

Golam Jalal Ahammed · Jing-Quan Yu
Editors

Plant Hormones under Challenging Environmental Factors

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*This book is dedicated to the Institute of
Vegetable Science of Zhejiang University for
advancing vegetable research and industry in
China.*

Preface

In nature, plants are often exposed to different environmental adversities. Although success in crop cultivation involves good care and management, some environmental factors at extreme states pose serious challenges that ultimately limit crop production. Abiotic stress is one of the primary causes of crop losses worldwide. The stress caused by abiotic factors alters normal plant metabolism leading to various negative effects on plant growth, development, and productivity. To survive, sessile plants have evolved a wide array of molecular programs to perceive environmental stimuli rapidly and adapt accordingly. In recent years, much progress has been achieved in unraveling the complex stress response mechanisms, particularly the involvement of different phytohormones in stress perception and signal transduction. Phytohormones are the most fascinating features of plant system that precisely regulate growth, development, and responses to stresses. In addition to normal regulatory functions, classical phytohormones such as auxins, cytokinins, gibberellins, abscisic acid, and ethylene could induce stress tolerance to various abiotic factors. New plant hormones such as jasmonates, salicylates, brassinosteroids, strigolactones, etc., have also been implicated in plant growth and stress adaptation. Although the in-depth mechanisms of phytohormone-mediated stress tolerance still remain largely unknown, plant growth regulators or hormone analogues are being used to manage different environmental adversities. Nonetheless, there is still a remarkable gap between theory and practice in terms of large-scale field application.

In this book, we tried to provide a unique compilation of the roles of phytohormones in the response of plants to abiotic stresses considering heat, cold, drought, salinity, flooding, soil acidity, heavy metals, light, and ozone as an individual environmental hazard in each chapter. The physiological and molecular mechanisms controlling phytohormone-mediated tolerance to a single abiotic stress and interactions among them are discussed for relevant cases. In the last chapter, genetic engineering aspects of phytohormone metabolism along with major challenges and future research directions are suggested. Much attention has been paid to adhere with the focus in each chapter that enabled the authors to avoid repetition of similar issues. It is worth mentioning that all authors of this book have recently contributed original research articles in the field of phytohormone research. The chapters are

written at the levels intended to be useful to students (senior undergraduate and postgraduate) and researchers in plant physiology, biochemistry, and biotechnology. Although minor editorial changes were adopted, author's justification was kept intact in each chapter. However, some errors may still exist in the book, and thus we would greatly appreciate reader's feedback for potential improvement in future edition. We wish to thank all the authors who joined this book project by contributing their valuable works. We extend our sincere thanks to Springer Science+Business Media, especially Mr. Zachary Romano (editor, biochemistry and molecular biology, Springer New York), Mr. Abbey (Xiaojin) Huang (assistant editor, medicine and biological sciences, Springer Beijing Office), and all the other staff members of Springer involved in this book project for their generous cooperation.

Hangzhou, Zhejiang, People's Republic of China

Golam Jalal Ahammed
Jing-Quan Yu

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Chapter 1

Role of Hormones in Plant Adaptation to Heat Stress

Golam Jalal Ahammed, Xin Li, Jie Zhou, Yan-Hong Zhou,
and Jing-Quan Yu

Abstract Heat stress is one of the devastating abiotic stresses that cause substantial crop loss around the world. The frequency and magnitude of heat stress are being intensified due to global climate change. Heat stress induces excessive production of reactive oxygen species that cause damage to lipids, proteins, and nucleic acids in plants. Plants have evolved various sophisticated mechanisms to sense heat stimuli and activate different defense responses rapidly to protect its vital cellular structures from heat-induced damage. Phytohormones are the endogenous messenger molecules that precisely mediate plant growth, development, and responses to various biotic and abiotic stresses including heat stress. With the advancement of molecular technologies, several hormones that were previously known only for their roles in plant growth and development have also been implicated in the heat stress response. To date, all major hormones such as abscisic acid, auxin, gibberellins, cytokinins, salicylic acid, jasmonic acid, ethylene, and brassinosteroids have been reported to play critical roles in response of plants to heat stress. In this chapter, we intend to review how various phytohormones are involved in plant adaptation to heat stress. Furthermore, we discuss the potential role of important plant hormones in the enhancement of heat tolerance. Hormone cross talk that mediates the response of plants to heat stress is also discussed.

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Keywords Heat stress response • Phytohormones • Hormone cross talk • Basal thermotolerance • Acquired thermotolerance • Heat shock proteins • Heat sensors • PIF4

1.1 Introduction

Plant growth and development are precisely regulated by coordination of both exogenous (environmental) and endogenous signals (Peleg and Blumwald 2011; Santner et al. 2009). External environmental factors such as light, temperature, moisture, and atmospheric carbon dioxide at optimum levels are essential for normal metabolic processes in plants. Among those various environmental factors, temperature is of great significance in the regulation of plant phenological development (Bahuguna and Jagadish 2015). A temperature beyond the “physiological optimum” that disturbs normal growth and development of a plant is generally considered as “high temperature” for that plant. Extremely high temperatures have the potential to cause “heat stress” in plants. However, the physiological threshold for the highest temperature that causes irreversible damage varies significantly across the plant kingdom even within genotypes. Heat-induced damages include changes in stability of proteins, enzymes, nucleic acids, biomembranes, and cytoskeletal structures (Asthir 2015). In order to maintain appropriate balance in metabolic processes, plants thoroughly or partially reprogram its transcriptome, proteome, metabolome, and lipidome (Mittler et al. 2012).

The frequency and magnitude of temperature extremes are being increased due to global climate change. Extreme seasonal heat which is considered as an important attribute of climate change could have detrimental impacts on agricultural productivity and food security by directly affecting crop growth and yield. In the tropics and subtropics, growing season temperatures will exceed the recorded highest seasonal temperatures of the last century by the end of the twenty-first century. Empirical data show that every 1 °C increase in seasonal temperature will likely to cause 2.5 to 16 % direct yield losses (Battisti and Naylor 2009). A recent study that analyzed national disaster-caused crop production losses (during 1964–2007) across the globe shows that drought and extreme heat dramatically decreased national cereal production by 9–10 %, and reduction in cereal yields was mainly due to extreme heat (Lesk et al. 2016). The authors estimated that a 1 °C increase in seasonal mean weather associated with extreme heat disasters results in a yield sensitivity of 6–7 %. Thus, rising temperature represents severe risks of food insecurity. It is anticipated that increasing heat stress will cause complete extinction of many species (Field et al. 2014). Therefore, climate change will obviously intensify the deleterious effect of heat stress on crop yield in the future.

As plants cannot relocate, they have to experience brutality of environmental extremes *in situ*. Extreme high temperature severely affects different biochemical processes in plants that are sensitive to heat shock. Heat stress causes changes in respiration and photosynthesis leading to a decreased plant productivity. Heat stress-

induced injuries are associated with over production of reactive oxygen species (ROS) that cause cellular damage. Through evolutionary success, plants have developed a complex but effective heat stress response (HSR) pathway that precisely regulates both short-term and long-term heat responses in order to minimize or prevent damage from heat stress. A better understanding of this complex heat response mechanism is important for future genetic manipulation of crops to ensure food security in the face of climate change (Hasanuzzaman et al. 2013; Kazan 2015).

Phytohormones are the endogenous messenger molecules that play a critical role in plant growth, development, and responses to various stresses. A number of previous reports provided solid evidence that phytohormones are actively involved in the response of plants to heat stress (Ahammed et al. 2014; Peleg and Blumwald 2011; Xia et al. 2015). Moreover, majority of the phytohormones provide physiological protection against heat stress. In addition to the individual role of a plant hormone, cross talk between multiple hormones precisely coordinates plant defense response to heat stress. Although significant advancement has been achieved in the molecular mechanisms of heat perception, the mechanisms that control phytohormone-mediated responses to heat stress still remain largely unknown. In this chapter, we briefly highlight the key progress on plant hormone research relating to the mechanisms of temperature sensing and subsequent thermal responses that are mediated by complex phytohormone networks. Additionally, we discuss the potential role of important phytohormones in the enhancement of heat tolerance.

1.2 Sensing Thermal Stimuli by Plants

In plants, changes in temperature are simultaneously perceived by all plant cells that are exposed to high temperature. Thus, at the cellular level, thermal perception and signaling may involve similar components across the plants (Mittler et al. 2012; Saidi et al. 2011). Plants have developed highly sophisticated mechanism that can even sense small changes (even 1 °C) in temperature (Kumar and Wigge 2010). Multiple pathways, regulatory networks, and cellular compartments are involved in the heat stress response (HSR) in plants (Mittler et al. 2012). The HSR is a highly conserved environmental and developmental program in plants and is an important component of the acclimation response. Using a wide array of thermosensors, plants precisely sense the absolute and gradual changes in diurnal and seasonal temperature (Bahuguna and Jagadish 2015).

All macromolecules in a plant cell such as protein complexes, membranes, and nucleic acids can simultaneously perceive heat stimuli from the surrounding atmosphere, and thus they serve as thermosensors through reversible physical changes such as increased membrane fluidity, partial melting of DNA and RNA strands, dissociation of proteins, etc. (Mittler et al. 2012; Richter et al. 2010). However, primary heat sensors or heat-responsive macromolecules must have the potential to perceive heat stimuli precisely followed by differential response to temperature change depending on the extent of variation. More importantly, they must possess

capability to differentially activate a unique signaling pathway that ultimately upregulates hundreds of HSR genes. As reviewed in detail elsewhere, at least four putative sensors are assumed to be involved in triggering the HSR (Mittler et al. 2012): such as (1) a plasma membrane channel for initiating an inward calcium flux, (2) a histone sensor localized in the nucleus (H2A.Z nucleosomes), (3) one unfolded protein sensor in the endoplasmic reticulum, and (4) one unfolded protein sensor in the cytosol.

Briefly, heat stress alters membrane stability and activates a plasma membrane (PM) calcium channel that causes an inward flux of calcium. In addition, lipid signaling is also stimulated by the alteration in membrane stability. Upon binding with the calmodulin CaM3, calcium activates various kinases and transcriptional regulators of the HSR, including heat shock transcription factors (HSFs), multiprotein-bridging factor 1c (MBF1c), WRKY, and dehydration-responsive element-binding transcription factors (DREB). PM-localized ROS-generating enzyme respiratory burst oxidase homolog D (RBOHD) is also activated by enhanced cytosolic calcium flow. ROS play a dual role; while NADPH oxidase-dependent ROS generation triggers redox signaling that activates MBF1c, HSFs, and mitogen-activated protein kinases (MAPKs), heat-induced excessive accumulation of ROS causes metabolic imbalance. The unfolded protein sensor in the cytosol functions through HSFs, whereas the unfolded protein sensor in the endoplasmic reticulum (ER) acts through the inositol-requiring enzyme 1 (IRE1) and transcription factors (TFs) such as basic leucine zipper bZIP17, bZIP28, and bZIP60. However, activation of the unfolded protein response (UPR) possibly requires specific calcium signals from the PM, indicating that UPR does not meet the characteristics of primary heat sensor in plants. The activation of the ER UPR pathway releases different bZIP TFs from the ER membrane, and then the released TFs enter the nuclei causing accumulation of ER chaperone transcripts and the activation of brassinosteroid (a steroidal plant hormone) signaling by inducing transcription of specific genes (Che et al. 2010; Mittler et al. 2012). The occupancy of the H2A.Z histone sensor localized in the nucleus is decreased following warming in *Arabidopsis*. H2A.Z-containing nucleosomes also coordinate the ambient temperature transcriptome, suggesting a temperature-dependent regulation of transcriptome by H2A.Z nucleosomes (Kumar and Wigge 2010). Perhaps, a complex signal transduction network integrates signals from all these different sensors involving calcium fluxes, calmodulin, calcium-dependent protein kinases (CDPKs), MAPKs, phosphatases, and transcriptional regulators such as HSFs, MBF1c, WRKY, DREB, and bZIP. Eventually, all these sensors can activate similar set of HSR genes that improve thermotolerance in plants (reviewed in Mittler et al. 2012). It is also becoming evident that phytohormones are involved in this signal transduction network. Nonetheless, the activation of the different pathway upon heat stress may vary depending on tissue type, more specifically in between vegetative and reproductive tissues. Although a significant progress has been achieved in the molecular mechanisms of heat perception, physiological relevance of plant thermal responses under a complex environmental condition is largely unknown (Bahuguna and Jagadish 2015).

1.3 The Concept of Basal and Acquired Thermotolerance

It is well conceived that gradual and abrupt fluctuations in temperature pose a serious threat to sustainable crop production and global food security. Short-term changes in temperature may trigger acclimation response, while gradual changes may activate adaption response in plants (Bahuguna and Jagadish 2015). The ability of a plant to tolerate high temperature without prior exposure to mild high temperature is generally referred as basal thermotolerance, whereas the adaptive capacity of a plant to survive under extremely high temperatures following preexposure to mild high temperature is considered as acquired thermotolerance. The ultimate survival of the plants depends on both basal and acquired thermotolerance (Ahuja et al. 2010). The requirement for certain regulatory and acclimation proteins may vary based on the kinds of tolerance such as basal and acquired thermotolerance (reviewed in Mittler et al. 2012). For instance, transcriptional regulator MBF1c or the ROS-detoxifying enzyme catalase is required for basal thermotolerance, while they are not required for acquired thermotolerance. However, some HSFs and the disaggregating chaperone heat shock protein 101 (HSP101) may be required for both basal and acquired thermotolerance. In *Arabidopsis*, pre-acclimation of seedlings (38 °C for 3 h) significantly increases thermotolerance at 45 °C, and accumulation of HSP101 is found positively associated with seedling survival and post-stress root growth. It is suggested that the modulation in HSP101 expression and associated thermotolerance appear to be interrelated and might be evolved adaptively in natural populations of *A. thaliana* (Zhang et al. 2015).

1.4 Hormones Involved in the Response of Plants to Heat Stress

The involvement of phytohormones in the plant response to heat stress can be investigated in many ways. Exposure approaches include short-term heat shock with extremely high-temperature, heat acclimation study by exposing plants to a mild heat stress before imposing extreme heat stress and also long-term high-temperature treatment. In most researches that are limited to the analysis of biochemical and physiological parameters, endogenous content of one or multiple hormones following heat stress is quantified together with the evaluation of different growth parameters such as biomass production and photosynthesis. This type of study is often accompanied with pre- or post-application of exogenous hormones predominantly on foliar portion. Thus, changes in certain phytohormone level and/or plant tolerance in response to high temperature are indicated as potential involvement of that hormone in the plant response to heat stress. Advanced studies that employ sophisticated molecular techniques coupled with functional analysis through generating impaired mutants or overexpression plants for a specific gene involved in hormone biosynthesis or signaling have greatly unraveled complex hormone signaling pathway under heat stress.

Nonetheless, the response remarkably varies with the stage (vegetative or reproductive) of plant when heat stress is imposed (Mittler et al. 2012).

Heat stress alters hormone homeostasis, stability, content, biosynthesis, and compartmentalization in plants (Maestri et al. 2002). With the advancement of molecular technologies, several hormones that were previously known only for their roles in plant growth and development have also been implicated in the response of plants to heat stress (Dobra et al. 2015). In addition, participation of multiple hormones that fine-tunes the plant response to heat stress has made hormonal cross talk much more complex. To date, all major hormones such as abscisic acid (ABA), auxin, gibberellins (GAs), cytokinins, salicylic acid (SA), jasmonic acid (JA), ethylene, and brassinosteroids (BRs) have been reported to play critical roles in the response of plants to heat stress (Mittler et al. 2012; Peleg and Blumwald 2011; Xia et al. 2015; Zhou et al. 2014). Moreover, requirement for certain hormone and/or its associated signaling varies depending on the kinds of thermotolerance. For instance, SA-dependent signaling improves basal thermotolerance; however, SA signaling is not required for acquired thermotolerance in *Arabidopsis thaliana* (Clarke et al. 2004). In the following section, involvement of different phytohormones in the response of plants to heat stress is discussed mentioning some specific circumstances that are unique to hormone-mediated response to heat stress. The mechanism of hormone perception and their roles in growth and development under normal temperature are not emphasized in this chapter.

1.4.1 Abscisic Acid

About one and a half decade ago, a study conducted by Larkindale and Knight (2002) showed that heat stress tolerance involves participation of multiple hormones such as ABA, ethylene, and SA in *Arabidopsis*. They noticed that the ethylene-insensitive mutant *etr-1*, the ABA-insensitive mutant *abi-1*, and the SA-deficient *NahG* (constitutively expressing the *Pseudomonas putida* SA hydroxylase transgene *NahG* that inhibits SA accumulation) transgenic plants are sensitive to heat stress. However, application of ABA, SA, and 1-aminocyclopropane-1-carboxylic acid (a precursor to ethylene) protected wild-type *Arabidopsis* plants from heat-induced oxidative damage. Using calcium channel blockers and calmodulin inhibitors, they found that calcium is required for protecting plants from heat-induced oxidative stress especially during/after recovery. In addition, *Arabidopsis* mutants, impaired with ABA biosynthesis and signaling, show decreased basal and acquired thermotolerance (Larkindale et al. 2005). In one of our earlier studies, we also noticed that ABA-deficient mutant *notabilis (not)* tomato genotype is sensitive to heat stress (42 °C for 24 h) as evidenced by decreased photochemical efficiency (Fv/Fm) and increased lipid peroxidation compared with wild-type Ailsa Craig (Li et al. 2015). ABA levels are rapidly and transiently increased following exposure of plants to heat stress as early as 10 min in *Pisum sativum*, which might indicate its involvement in heat sensing and acclimation (Liu et al. 2006).

An optimum balance between GA and ABA is required to control the seed dormancy and germination. However, both ABA and GA are regulated by H_2O_2 that mediates enhancement of ABA catabolism and biosynthesis of GA during seed germination. Analysis of ABA catabolism mutants revealed that endogenous ABA content is inversely correlated with GA biosynthesis. High concentration of ABA inhibits seed germination by repressing GA biosynthesis, while exogenously applied GA can only overcome inhibition of germination that is induced by low level of ABA (Liu et al. 2010). In *Arabidopsis*, high temperature inhibits germination by stimulating ABA levels, and ABA in turn represses both GA synthesis and signaling. Under high temperature, the increase in ABA level in imbibed seeds is achieved by upregulation of ABA biosynthetic genes such as *ABA1/ZEP*, *NCED2*, *NCED5*, and *NCED9*, while GA levels remain low due to suppression of GA 20-oxidase genes, *GA20ox1*, *GA20ox2*, and *GA20ox3*, and GA 3-oxidase genes, *GA3ox1* and *GA3ox2*. Furthermore, ABA-deficient *aba2-2* mutant seeds showed increased expression of GA synthesis genes, but suppressed expression of GA negative regulator gene *SPINDLY (SPY)* under high temperature, indicating that ABA levels are important for controlling GA levels in seeds under high temperature (Toh et al. 2008). In *Arabidopsis*, five *NCED* genes such as *NCED2*, *NCED3*, *NCED5*, *NCED6*, and *NCED9* are involved in ABA biosynthesis. Among them *NCED9* plays a major role, while *NCED5* and *NCED2* play relatively minor roles in high-temperature-induced ABA synthesis and subsequent inhibition of seed germination (Toh et al. 2008).

One of the most adverse effects of high temperature is noticed during grain filling stage as high temperature limits the rate of dry matter transport from vegetative organ to kernel. Changes in hormonal levels (imbalance) in grain were found to be associated with heat stress-induced disruption of grain development (Asthir 2015; Asthir and Bhatia 2014). In wheat, every 1 °C increase in temperature above 18 °C results in 3–5 % single-grain mass fall. High temperature disrupts starch biosynthesis resulting in increased free sugar accumulation and enhanced nitrogen metabolism in wheat. Exogenous application of GA_3 and ABA promotes starch accumulation under heat stress by increasing the activities of glutamate-oxaloacetate transaminase and glutamate-pyruvate transaminase (Asthir and Bhatia 2014). Although exogenous ABA does not influence endogenous ABA concentration at normal temperatures, application of ABA to ears followed by imposition of heat stress (45 °C for 2 h) significantly doubled ABA concentration in grain, suggesting an active involvement of ABA in thermoprotection.

Regulation of stomatal aperture is an important adaptation strategy in response to heat stress. Upon heat stress, tobacco (*Nicotiana tabacum* L.) plants transiently increase stomatal conductance which is mediated by an enhanced catabolism of ABA. Thus, an elevation of temperature beyond physiological optimum initially stimulates transpiration that helps plant to decrease its leaf temperature; however, enhanced transpiration causes water deficit that eventually induces ABA level for stomatal closure (Mackova et al. 2013).

As mentioned earlier the physiological threshold for the highest temperature that causes irreversible damage to cellular macromolecules varies significantly across

the plant kingdom. For instance, *Portulaca oleracea* that is widely distributed in tropical region can survive above 35 °C and 90% relative humidity for several days, while such condition is unbearable for survival of *Arabidopsis*. *P. oleracea* applies multiple strategies for survival that include ABA-mediated regulation of stomatal conductance and respiration (Yang et al. 2012). Interestingly, depleting ABA content due to increasing ABA-8'-hydroxylase results in greater stomatal conductance and respiration rates in *P. oleracea*. Moreover, plant tolerance to heat stress varies with the species, even though they are habituated in the same geographical location. For instance, Mediterranean plants such as rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), and lemon balm (*Melissa officinalis* L.) show different tolerance to heat stress recurrence, although they exhibit similar tolerance to single heat stress event (Asensi-Fabado et al. 2013). Compared to rosemary and sage that show some sort of acclimation response with decreased JA content in rosemary and reduced ABA level in sage following stress reiteration, lemon balm is found most sensitive showing decreased relative water content, but enhanced levels of α -tocopherol and SA. Even when heat stress was combined with water deficit, rosemary and sage were much more resistant than lemon balm; although the hormonal content was not changed in sage, ABA and SA levels in rosemary were increased and decreased, respectively following repeated stress exposure, indicating that changes in hormonal levels that impact plant tolerance following stress imprints are species specific.

1.4.2 Auxins

In addition to well-established role of auxin in cell division and elongation, its role as a key regulator of adaptive growth response to high temperature has emerged during the last decade. As an adaptive response to high temperature, seedlings may elevate the photosynthetic and meristematic tissues away from heat-adsorbing soil by elongating hypocotyls and thus providing better advantage of the cooling effect of moving air (Gray et al. 1998). In accordance with this principle, *Arabidopsis* seedling elongates its hypocotyl upon mild heat stress; however, inhibition of auxin biosynthesis and mutation relating to auxin response or transport all compromise high-temperature-induced hypocotyl elongation. Similarly, BR biosynthetic or signaling mutants such as *de-etiolated2-1* (*det2-1*), *brassinosteroid insensitive1-5* (*bri1-5*), and *dwarf7-1* (*dwf7-1*) also fail to show increased hypocotyl elongation in response to high temperature (Gray et al. 1998; Maharjan and Choe 2011). On the other hand, exogenous application of auxin upregulates expression of the BR biosynthetic gene *DWARF4*, indicating some functional interactions between auxin and BR to regulate temperature-induced hypocotyl elongation (Gray et al. 1998). It is suggested that temperature-induced synthesis of free auxin may stimulate BR biosynthesis via upregulating *DWARF4* that eventually regulates hypocotyl growth under high temperature (Maharjan and Choe 2011).

Auxin accumulation coupled with induction of *YUCCA* (flavin-containing monooxygenase) genes is rapidly increased upon exposure of *Arabidopsis* to mild high temperature (28–29 °C). Although all parts of *Arabidopsis* are capable of synthesizing auxin, its regulatory action also depends on polar auxin transport. For instance, upon mild heat stress, transcripts of auxin biosynthetic genes *YUCCA8* and *YUCCA9* are induced more significantly in cotyledons compared to that in hypocotyls, indicating that cotyledons serve as main auxin source under high-temperature stress, which is then transported to hypocotyls. When auxin transport is blocked using the inhibitor of polar auxin transport 1-naphthylphthalamic acid, high-temperature-induced hypocotyl elongation response is abolished, suggesting that auxin transport is essential for the response to increased temperature (de Wit et al. 2014; Stavang et al. 2009). In addition, petioles of *Arabidopsis* become elongated following exposure to high temperature, even adult plants show hyponastic leaves with long petiole and small lamina. *Arabidopsis* seedlings that are grown in 28 °C have lower number of stomata, but possess better cooling ability, possibly due to less compact shoot architecture and leaf hyponasty that facilitate transpiration. Nonetheless, long-term exposure of plants to increased temperature induces early flowering as an escape strategy in *Arabidopsis* (reviewed in de Wit et al. 2014).

In addition to phytohormones, high-temperature (28 °C)-induced hypocotyl elongation response requires a basic helix-loop-helix transcription factor, PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) (Koini et al. 2009). In *Arabidopsis*, PIF4-deficient mutants (*pif4* mutants) do not show elongation responses or leaf hyponasty when transferred to high temperature. Moreover, *pif4* mutants fail to induce auxin-responsive gene *IAA29* upon mild heat stress. PIF4 regulates expression of auxin biosynthetic genes as well as endogenous levels of auxin that might mediate stem elongation under high temperature (Franklin et al. 2011). A family of *SMALL AUXIN UP RNA (SAUR)* genes is expressed under high temperature in a PIF4-dependent manner to trigger high-temperature-induced elongation response. Box et al. (2015) have shown that in addition to phytohormone and PIF4, transcriptional regulator *EARLY FLOWERING3 (ELF3)* controls elongation growth in a temperature-dependent manner in *Arabidopsis*. As ELF3 disappears at 27 °C (high temperature for *Arabidopsis*), it is quite likely that the locus harboring ELF3 is involved in a gene-by-environment interaction (Box et al. 2015). The gating of growth at night is relieved by high temperature which indicates the significance of temperature-dependent repression of growth in *Arabidopsis*. Until the recent past, PIF4 was considered as the key molecular player that integrates environmental signal (such as heat stimuli) and endogenous signal (such as auxin). Very recently, Wang et al. (2016) showed that HSP90 which is considered as key molecular chaperone during heat stress is also involved in mild high-temperature increase-dependent and auxin-mediated growth response in *Arabidopsis*. The authors showed that auxin receptor TIR1 (TRANSPORT INHIBITOR RESPONSE1) is a HSP90 client and HSP90-SGT1 (SUPPRESSOR OF G2 ALLELE SKP1, a protein required for auxin response) chaperone system is essential for ambient temperature increase response in plants. In addition to elongated hypocotyl response, HSP90 was also found to be involved in ambient temperature increase-induced primary root growth

and lateral root formation (Wang et al. 2016). Interestingly, inhibition of HSP90 by geldanamycin significantly prevented 29 °C-induced upregulation of auxin-responsive genes such as *GH3.17*, *IAA19*, and *IAA5*, but did not alter the expression of auxin biosynthetic gene *YUCCA8*, indicating that HSP90 regulates temperature growth response by stimulating auxin signaling rather than modulating auxin biosynthesis. They also found that HSP90 plays a critical role in stabilizing TIR1 (an unstable protein) which results in enhanced accumulation of the TIR1/AFBs (auxin co-receptors). Finally, they concluded that HSP90 and SGT1 integrate temperature and auxin signaling in order to regulate plant growth in a rising ambient temperature. As plants gradually adapt to temperature increase in the real world, this finding may have significant implication in understanding of acquired thermotolerance.

Compared with vegetative stage, reproductive development of plants is more prone to high temperature (Sobol et al. 2014). Due to climate warming, global minimum night temperatures are increasing much rapidly than maximum day temperatures, which has significant negative effect on global rice yield (Shi et al. 2013). The decreased yield caused by high night temperature is partly due to increased respiration rate and membrane damage, decreased pollen germination, poor translocation of photoassimilates to grain, low seed set, and reduced grain weight. In heat-tolerant rice variety (*Oryza sativa* L. genotype N22), a concomitant induction of HSPs, calcium signaling proteins, and efficient protein modification and repair mechanism mediate enhanced tolerance to high night temperature (28 °C) especially during early grain filling stage (Shi et al. 2013). During the early phase of anther development, the occurrence of high temperature results in proliferation arrest and premature degradation of anther wall cells. Moreover, high temperature inhibits DNA proliferation in mitochondria, chloroplast, and nuclei of developing panicle. Notably, endogenous auxin levels in the developing anther and panicles are remarkably decreased under high temperature due to repressed expression of auxin biosynthetic genes (*YUCCA2*, *YUCCA6*) in *Arabidopsis* and barley. Exogenous application of auxin under high temperature induces anther cell proliferation and can reverse high temperature-induced male sterility, indicating that heat-induced tissue-specific reduction of auxin is the major cause of heat-related damage during reproductive development (Sakata et al. 2010; Oshino et al. 2011).

1.4.3 Cytokinins

Cytokinins are one of the major plant hormones that regulate numerous aspects of growth and development. Although the role of cytokinin in various developmental processes has been well characterized, our knowledge on its effect on plant stress tolerance is still fragmentary. This is possibly because of complex cross talk between cytokinin and stress signaling especially response related to abiotic stress tolerance (Zwack and Rashotte 2015). In many developmental processes, cytokinin and GA act antagonistically. Although most of the studies profoundly suggest a GA-regulated cytokinin action, evidence relating to cytokinin regulating GA activity cannot be

ignored. In tomato, cytokinins inhibit GA-dependent hypocotyl elongation as well as leaf serration (Fleishon et al. 2011). One of the most important mechanisms that plants deploy to minimize heat injury is by cooling leaf, the most important organ that performs photosynthesis. Leaf cooling is actually achieved by increasing transpiration under heat stress. In this response cytokinins play a critical role by stimulating stomatal opening that facilitates transpiration. Cytokinins can induce a number of heat-responsive proteins including small heat shock proteins (sHSPs) and glycine-rich protein under heat stress. Overexpression of cytokinin biosynthetic gene *ISOPENTENYLTRANSFERASE (IPT)* increases endogenous cytokinin levels that eventually enhance heat stress tolerance in grass (Xing et al. 2009). In addition to upregulation of endogenous cytokinin level by overexpressing *IPT*, exogenous application of cytokinin can also improve tolerance to heat stress in bent grass (Xu and Huang 2009). Transgenic tobacco plants overexpressing the *CYTOKININ OXIDASE/DEHYDROGENASE 1 (CKX1)* gene of *Arabidopsis thaliana* L. show reduced and delayed stomatal response, but maintain a lower leaf temperature (Mackova et al. 2013). In addition, overexpression of *CKX1* in roots using the *WRKY6* promoter results in enhanced drought and heat tolerance in tobacco.

As mentioned earlier, the most devastating effect of heat stress is observed when stress occurs during reproductive stage particularly during anthesis period. Stress at anthesis causes floral abortion, resulting in severe yield loss. For instance, exposure of flower primordia of passion fruit (*Passiflora edulis*) to hot ambient temperatures causes total floral abortion (Sobol et al. 2014). Two growth hormones such as GAs and cytokinins play different roles in this condition. Application of GA₃ triggers floral abortion, but application of cytokinins improves heat tolerance by substantially minimizing floral abortion. More importantly, heat-tolerant passion fruit genotypes have been found to contain high levels of cytokinins in their leaves. In *Arabidopsis*, transgenic plants with low cytokinin levels are prone to heat stress, while transgenic plants with high cytokinin levels show increased heat tolerance during flowering. Moreover, application of exogenous cytokinins on wild-type *Arabidopsis* plants also demonstrates a protective role of cytokinins against hot air temperature during flower development stage. Cytokinins might either reduce endogenous GAs levels to improve heat tolerance, or it may simply affect downstream branches of GA signaling pathway without altering active GAs levels (Fleishon et al. 2011; Sobol et al. 2014). However, such protective effect of cytokinin is quite contradictory compared with its role in other stresses such as drought or water deficit. *Arabidopsis* mutant with low level of cytokinins showed increased tolerance to water stress, in which low levels of cytokinins perhaps increase ABA sensitivity that eventually promotes drought tolerance in plants (Nishiyama et al. 2011).

Studies have revealed that rapid perception of heat stimuli and subsequent transduction of its signal are crucial for activating plant defense system timely. Nonetheless, these phenomena are mediated by a complex signaling network where phytohormones play an important role. For instance, heat stress activates cascade of HSFs where HSF2 serves as a master regulator of the HSR (Liu and Charnig 2013). Activation of HSFs rapidly upregulates expression of various genes including those involved in the synthesis of HSPs that serve as molecular chaperons by preventing