandez et al. 2007), 636  
oxidative (Baxter et al. 2007), and heavy metals 637  
(Le Lay et al. 2006). But, less work has been done 638  
in the case of heat stress. A recent metabolome 639  
analysis showed common metabolites in response 640  
to cold and other stresses and demonstrated a 641  
prominent role for the DREB1/CBF transcrip- 642  
tional network in the cold-response pathway 643  
(Maruyama et al. 2009). Comparative metabolite 644  
analysis has been carried out using GC-MS 645  
(Kaplan et al. 2004) and GCTOF-MS (Weinkoop 646  
et al. 2008) between *Arabidopsis* plants resp- 647  
onding to heat and cold shocks. Many metabolites 648  
produced in response to heat shock overlapped 649  
with those produced in response to cold shock 650  
also. Many metabolite levels changed specifically 651

AU8

652 in response to cold than to heat. This response  
653 points out a strong impact of cold stress on plant  
654 metabolism. Wang et al. (2004) reported that a  
655 combination of drought and heat stress results in  
656 decrease of the growth and productivity of the  
657 crops when compared with each of the different  
658 stresses applied individually. Integrated  
659 metabolome and transcriptome results were  
660 applied by Yamakawa and Hakata (2010) to ana-  
661 lyze rice developing caryopses under high-  
662 temperature conditions. Molecular events underly-  
663 ing pollination-induced and pollination-  
664 independent fruit sets were carried out by Wang  
665 et al. (2009a, b) and also the effects of DE-  
666 ETIOLATED1 downregulation in tomato fruits  
667 (Enfissi et al. 2010). Heat stress induced the accu-  
668 mulation of key metabolites like alanine, allantoin,  
669 arachidic acid, 2-ketoisocaproic acid, myo-  
670 inositol, putrescine, and rhamnose, while it  
671 decreased fructose-6-phosphate (Luengwilai et al.  
672 2012). Moreover, these results suggested that a  
673 metabolic network of compatible solutes including  
674 proline, monosaccharides, galactinol, and raffi-  
675 nose has an important role to play in temperature  
676 stress tolerance (Alcazar et al. 2010).

677 Information resources related to metabolic  
678 profiling are available and updated and provide  
679 data archives for metabolome data sets and analyt-  
680 ical platforms such as LC-MS-based metabolome  
681 database ([http://appliedbioinformatics.wur.nl/  
682 moto/](http://appliedbioinformatics.wur.nl/moto/)) (Moco et al. 2006), KOMICS (Iijima et al.  
683 2008), Plant MetGenMAP (Joung et al. 2009),  
684 Metabolome Express ([https://www.metabolome-  
685 express.org/](https://www.metabolome-express.org/)) (Carroll et al. 2010; Ferry-Dumazet  
686 et al. 2011), MeRy-B ([http://www.cbib.u-bor-  
687 deaux2.fr/MERYB/](http://www.cbib.u-bordeaux2.fr/MERYB/)) (Ferry-Dumazet et al.  
688 2011), KaPPA-View4 SOL (Sakurai et al. 2011),  
689 MetaCrop 2.0 ([http://metacrop.ipk-gatersleben.  
690 de](http://metacrop.ipk-gatersleben.de)) (Schreiber et al. 2012), and PRIME ([http://  
691 prime.psc.riken.jp/](http://prime.psc.riken.jp/)) (Sakurai et al. 2013). Apart  
692 from this, several individual species-wise  
693 databases are available at Gramene database like  
694 RiceCyc, MaizeCyc, BrachyCyc, SorghumCyc,  
695 and Sol Genomics Network (SGN). These  
696 databases play crucial roles as information  
697 resources and repositories of large-scale data sets  
698 and also serve as tools for further integration of  
699 metabolic profiles containing comprehensive data

acquired from other omics research (Akiyama 700  
et al. 2008). Following these successes, several 701  
multi-omics-based systems analyses have been 702  
used for understanding plant cellular systems. 703

## 2.4 Phenomics 704

Phenomics is the systematic study of the physical 705  
and morphological properties of organism as they 706  
change in response to genetic mutation and envi- 707  
ronmental influences. Traditional methods of 708  
measuring growth and other morphological 709  
features are time consuming and costly and 710  
involve many genotypes and the destructive har- 711  
vest of plants. Phenomics has been considered as 712  
one of the important techniques to screen the 713  
germplasm and to utilize the available morpho- 714  
logical variation in breeding programs aimed at 715  
heat-stress tolerance. Therefore, phenomics as a 716  
technique remains critical in the post-genomics 717  
era. Phenomics approach also enables us to under- 718  
stand the precise molecular mechanism involved 719  
in conferring tolerance against different kinds of 720  
abiotic stresses. This has stimulated the research 721  
in several institutions to invest in developing 722  
technologies and platforms able to speed up the 723  
phenotyping process. The investments started ear- 724  
lier in the private sectors, and more recently this 725  
has been embraced by public research institutions 726  
that are developing an international collaboration 727  
network ([www.plantphenomics.com](http://www.plantphenomics.com)). There are a 728  
large number of initiatives launched (International 729  
Plant Phenomics Network, Deutsche Plant 730  
Phenomics Network, and European Plant 731  
Phenomics Network) to create phenotyping 732  
facilities to screen populations using high- 733  
throughput methods located in Australia, 734  
Germany, France, Canada, Italy, and many more 735  
(Furbank 2009; Finkel 2009). Large phenotyping 736  
platforms represent technologies that are mainly 737  
based on nondestructive image analyses of plant 738  
tissues or structural and functional features 739  
obtained by advanced technologies (Nagel et al. 740  
2009; Yazdanbakhsh and Fisahn 2009). In other 741  
labs, glass houses and greenhouses can be fitted 742  
with cameras, and plants may be carried on the 743  
conveyor belts to the imaging stations. Such 744

745 facilities exist in several laboratories around the  
746 world (CropDesign, Belgium; The Plant Acceler-  
747 ator, Australia; PhenoPhab, Holland; Metapontum  
748 Agrobios, Italy; IPK, Germany) and have the  
749 advantage of acquiring 3-D images. Using high-  
750 throughput phenomics platforms, various  
751 parameters like water-deficit responses can be  
752 studied (Sadok et al. 2007; Berger et al. 2010).  
753 So far, only a handful of studies have been carried  
754 out in the phenomics area in response to heat  
755 stress in the crop plants (Sharma et al. 2012; Yeh  
756 et al. 2012). However, the application of  
757 phenomics will really become useful and impor-  
758 tant if specific questions are asked to these  
759 platforms.

760 Plants show numerous responses to heat stress  
761 regarding carbon metabolism and water balance,  
762 but unfortunately no single key physiological trait  
763 that relates to a genetic base for heat-stress toler-  
764 ance has been identified (Allakhverdiev et al.  
765 2008; Wolkovich et al. 2012). It is known that  
766 the reproductive processes are the most sensitive  
767 to heat stress in many species. Heat stress signifi-  
768 cantly affects cellular homeostasis including both  
769 protein and membrane stability. These responses  
770 include basal thermotolerance, short- and long-  
771 term acquired thermotolerance, and thermo-  
772 tolerance to moderately high temperatures. High  
773 temperatures adversely affect the seed germina-  
774 tion, growth, photosynthetic efficiency, core  
775 metabolic processes, pollen viability, respiration,  
776 water relations, and protein and membrane stabil-  
777 ity. Different species and cultivars may vary their  
778 tolerance to high temperatures with the stage of  
779 development, but all vegetative and reproductive  
780 stages are highly affected by heat stress (Hall  
781 1992). Different phenological stages of plants dif-  
782 fer in their sensitivity to high temperature. During  
783 vegetative stage, high day temperature can dam-  
784 age leaf gas-exchange properties. High night  
785 temperatures make the pollen sterile. But this  
786 depends on species and the genotype under  
787 study. Sharma et al. (2012) identified 41  
788 contrasting lines in terms of heat tolerance by  
789 mass screening of 1,274 wheat cultivars of diverse  
790 origin. This contrasting set of cultivars was then  
791 used to compare the ability of chlorophyll fluores-  
792 cence parameters to detect genetic difference in

heat tolerance. This identification may aid future 793  
studies to understand the genetic and physiologi- 794  
cal nature of heat-stress tolerance (Sharma et al. 795  
2012). The temperature and duration of heat-stress 796  
treatments resulting in changes in growth and 797  
development of seeds, seedlings, mature leaves, 798  
panicles or spikes, and fruits have been used in 799  
crop thermotolerance studies (Rahman et al. 2007; 800  
Seepaul et al. 2011). But, high-throughput pheno- 801  
typing analyses are necessary for deepening our 802  
understanding of the molecular genetics of 803  
thermotolerance. 804

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### 3 Heat-Shock Proteins

805

Heat-shock response (HSR) is a highly 806  
conserved reaction caused by exposure of an 807  
organism or tissue or cells to sudden high- 808  
temperature stress. High-temperature stress is 809  
characterized by rapid induction and transient 810  
expression of conserved heat-shock transcripts 811  
and other regulators. Among five conserved 812  
families of Hsps (Hsp100, Hsp90, Hsp70, 813  
Hsp60, and sHsp), the small heat-shock proteins 814  
(sHsps) are found to be most prevalent in plants, 815  
the expression of which can increase up to 200- 816  
fold under heat stress. Different classes of molec- 817  
ular chaperones appear to bind to specific nonna- 818  
tive substrates and states. Molecular Hsps/ 819  
chaperones are located in the cytoplasm and 820  
cell organelles such as the nucleus, mitochondria, 821  
chloroplasts, and ER (Wang et al. 2004). The 822  
mechanism by which Hsps contribute to heat 823  
tolerance is still enigmatic though several roles 824  
have been ascribed to them. Many studies assert 825  
that Hsps are molecular chaperones ensuring the 826  
native configuration and functionality of cell 827  
proteins under heat stress. During stress, many 828  
enzymes and structural proteins undergo deleter- 829  
ious structural and functional changes. There- 830  
fore, it is vital to maintain proteins in their 831  
functional conformations, preventing aggrega- 832  
tion of nonnative proteins and refolding of dena- 833  
tured proteins. It is also important to remove 834  
nonfunctional and harmful polypeptides arising 835  
from misfolding, denaturation, or aggregation for 836  
cell survival under stress. Thus, the different 837

838 classes of Hsps/chaperones cooperate and play  
 839 complementary and sometimes overlapping roles  
 840 in the protection of proteins from stress (Bowen  
 841 et al. 2002). When denatured or misfolded  
 842 proteins form aggregates, they can be resolu-  
 843 bilized by Hsp100/Clp followed by refolding or  
 844 degraded by proteases (Schöffl et al. 1998).  
 845 Some Hsps/chaperones (Hsp70, Hsp90) accom-  
 846 pany the signal transduction and transcription  
 847 activation that lead to the synthesis of other  
 848 members of Hsps/chaperones. Similar observa-  
 849 tions have been reported with plant chaperones.  
 850 It has been shown that Hsp18.1 from *Pisum*  
 851 *sativum* could stably bind to heat-denatured pro-  
 852 tein and maintained it in a folding-competent  
 853 state for further refolding by Hsp70/Hsp100  
 854 complexes (Mogk et al. 2003). A recent study  
 855 has shown that Hsp70 and Hsp90 have roles to  
 856 play in protecting the enzymes at higher  
 857 temperatures (Reddy et al. 2010, 2011). HSR in  
 858 plants was investigated in depth, and the pres-  
 859 ence of multiple signaling pathways was pro-  
 860 posed (Kotak et al. 2007; von Koskull-Doring  
 861 et al. 2007). Many studies noted upregulation of  
 862 transcripts including high-molecular-weight  
 863 Hsps like Hsp101, Hsp70s, and small Hsps  
 864 (Sarkar et al. 2009; Mittal et al. 2009; Chauhan  
 865 et al. 2011) and also identified additional  
 866 transcripts such as DREBs, galactinol synthases  
 867 and other enzymes in the raffinose oligosaccha-  
 868 ride pathway, and oxidative stress enzymes  
 869 (Frank et al. 2009; Suzuki et al. 2011).  
 870 Genome-wide survey for Hsps and Hsfs using  
 871 the tools of bioinformatics helps us to find out  
 872 not only the number of genes present in a genome  
 873 but also their chromosomal location. Software  
 874 tools also help us to find their subcellular  
 875 locations and the upstream promoter sequences  
 876 along with their predicted functions.

877 The regulation of heat-shock gene expression  
 878 in eukaryotes is mediated by the heat-shock tran-  
 879 scription factors (Hsfs), which are highly  
 880 conserved throughout the eukaryotic kingdom  
 881 (Scharf et al. 2012). Plant *Hsfs* have unique  
 882 characteristics and the existence of heat-stress-  
 883 induced *Hsf* genes might have a major role to  
 884 play in the modulation of transcription during  
 885 long-term heat-shock response (Chauhan et al.

2011). Temperature stress-response signal 886  
 transduction pathways and defense mechanisms 887  
 involving Hsfs and Hsps are thought to be inti- 888  
 mately associated with reactive oxygen species 889  
 (ROS) production (Frank et al. 2009). Heat- 890  
 shock transcription factor-dependent expression 891  
 of antioxidant enzymes such as ascorbate 892  
 peroxidases in *Arabidopsis* (Frank et al. 2009) 893  
 suggested that Hsfs might be involved not only in 894  
 Hsp regulation but also in the regulation of oxi- 895  
 dative stress (Reddy et al. 2009). Recent research 896  
 revealed the involvement of noncanonical tran- 897  
 scription factors in HSR; for instance, bZip28, a 898  
 gene-encoding membrane, tethered TF, which 899  
 was induced by HS, and the bzip28 null mutant 900  
 became hypersensitive to HS (Gao et al. 2008). 901  
 Besides Hsps, there are other plant proteins 902  
 including ubiquitin, LEA proteins, and cytosolic 903  
 Cu/Zn-SOD and Mn-SOD whose expressions are 904  
 stimulated upon heat stress. A number of 905  
 osmotin-like proteins induced by heat and nitro- 906  
 gen stresses, collectively called Pir proteins, have 907  
 also been found to be overexpressed in many 908  
 plant cells under heat stress conferring them 909  
 resistance. Microarray expression data in 910  
*Hordeum vulgare* revealed that most of the 911  
*sHsp* and *Hsf* genes are differentially regulated 912  
 during drought and temperature stresses at differ- 913  
 ent plant developmental stages suggesting con- 914  
 siderable cross talk between stress and non-stress 915  
 regulatory networks. In silico *cis*-regulatory 916  
 motif analysis of *Hsf* promoters showed an 917  
 enrichment with abscisic acid-responsive *cis*- 918  
 elements (ABREs), implying regulatory role of 919  
 ABA in mediating transcriptional response of 920  
*HvsHsf* genes. 921

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#### 4 Heat-Shock Promoters 922

The need for inducible or specific promoters can 923  
 be a key tool in plant biotechnology, and their need 924  
 will increase as we attempt to transfer and validate 925  
 genes associated with abiotic stress tolerance. In 926  
 the last decade, several candidate genes, pathways, 927  
 and strategies have been identified by various 928  
 groups across the globe and provided insights in 929  
 plant heat-stress adaptation. Strong constitutive 930

931 promoters are routinely used in plant transforma- 980  
932 tion with a regulated expression of heat-stress- 981  
933 responsive genes. But such use of constitutive 982  
934 promoters is resulting in serious penalties on 983  
935 plant growth and development with overall nega- 984  
936 tive performance of transgenics (Sakuma et al. 985  
937 2006a, b). Since constitutive promoters are ham- 986  
938 pering the final productivity, it is important for us 987  
939 to identify and isolate heat-stress-inducible 988  
940 promoters and use them while developing trans- 989  
941 genic crops. However, the regulated expression of 990  
942 transgenes in plants in a tissue-specific manner and 991  
943 at a specific developmental stage remains a chal- 992  
944 lenging task. The isolation and characterization of 993  
945 heat-stress-responsive promoters and their regu- 994  
946 latory regions will have more biotechnological 995  
947 applications as these promoters could be used to 996  
948 engineer the target genes to express only at the site 997  
949 of stress. A powerful approach for measuring the 998  
950 activity of any heat-shock promoter is by fusing 999  
951 the promoter of heat-shock gene to reporter genes 1000  
952 such as GFP or GUS. This allows measuring the 1001  
953 developmental and tissue-specific expression of 1002  
954 genes with or without heat stress (Khurana et al. 1003  
955 2013). It has been found that while some 1004  
956 transcripts exhibit translational repression, others 1005  
957 escape such repression and remain actively trans- 1006  
958 lated. But the underlying mechanisms that mediate 1007  
959 this control especially the identities of the regu- 1008  
960 latory RNA elements involved were poorly under- 1009  
961 stood. Using a computational and experimental 1010  
962 approach, Matsuura et al. (2013) identified a 1011  
963 novel *cis*-regulatory element in the 5'-UTR that 1012  
964 affects differential translation and has a *cis*-regu- 1013  
965 latory signature responsible for heat-stress-regu- 1014  
966 lated mRNA translation in *Arabidopsis*. A 1015  
967 comprehensive transcriptome analysis by using 1016  
968 microarrays revealed the relationships among 1017  
969 stress-regulated transcripts and enabled the predic- 1018  
970 tion of their *cis*-regulatory elements in temperature 1019  
971 stress-inducible genes (Weston et al. 2008). In 1020  
972 addition, characterization of the transcriptional 1021  
973 dynamics during seed development under different 1022  
974 stress conditions enabled the prediction of their 1023  
975 *cis*-regulatory elements (Weston et al. 2008). Ma 1024  
976 and Bohnert (2007) showed a clear correlation 1025  
977 between expression profiles and the 5'*cis*-regu- 1026  
978 latory motifs of stress-regulated genes. These 1027  
979 analyses indicated that stress-regulated genes are

controlled by a complicated regulatory network 980  
and cross talk between pathways. This type of 981  
network has been proposed based on 982  
transcriptome data using different bioinformatics 983  
approaches (Long et al. 2008). The basic structure 984  
and promoter recognition of Hsfs are highly 985  
conserved throughout the eukaryotic kingdom 986  
(Scharf et al. 2012). Expression of *cis*-motifs 987  
containing these *Hsf* genes might be regulated 988  
by *Hsfs* themselves, via formation of a regulatory 989  
network as proposed by Nover et al. (2001). The 990  
expression of *Hsps* during stress treatments and 991  
different developmental stages will depend on the 992  
*cis*-motifs present in the respective *Hsp* and *Hsf* 993  
promoters which will subsequently bind to differ- 994  
ent transcription factors particularly *Hsfs* as 995  
demonstrated by transient reporter assays in 996  
sunflower embryos (Almoguera et al. 2002). 997  
Hsp18.2 promoter fused to the *GUS* gene trans- 998  
genic *Arabidopsis* plants showed that heat stress 999  
induced the *GUS* gene activity in almost all the 1000  
organs of the plant (Takahashi et al. 1992). Simi- 1001  
larly, heat-shock-induced GUS activity was 1002  
observed in transgenic *Arabidopsis* when the pro- 1003  
moter of *Hsp81* gene was used (Yabe et al. 1994). 1004  
Crone et al. (2001) did a detailed analysis of the 1005  
expression of the GmHsp17.5E promoter in all 1006  
the organs and tissues of the flower and found that 1007  
promoter is differentially expressed after heat 1008  
shock in different floral tissues. Hsfs bind to 1009  
heat-stress elements (HSEs) with the core 1010  
sequence nGAAnnTTCn or nTTCnnGAAn and 1011  
form trimers, thereby regulate downstream gene 1012  
expression (Wu 1995). Despite the occurrence of 1013  
heat-shock elements in the promoters of heat- 1014  
stress-inducible genes, a set of *Hsp* genes are 1015  
expressed during seed development (Kotak et al. 1016  
2007). Atsp90-1 promoter region contributes in a 1017  
combinatorial manner to regulate the expression 1018  
in development and stress conditions (Harala- 1019  
mpidis et al. 2002). The heat-stress induction of 1020  
*Arabidopsis* HsfA3 is regulated directly by 1021  
DREB2A, a transcription factor functioning in 1022  
drought stress responses (Sakuma et al. 2006a, 1023  
b). Accordingly, the DRE has been identified in 1024  
the promoters of a cluster of heat-inducible genes 1025  
(Larkindale and Vierling 2008). 1026

Promoters of heat-shock protein (*Hsp*) genes 1027  
are good candidates for inducible expression, 1028

1029 since they are rapidly and highly induced under  
1030 heat-stress conditions. Besides, their induction can  
1031 be accurately controlled by varying the tempera-  
1032 ture and duration of induction. Several detailed  
1033 studies have been performed using the reporter  
1034 gene expression driven by plant small Hsp  
1035 promoters in different hosts. The AtHsp18.2 pro-  
1036 moter has been successfully used in *Arabidopsis*  
1037 (Takahashi et al. 1992) and in other species, such  
1038 as *N. plumbaginifolia* (Moriwaki et al. 1999) and  
1039 *N. tabacum* hairy roots (Lee et al. 2007). Besides,  
1040 the inducibility of soybean GmHsp17.3B pro-  
1041 moter was studied in the moss *Physcomitrella*  
1042 *patens* (Saidi et al. 2007). *Arabidopsis* Hsp18.2  
1043 promoter was used to drive the expression of  
1044 GUS gene in *N. tabacum* BY-2 cells, and maxi-  
1045 mum activity of protein was obtained under the  
1046 heat stress (Shinmyo et al. 1998). Khurana et al.  
1047 (2013) studied the wheat sHsp26 promoter activity  
1048 in transgenic *Arabidopsis* and observed consis-  
1049 tently high levels of *GUS* gene expression under  
1050 different abiotic stress conditions especially in  
1051 heat stress. However, the mechanisms that regulate  
1052 Hsp expression during seed maturation remain  
1053 largely unknown. In addition to their direct  
1054 functions in acquired stress tolerance and develop-  
1055 ment, Hsps/chaperones function synergistically  
1056 with other components, thus help in decreasing  
1057 cellular damage. If the range of promoters is  
1058 wide, then it is possible to introduce multiple  
1059 transgenes into plants that are expressed differen-  
1060 tially in response to various environmental  
1061 stresses. Identification of heat-stress-inducible  
1062 promoters from crop plants would be of immense  
1063 help in generating transgenic plants with improved  
1064 agronomic performance.

---

1065 **5 Heat-Stress-Tolerant**  
1066 **Transgenic Crops Generated**  
1067 **Through Expression of Hsps**

1068 Most crops are affected by daily/seasonal  
1069 fluctuations in day and/or night temperatures.  
1070 Conventional breeding for high-temperature  
1071 stress tolerance has not been successful so far.  
1072 This could be due to lack of our understanding on  
1073 the genetic mechanisms associated with heat

1074 stress, suitable source of genes, and complex  
1075 nature of the HS trait. This complexity is now  
1076 being dissected out including features like heat-  
1077 shock elements (HSEs), heat-shock factors  
1078 (HSFs), possible receptors of the heat-shock  
1079 response, signaling components, and chromatin  
1080 remodeling aspects (Proveniers and van Zanten  
1081 2013). Several groups have altered the levels of  
1082 sHsps in bacterial systems and shown that when  
1083 overexpressed in bacterial cells, Hsps have a role  
1084 in conferring thermotolerance. The overexp-  
1085 ression of *OsHsp16.9* in *E. coli* conferred  
1086 thermotolerance. Yeh et al. (2012) constructed  
1087 deletion mutants of this sHsp to find out the  
1088 regions associated with heat-stress tolerance.  
1089 They overexpressed the constructs in *E. coli*  
1090 (Yeh et al. 2012) and found out that the deletion  
1091 of amino acid residues 30–36 (PATSDND) in the  
1092 N-terminal domain or 73–78 (EEGNVL) in the  
1093 consensus-II domain of *OsHsp16.9* caused the  
1094 loss of chaperone activities and also rendered  
1095 the *E. coli* incapable of surviving at 47.5 °C.  
1096 When three sHsps were introduced into *E. coli*,  
1097 they acquired thermotolerance and were able to  
1098 protect malate dehydrogenase (MDH) from  
1099 in vitro thermal aggregation (Pike et al. 2001).  
1100 The survivability of *E. coli* B121 (DE3) cells  
1101 transformed with a recombinant plasmid  
1102 containing different Hsps was compared with  
1103 the control *E. coli* cells (transformed with the  
1104 PET28a vector) under heat and different abiotic  
1105 stresses. The PgHsp transformed cells showed  
1106 thermotolerance at 47.5 °C, a treatment that  
1107 was lethal to the untransformed bacterial cells.  
1108 When the cell lysates from transformed and  
1109 untransformed were heated at 55 °C, the amount  
1110 of protein denatured in the PgHsps-B121 DE3  
1111 cells was 50 % less than that of the PET28a  
1112 vector (control) cells (Reddy et al. 2010, 2011).  
1113 Furthermore, genetically modified *E. coli*  
1114 expressing DcHsp17.7 exhibited a higher salt  
1115 stress tolerance than control *E. coli* (Song and  
1116 Ahn 2011). These results suggest that expression  
1117 of Hsps confers abiotic stress tolerance to *E. coli*  
1118 cells and may play a role in the plant's adaptation  
1119 to harsh environments.

1120 The involvement of Hsps in regulating thermo-  
1121 tolerance has been further carried out in higher

1122 plants by downregulating their expression levels  
1123 using either antisense or RNAi approach. Mutants  
1124 of *Zea mays* and *A. thaliana* plants under-  
1125 expressing their respective Hsp100 proteins were  
1126 observed to lack both basal and induced  
1127 thermotolerance (Hong and Vierling 2000, Nieto-  
1128 Sotelo et al. 2002). Yang et al. (2006) showed that  
1129 the tomato plants silenced for Hsp100/ClpB protein  
1130 were impaired in thermotolerance. Acquisition of  
1131 thermotolerance has been found to be negatively  
1132 affected in Hsp70 antisense *A. thaliana* plants (Lee  
1133 and Schoffl 1996). Mutants of *Zea mays* and  
1134 *Arabidopsis* with low levels of their respective  
1135 Hsp100 proteins were observed to lack both basal  
1136 and induced thermotolerance. Plants lacking Hsa32  
1137 do not survive HS treatment even after a  
1138 pretreatment at a sublethal temperature (Chang  
1139 et al. 2006). Heat-inducible transactivator HsfA2  
1140 with low levels of expression results in an increased  
1141 sensitivity of the mutant plants to heat stress  
1142 (Chang et al. 2007). Genome-wide transcriptome  
1143 analysis of HsfA1a, A1b, and A2 knockout mutants  
1144 in *Arabidopsis* suggests that HsfA1a and A1b play  
1145 important roles in the initial phase of heat-stress  
1146 response, but HsfA2 functions under prolonged  
1147 heat-stress conditions and during the recovery  
1148 phase (Schramm et al. 2006; Nishizawa et al.  
1149 2006). The heat-stress-induced expression of  
1150 HsfA2 in *Arabidopsis* is not influenced by  
1151 HsfA1a or HsfA1b (Busch et al. 2005). The  
1152 *HsfA2* gene is also induced by high light intensity  
1153 and H<sub>2</sub>O<sub>2</sub> (Nishizawa et al. 2007). It is also closely  
1154 related to the regulation of *ASCORBATE PEROX-*  
1155 *IDASE 2 (APX2)* encoding a key enzyme in oxida-  
1156 tive stress response, indicating that HsfA2 plays  
1157 diverse roles under various environmental stresses.  
1158 Conversely, upregulation of Hsps has been  
1159 achieved in a large number of plant species. Trans-  
1160 genic carrot cell lines and plants in which carrot  
1161 *sHsp17.7* was overexpressed resulted in enhanced  
1162 survival of transgenic tissues at high temperature  
1163 (Malik et al. 1999). Transgenic tobacco plants  
1164 overexpressing tobacco sHsps result in higher cot-  
1165 yledon opening rate (Park and Hong 2002). Simi-  
1166 larly, transgenic tomato plants overexpressing  
1167 tomato *HsfA1* gene showed increased thermo-  
1168 tolerance. Tomato mitochondrial *Hsp* gene  
1169 overexpressed in tobacco showed that transgenics

were more thermotolerant at 48 °C than the 1170  
transgenics produced with the antisense construct 1171  
of the same gene (Sanmiya et al. 2004). Transgenic 1172  
rice overexpressing *OsHsp17.7* gene showed 1173  
increased thermotolerance and greater resistance 1174  
to UV-B stress than untransformed control plants 1175  
(Murakami et al. 2004). Constitutive expression of 1176  
*RcHsp17.8* in transgenic *Arabidopsis* conferred 1177  
higher thermotolerance and resistance to salt, 1178  
drought, and osmotic stresses (Jiang et al. 2009). 1179  
Overexpression of *CaHsp26* in transgenic tobacco 1180  
protected PSII and PSI during chilling stress under 1181  
low irradiance (Guo et al. 2007). When transgenic 1182  
*A. thaliana* plants were generated with overexp- 1183  
ression of high-molecular-weight Hsps, trans- 1184  
genics survived at temperatures as high as 45 °C 1185  
(1 h) and they showed vigorous growth after 1186  
relieving the plants from stress, while vector- 1187  
transformed control plants could not regain growth 1188  
during the post-stress recovery period (Queitsch 1189  
et al. 2000). Similarly, transgenic rice lines 1190  
overexpressing *AtHsp100* exhibited regrowth in 1191  
the post-high-temperature-stress recovery phase, 1192  
while the untransformed plants could not recover 1193  
to the similar extents (Katiyar-Agarwal et al. 1194  
2003). Recent study in maize demonstrated that 1195  
small *Hsp* gene, *ZmsHsp*, might have a function in 1196  
cytokinin response (Cao et al. 2010). Also, 1197  
*MsHsp23* gene in the tall fescue (*Festuca* 1198  
*arundinacea*) transgenics protected the leaves 1199  
from oxidative damage through chaperon and anti- 1200  
oxidant activities. These results suggest that 1201  
*MsHsp23* confers abiotic stress tolerance in trans- 1202  
genic tall fescue and may be useful in developing 1203  
stress tolerance in other crops also (Lee et al. 1204  
2012). Overexpression of *ZmHSP16.9* in trans- 1205  
genic tobacco conferred tolerance to both heat 1206  
and oxidative stresses and increased the seed ger- 1207  
mination rate, root length, and antioxidant enzyme 1208  
activities when compared with wild-type plants 1209  
(Sun et al. 2012). Transgenic *Arabidopsis* plants 1210  
overexpressed with *WsHsp26* were tolerant under 1211  
continuous high temperature and produced bold 1212  
seeds under high temperature, having higher ger- 1213  
mination rate than wild type (Chauhan et al. 1214  
2012). The list of transgenic plants raised for high- 1215  
temperature tolerance using Hsps is shown in the 1216  
Table 1. 1217

**Table 1** Comprehensive details on plant transgenics raised by using Hsp genes for high-temperature tolerance

Gene	Protein	Source	Target plant	Function	Reference
<i>Hsf1</i>	Hsf	<i>A. thaliana</i>	<i>A. thaliana</i>	Thermotolerance and also constitutive expression of the Hsp genes	Lee et al. (1995)
<i>Hsf3</i>	Hsf	<i>A. thaliana</i>	<i>A. thaliana</i>	Increase in basal thermotolerance and thermoprotective processes	Prandl et al. (1998)
<i>HsfA1</i>	Hsf	<i>S. lycopersicon</i>	<i>S. lycopersicon</i>	Advantage for growth and fruit ripening processes under high temperature	Mishra et al. (2002)
<i>Hsf3</i>	Hsf	<i>A. thaliana</i>	<i>A. thaliana</i>	Lower threshold temperature	Panchuk et al. (2002)
<i>HsfA2</i>	Hsf	<i>A. thaliana</i>	<i>A. thaliana</i>	Mutants displayed reduced basal and acquired thermotolerance, while the overexpression lines displayed increased tolerance	Li et al. (2005)
<i>HsfA2e</i>	Hsf	<i>O. sativa</i>	<i>A. thaliana</i>	Enhanced thermotolerance	Yokotani et al. (2005)
<i>HsfA2</i>	Hsf	<i>A. thaliana</i>	<i>A. thaliana</i>	Increased thermotolerance but also salt/osmotic stress tolerance and enhanced callus growth	Ogawa et al. (2007)
<i>HsfA2e</i>	Hsf	<i>O. sativa</i>	<i>A. thaliana</i>	Enhances tolerance to environmental stresses	Yokotani et al. (2008)
<i>Hsf7</i>	Hsf	<i>O. sativa</i>	<i>A. thaliana</i>	Response to high temperature	Liu et al. (2009)
<i>HsfC1b</i>	Hsf	<i>O. sativa</i>	<i>O. Sativa</i>	Osmotic stress and is required for plant growth under non-stress conditions	Schmidt et al. (2012)
<i>DnaK</i>	Hsp70	<i>Aphanothece halophytica</i>	<i>O. sativa</i> and <i>N. tabacum</i>	Increased seed yield and total plant biomass in high temperature and salt stress	Uchida et al. (2008)
<i>Hsp70</i>	Hsp70	<i>Trichoderma harzianum</i>	<i>A. thaliana</i>	Enhanced tolerance to heat stress	Montero-Barrientos et al. (2010)
<i>mtHsp70</i>	Hsp70	<i>O. sativa</i>	<i>O. Sativa</i>	Suppresses programmed cell death	Qi et al. (2011)
<i>Hsc70-1</i>	Hsp70	<i>A. thaliana</i>	<i>A. thaliana</i>	More tolerant to heat shock	Sung and Guy (2003)
<i>Hsp101</i>	HSP100	<i>A. thaliana</i>	<i>A. thaliana</i>	Sudden shifts to extreme temperature better than the controls	Quietsch et al. (2000)
<i>Hsp101</i>	Hsp100	<i>A. thaliana</i>	<i>O. sativa</i>	Enhanced tolerance to high temperature	Katiyar-Agarwal et al. (2003)
<i>Hsp17.7</i>	Hsp17.7	<i>D. carota</i>	<i>D. carota</i>	Increased thermotolerance	Malik et al. (1999)
<i>mtsHsp</i>	sHsp	<i>S. lycopersicon</i>	<i>N. tabacum</i>	Thermotolerance	Sanmiya et al. (2004)
<i>Hsp21</i>	sHsp	<i>S. lycopersicon</i>	<i>S. lycopersicon</i>	Temperature-dependent oxidative stress	Neta-Sharir et al. (2005)
		<i>sHsp17.7</i>	sHsp	<i>O. sativa</i>	<i>O. Sativa</i>
Drought		tolerance in transgenic rice seedlings	Sato and Yokoya (2008)		
<i>Hsp16.9</i>	sHsp	<i>Zea mays L.</i>	<i>N. tabacum</i>	Enhanced tolerance to heat and oxidative stress	Sun et al. (2012)
<i>Hsp17.5</i>	sHsp	<i>Nelumbo nucifera</i>	<i>A. thaliana</i>	Improved basal thermotolerance	Zhou et al. (2012)
<i>Hsp26</i>	sHsp	<i>Capsicum annuum</i>	<i>N. tabacum</i>	Protection of PSII and PSI during chilling stress under low irradiance	Guo et al. 2007

(continued)

t.26 **Table 1** (continued)

t.27	Gene	Protein	Source	Target plant	Function	Reference
t.28	<i>Hsp17.8</i>	sHsp	<i>Rosa chinensis</i>	<i>A. thaliana</i>	Increased tolerance to heat, salt, osmotic, and drought stresses	Jiang et al. (2009)
t.29			<i>sHsp17.7</i>	sHsp	<i>O. sativa</i>	<i>O. sativa</i>
			Increased	thermotolerance	Murakami et al. (2004)	
	<i>sHsp26</i>	sHsp	<i>Triticum</i>	<i>A. thaliana</i>	Seed maturation and germination and imparts tolerance to heat stress	Chauhan et al. (2012)
	<i>sHsp18</i>	sHsp	<i>Opuntia streptacantha</i>	<i>A. thaliana</i>	Increased the seed germination rate under salt, osmotic, and ABA treatments	Salas-Muñoz et al. (2012)

## 1218 **6 Conclusions**

1219 It appears that a wide range of “omics” studies are  
 1220 currently in progress using numerous methodo-  
 1221 logies, plant species, and stress conditions. As  
 1222 more results are published, it is becoming increas-  
 1223 ingly clear that high-temperature stress causes dis-  
 1224 tinct molecular responses in plant tissues. As more  
 1225 data are generated in such studies, it provides  
 1226 suitable candidates for selective breeding prog-  
 1227 rams aimed at enhancing stress tolerance in  
 1228 ecologically and economically important plant  
 1229 species. Plant cells are fundamentally different to  
 1230 those of mammalian species, and these biological  
 1231 differences cause inherent difficulties in plant  
 1232 functional genomic studies. Advances in techni-  
 1233 ques and approaches will change the way plant  
 1234 heat-stress omics studies are conducted in future.  
 1235 Genomics, transcriptomics, proteomics, and  
 1236 metabolomics investigate different facets of a  
 1237 given scientific issue, such as heat-stress tolerance,  
 1238 but complement each other. Integration of pheno-  
 1239 typic, genetic, transcriptomic, proteomic, and  
 1240 metabolomic data will enable accurate and  
 1241 detailed gene network reconstruction. This will  
 1242 ultimately result in the elucidation of the molecu-  
 1243 lar pathways involved in complex phenotypic  
 1244 traits. A better understanding of genetic and cellu-  
 1245 lar mechanisms behind heat-stress tolerance would  
 1246 facilitate generation of transgenic plants with  
 1247 desired traits with little or no undesired/unforeseen  
 1248 effects. Bioinformatics tools are also helping us to  
 1249 obtain genome-wide data on the number of Hsps  
 1250 and Hsfs and their regulations. Taken together, the  
 1251 omics data and the information generated using

the tools of bioinformatics would help us to  
 understand better about heat-stress tolerance in  
 crop plants. Future knowledge of tolerance compo-  
 nents and the identification of QTLs and cloning  
 of responsible genes may allow transformation of  
 plants with multiple genes and production of highly  
 stress-tolerant transgenic crop plants.

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