Ranjeet Ranjan Kumar
Shelly Praveen
Gyanendra Kumar Rai *Editors*

Thermotolerance in Crop Plants

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Editors
Ranjeet Ranjan Kumar
Division of Biochemistry
Indian Agricultural Research Institute
New Delhi, India

Shelly Praveen
Division of Biochemistry
Indian Agricultural Research Institute
New Delhi, India

Gyanendra Kumar Rai
School of Biotechnology
Sher-e-Kashmir University of Agricultural
Sciences and Technology of Jammu
Jammu, Jammu and Kashmir, India

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Preface

Global climate change has now become reality rather than myth, as evident from the decrease in the production and productivity of agriculturally important crops. The major challenges before us are—how to feed the burgeoning population and how to protect them from the menace of malnutrition. Abiotic stress has severely affected the quantity and quality of the grains. Out of all the abiotic stresses, heat stress has been considered as most damaging for the crop plants. Even an increase of 1 °C in temperature has been reported to reduce the crop yield by ~4%. We have very limited information about various factors responsible for modulating the tolerance level of the plant. Genetic, physiological, biochemical, and molecular approaches are considered as a perquisite for countering the effect of stress in plants. This book entitled *Thermotolerance in Crop Plants* covers different aspects of stress tolerance in crop plants starting from the severity of the problem on quantity and quality of yield under present threat of global climate change to other mechanistic dimensions like—physiochemical and molecular mechanism underlying thermotolerance, signaling mechanism under heat stress, role of heat shock proteins (HSPs) in modulating the thermotolerance, different approaches used in the past to develop heat stress-tolerant crop plants, list of developed thermotolerant agriculturally important crop plants, redox homeostasis under heat stress, effect of exogenous application of phytohormones on tolerance level of the plant, carbon partition and distribution under heat stress, omics approaches for the development of climate smart-crop, etc. This insightful book gives lucid information about various mechanisms underlying heat stress tolerance in plants and approaches to modulate the thermotolerance with future possibilities of developing climate smart crop. This book will be useful for the life science students (MSc and PhD), researchers working in the area of manipulating abiotic stress tolerance in crop plants, scientists, professors, etc.

New Delhi, India	Ranjeet Ranjan Kumar
New Delhi, India	Shelly Praveen
Jammu, India	Gyanendra Kumar Rai

The original version of this book has been revised: the affiliation of the third editor Dr. Gyanendra Kumar Rai has been updated. A correction to this book can be found at https://doi.org/10.1007/978-981-19-3800-9_15

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Editors and Contributors

About the Editors

Ranjeet Ranjan Kumar has completed his UG from the University of Agricultural Sciences, Bangalore, and PhD from Division of Biochemistry, IARI, New Delhi. His research area is "stress-genomics and nutritional fortification of millets." Dr Ranjan has elucidated several mechanisms and models of heat stress tolerance and remodeled the starch biosynthesis pathway in wheat. He has published more than 80 research papers, 5 books, 15 book chapters, and several popular articles, bulletins, and manuals in the areas of nutrition, stress tolerance, and basic principles of Genetic Engineering. Dr Ranjan has been recipient of various prestigious awards like Young Scientist Award, Young Scientist Associate Award, etc. and has recently been selected for the Associateship of the National Academy of Sciences, India.

Shelly Praveen has completed her PhD from the Indian Agricultural Research Institute, New Delhi. Her research area is nutritional enhancement and development of strategies for biotic stress tolerance. She has more than 27 years of research and teaching experience. She has elucidated several mechanisms of host–virus interaction and successfully demonstrated the management of economically important viral diseases of tomato through transgenic resistance. She has published more than 85 research papers in peer-reviewed journals with several books, book chapters, popular article, bulletins, and technical manuals. Her research contributions are reflected very well through various prestigious awards and her induction as a fellow of the National Academy of Sciences, India (NASI), and the National Academy of Agricultural Sciences (NAAS).

Gyanendra Kumar Rai obtained MSc and PhD from the University of Allahabad, Allahabad, UP, India. He is serving as Assistant Professor (Sr. Scale) in the Department of Biochemistry at School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu. His research areas include stress physiology, proteomics, molecular biology, and nutritional physiology. He has published more than 60 research papers, 5 books, 4 practical manuals, and more than 25 popular articles. Dr. Rai has been honored with various prestigious awards like Young Scientist Award, Young Scientist Associate Award, Best Teacher Award, Scientist of the Year Award, etc. He is Fellow of the Society of Applied Biotechnology (SAB) and member of Editorial board of the Indian Journal of Agricultural Biochemistry.

Contributors

Prakshi Aneja National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi, India

Sunder Singh Arya Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Suman Bakshi Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, India

Basharat A. Bhat School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, Jammu and Kashmir, India

Pradeep Bhati Borlaug Institute for South Asia (BISA)/CIMMYT, Ludhiana, Punjab, India

Narayan Bhatt Devate ICAR-Indian Agricultural Research Institute, New Delhi, India

Rajkumar Biradar Department of Botany, Sambhajirao Kendre Mahavidyalaya, Jalkot, Maharashtra, India

Neeraj Budhlakoti Division of Agricultural Bioinformatics, ICAR-Indian Agricultural Statistics Research Institute, New Delhi, India

Krishna Kumar Chaturvedi Division of Agricultural Bioinformatics, ICAR-Indian Agricultural Statistics Research Institute, New Delhi, India

Anil Dahuja Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India

Ranjan Das Department of Crop Physiology, Assam Agricultural University, Jorhat, Assam, India

Sangita Das Department of Crop Physiology, Assam Agricultural University, Jorhat, Assam, India

Soibam Helena Devi Department of Crop Physiology, Assam Agricultural University, Jorhat, Assam, India

Aditi Dwivedi National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi, India

Rahul Gajghate ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India

K. Gopalareddy ICAR-Indian Institute of Wheat and Barley Research, Karnal, India

Suneha Goswami Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India

Sayanti Guha Majumdar Division of Agricultural Bioinformatics, ICAR-Indian Agricultural Statistics Research Institute, New Delhi, India

Harikrishna ICAR-Indian Agricultural Research Institute, New Delhi, India

Pravin Jadhav Biotechnology Centre, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Sanjay J. Jambhulkar Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, India

Sourabh Karwa Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Anuj Kumar Division of Agricultural Bioinformatics, ICAR-Indian Agricultural Statistics Research Institute, New Delhi, India

Dinesh Kumar Barley Network Programme, ICAR-Indian Institute of Wheat and Barley Research, Karnal, India

Mahesh Kumar Department of Molecular Biology and Genetic Engineering, Dr. Kalam Agricultural College, Bihar Agricultural University, Bhagalpur, Bihar, India

Ranjeet Ranjan Kumar Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India

Sudhir Kumar Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Uttam Kumar Borlaug Institute for South Asia (BISA)/CIMMYT, Ludhiana, Punjab, India

Antim Kundu Chaudhary Charan Singh Haryana Agricultural University, Hisar, India

Sayali Magar Biotechnology Centre, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

H. M. Mamrutha ICAR-Indian Institute of Wheat and Barley Research, Karnal, India

Dwijesh Chandra Mishra Division of Agricultural Bioinformatics, ICAR-Indian Agricultural Statistics Research Institute, New Delhi, India

Mangesh Moharil Biotechnology Centre, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Merentoshi Mollier Department of Crop Physiology, Assam Agricultural University, Jorhat, Assam, India

Gopika Mote Biotechnology Centre, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Muntazir Mushtaq School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, Jammu and Kashmir, India

Sneh Narwal Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India

Sujata Pachoni Department of Crop Physiology, Assam Agricultural University, Jorhat, Assam, India

Jasdeep C. Padaria National Institute for Plant Biotechnology (NIPB), New Delhi, India

Madan Pal Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Shelly Praveen Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India

Gyanendra Kumar Rai School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, Jammu and Kashmir, India

Pradeep Kumar Rai Advance center for Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Udheywala, Jammu and Kashmir, India

Ved Prakash Rai Agricultural Research Station, Navsari Agricultural University, Tanchha, Bharuch, Gujarat, India

Aashish Ranjan National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi, India

Akshay Sakhare Indian Rice Research Institute, Hyderabad, Telangana, India

Sindhu Sareen Indian Institute of Wheat and Barley Research, Karnal, Haryana, India

Md. Shamim Department of Molecular Biology and Genetic Engineering, Dr. Kalam Agricultural College, Bihar Agricultural University, Bhagalpur, Bihar, India

P. Shashikumara ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India

Sonia Sheoran Department of Crop Improvement, ICAR-Indian Institute of Wheat and Barley Research, Karnal, India

Ajeet Singh Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India

Bhupinder Singh Centre for Environment Science and Climate Resilient Agriculture (CESCRA), Indian Agricultural Research Institute, New Delhi, India

G. P. Singh ICAR-Indian Institute of Wheat and Barley Research, Karnal, India

Monika Singh G.L. Bajaj Institute of Technology and Management, Greater Noida, Nagar, Uttar Pradesh, India

Deepti Srivastava Department of Agriculture, Integral Institute of Agricultural Science and Technology, Integral University, Lucknow, Uttar Pradesh, India

Vinutha T Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India

Pranita Thakur Biotechnology Centre, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

Chandrapal Vishwakarma Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi, India

Manish Kumar Vishwakarma Borlaug Institute for South Asia-CIMMYT, New Delhi, India

Heat Stress in Wheat: Adaptation Strategies

1

P. Shashikumara, Rahul Gajghate, Narayan Bhatt Devate,
Harikrishna, H. M. Mamrutha, K. Gopalareddy, and G. P. Singh

Abstract

Globally wheat productivity is affected due to unfavorable effects of different biotic and abiotic stresses. Combating these stresses to minimize the yield losses in wheat crop is the major area of concern to ensure food security. Among the different abiotic stresses, increase in ambient temperature is one of the major threats to global wheat production. Wheat being second most important crop in the world accounts for 20% of global protein. Heat stress impairs physiological functions, hormonal productions and reduces photosynthetic efficiency, metabolic activities, and pollen viability resulting in loss in grain yield. To cope up with heat stress, wheat plant evolves various adaptive strategies which includes morphological, physiological, molecular, epigenetic, and biochemical. The current chapter presents inclusive information on importance of heat stress, its effect on various growth stages and the different strategies adapted by wheat crop to cope up with heat stress, which helps the wheat researchers in developing climate resilient wheat.

Keywords

Wheat · Abiotic stress · Heat stress · Adaptation strategies · Heat stress priming

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P. Shashikumara · R. Gajghate
ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India

N. Bhatt Devate · Harikrishna
ICAR-Indian Agricultural Research Institute, New Delhi, India

H. M. Mamrutha · K. Gopalareddy · G. P. Singh (✉)
ICAR-Indian Institute of Wheat and Barley Research, Karnal, India

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1.1 Introduction

Wheat (*Triticum* spp.) is the most widely cultivated rabi cereal and staple crop in many countries across the globe. As per FAO estimation, an additional 198 million tonnes of wheat are required by 2050 to meet the ever-growing future wheat demand (Sharma et al. 2015a, b). However, wheat growing areas are frequently exposed to unpredictable temperature fluctuations during critical crop growth stages, thereby affecting grain yield significantly. The temporal variation in temperature during wheat crop season has already been reported to affect the productivity in many parts of the globe (Fontana et al. 2015; Mueller et al. 2015). Low latitude wheat growing zones with around 100 million hectares of wheat area is more predominantly heat prone across the globe (Braun et al. 2010). A total of 30 different wheat crop models were tested by Asseng et al. (2014) with crop growing mean temperatures ranged 15 °C–32 °C, which includes the artificial heating. Their analysis suggests that the higher temperatures already affected wheat grain yield in majority wheat growing areas. During the period between 1981 and 2010, average yield reductions ranged 1–28% across 30 world sites for every 2 °C rise in temperature; this yield reduction increased and ranged 6–55% for every 4 °C rise in temperature. They also estimated that the global wheat production will be reduced by 6% for every rise in 1 °C. Higher yield variability due to higher temperature was observed in low latitudes as compared to high latitudes. Singh et al. (2011) reported yield reduction of 400 kg per hectare if the March–April month mean temperature rises even 1 °C in India. Elevated temperature during grain filling stage resulted in terminal heat stress and yield reduction in wheat. When the mean temperature reaches above 31 °C during grain filling stage, then the terminal heat stress occurs in wheat.

Heat stress is a complex phenomenon, which affects growth and development, impaired physiological functions (Mondal et al. 2013). The heat stress in wheat alters the hormonal productions (Krasensky and Jonak 2012), photosynthetic efficiency (Ashraf and Harris 2013), metabolic activities (Farooq et al. 2011), and plant water relations (Hasanuzzaman et al. 2013), pollen tube development and pollen mortality (Oshino et al. 2011). Heat stress also causes the enhanced production of reactive oxygen species (Wang et al. 2011) and ethylene (Hays et al. 2007). Increasing temperature will have impact on sustaining global wheat production and in turn food security, particularly during the period of climate change (Tripathi et al. 2016). Elevated temperature affects seed germination resulting in poor plant stand, decreased photosynthesis, membrane instability, senescence, reduced pollen viability and finally less grains with reduced grain size (Asseng et al. 2011). The severity of such effects greatly depends on the cultivars and phenological stages. Heat stress during flowering causes pollen and anther sterility leading to reduced grain number, while high temperature coincides with grain filling stage, which leads to reduced grain weight and in turn reduced yield (Mondal et al. 2013). Temperature stress due to change in the climate pattern necessitates to determine the heat stress effects and possible ways of improvement for sustainable wheat production under heat stress prone environments.

Adoption of climate smart practices is necessary to control the damages caused by the elevated temperatures during crop growth. Adaptation strategies towards climate change are needed to minimize the crop damages (Niles et al. 2015). Plants have different adaptive mechanisms under heat stress including avoidance, escape, and tolerance. Development of heat-tolerant wheat cultivars by the utilization of diverse genetic sources is the most sustainable and eco-friendly strategy to mitigate the heat stress effects (Ortiz et al. 2008). Choice of cultivars and adjustments in sowing dates are two important adoptive measures under heat prone environments (Deryng et al. 2014). Other adaptation strategies include antioxidants defense (Caverzan et al. 2016) and osmo-protectants (Kaushal et al. 2016) along with several molecular adaption mechanisms and management practices like surface cooling by irrigation (Lobell et al. 2008).

Thorough understanding of the genetic, molecular, physiological, and biochemical mechanisms through genomic, transcriptomic, proteomics, and metabolomics analysis would help in the mitigation of heat stress in wheat. Identification of major heat responsive proteins and genes is important to integrate them in crop breeding programs. Functional validation of major heat responsive genes/proteins will also pave the way for the development of heat-tolerant varieties in wheat breeding programs. Thus, the present chapter focuses on compiling the information on significant effect of heat stress on different growth stages of wheat and the strategies adapted for overcoming the stress effect.

1.2 Effect of Heat Stress on Wheat Crop

The wheat crop under field may encounter either with sudden or slow increase in temperature and they have negative influence on various growth stages of wheat (McClung and Davis 2010; Grant et al. 2011). The high temperature effect on wheat crop depends on duration and extent of heat stress and also on pheno stage of the crop (Ruelland and Zachowski 2010). Some of the major effect of heat stress on various growth stages and on quality of wheat grains are discussed below.

1.2.1 Effect of Heat Stress on Vegetative Phase

The primary effect of heat stress is the impairment of seed germination and poor crop stand establishment (Johkan et al. 2011; Hossain et al. 2013). High temperature of around 45 °C severely degenerates mitochondria, changes the protein expression profiles, reduces ATP accumulation, and oxygen uptake in imbibing wheat embryos, resulting in loss of seed quality relating to seed mass, vigor, and germination which later reduces crop stands (Balla et al. 2012; Hampton et al. 2013). Warm environment produces lower biomass compared to plants grown under optimum or low temperature. Day and night temperature around 30 and 25 °C, respectively, has severe effects on leaf development and productive tiller formation in wheat. It is reported that high temperatures are generally involved in regulation of leaf appearance rates and leaf-elongation rates along with decreasing leaf-elongation duration (Rahman et al. 2009). The plant height is significantly reduced which adds for reduced biomass under high temperature. Though vegetative stages are affected by heat stress, many a times cooler temperatures at reproductive period will make up its loss (Mamrutha et al. 2020).

Photosynthesis is the primary traits influenced by heat stress and mainly affects rubisco, rubisco activase activity, functioning of photosystems and thylakoid membrane (Iwai et al. 2010; Mathur et al. 2014). The availability of high O_2 concentration promotes photorespiration under high temperature. The change in solubility of O_2 and CO_2 gases was observed under heat stress condition causing increase in flag leaf photorespiration in wheat (Almeselmani et al. 2012). Extended duration of heat stress results in gradual senescence while intensive heat stress for short period of time leads to protein denaturation and aggregation, causing death of the plant (Pandey et al. 2019). High temperature ($>$34 °C) accelerates the leaf senescence due to reduction in biosynthesis of chlorophyll (Hasanuzzaman et al. 2013). It affects the water relation and content in the plant, cell dehydration is observed under heat stress due to reduction in osmotic potential (Ahmad et al. 2010).

1.2.2 Effect of Heat Stress on Reproductive Phase

The incidence of reproductive stage heat stress has been found to be more detrimental in wheat production (Nawaz et al. 2013; Mamrutha et al. 2020). One degree rise in average temperature during reproductive phase can cause severe yield loss in wheat (Bennett et al. 2012; Yu et al. 2014). The optimum temperature for flowering and grain filling ranges from 12 °C to 22 °C (Sharma et al. 2019). Anthesis stage is considered very crucial with respect to heat stress because the induction of heat stress just before and at this stage showed significant increase in floral abortion and lower number of seeds (Gupta et al. 2013; Asthir and Bhatia 2014; Sharma et al. 2016). Both micro-sporogenesis and mega-sporogenesis are affected under heat stress, resulting in lower seed-set. Early stage of gametogenesis is harmed when heat stress occurs during meiosis (Saini et al. 1983; Kaur and Behl 2010). The grain development phenomenon depends upon the grain filling rate and duration which is highly sensitive to heat stress (Gourdji et al. 2013; Lobell and Gourdji 2012). 1–2 °C rise in temperature lowers seed weight due to decrease in grain filling duration, thus resulting in production of smaller seed size (Sharma et al. 2019). Short-term heat stress during grain filling may result in grain yield loss up to 23%. In heat stress, grain number is decreased causing reduction in harvest index (Mason et al. 2010).

1.2.3 Effect of Heat Stress on Grain Quality Traits

Heat stress associated decrease in assimilate production and remobilization result in reduced grain quality (Lizana and Calderini 2013). Starch is the major constituents of wheat and is made up of amylose and amylopectin. Amylose content is key parameter to mark starch quality. Starch characteristics are affected by variation in amylose content and high temperature is associated with increase in amylose content and amylose:amylopectin ratio (Sharma et al. 2015a, b). ADP-Glucose Pyrophosphorylase (AGPase) and starch synthase are key enzymes involved in starch biosynthesis, at high temperature, there is decrease in starch content in grain up to one-third of total endosperm starch, which is caused due to decrease in efficiency of these two enzymes (Liu et al. 2011). Under heat stress, there is increase in total soluble sugar and protein (Asthir and Bhatia 2014) content with increase in essential amino acids fractions, leaf nitrogen content, and sedimentation index as kernel size is smaller (Iqbal et al. 2017). It is observed that heat stress during grain filling can significantly alleviate protein concentration while lowering the functionality of protein and this augment seems to be higher when high temperatures are imposed in early stages of grain filling (Corbellini et al. 1997; Vijayalakshmi et al. 2010). Comparison of milled grains from control and high temperature treated plants revealed alterations in peaks assigned to polysaccharides and proteins (Corbellini et al. 1998; Castro et al. 2007; Daniel and Triboi 2001).

1.3 Heat Stress Adaptation Strategies

1.3.1 Morphological Adaptation Strategies

Abiotic stresses, such as drought, heat, cold, salinity, and heavy metals are severely affecting the plant growth, development, and yield of the crop (Raghavendra et al. 2020; Jain et al. 2014). Meanwhile, plants try to adapt to such adverse conditions through modifying their morphological structure to sustain in the existing environment (Hossain et al. 2016; Sah et al. 2016).

The plant will show morphological modification to overcome combined effects of heat and drought stress. The rapid ground cover will withhold evaporation of water from the soil under plant canopy. This increases water availability to maintain evapotranspiration, keeps cooler plant canopy under heat stress (Cossani and Reynolds 2012; Khan and Kabir 2014). Early emergence of seedlings and early ground cover are important adaptive traits used for selection of earliness in wheat germplasm (Mondal et al. 2017). Leaf rolling and leaf thickening through changes in lipid composition are important survival mechanisms under prevailing heat stress (Nawaz et al. 2013). The presence of leaf hairs and waxy covering on leaf and stem surface avoids heat stress. These leaf modifications lead to reduced exposure to solar radiations, smaller leaf boundary evacuate heat to ambient more rapidly and increases transpiration leaf cooling of the plant under heat stress condition (Hasanuzzaman et al. 2013). Flag leaf area and awn length are also effective traits correlated with grain yield under heat stress conditions (Munjal and Suresh 2020). The early maturing varieties can escape terminal heat stress situations with minimum yield loss under heat stress. For late sown conditions days to heading, days to maturity, plant height, number of effective tillers per plant, and biological yield are important yield attributing parameters which need to be considered in wheat.

1.3.2 Physiological Adaptations

Under high temperature condition, plants show tendency to reduce cell size, closure of stomata, and increased trichomes density in leaves (Anon et al. 2004). Cell membrane is highly susceptible and affected by increasing temperature. The thermotolerant wheat genotype maintains cell membrane stability, avoiding the leakage of electrolytes thereby protecting membrane from disintegration due to high temperature (Blum and Ebercon 1981). Estimation of osmotic potential of the cell gives the idea on cell membrane stability. The scavenging reactive oxygen species which are produced due to oxidative stress are important activities to avoid cell membrane breakdown. The tolerant plant will have good deep root system and enhanced transpiration ability to keep cooler plant canopy under heat stress. Through use of infrared gun, we can estimate canopy temperature (CT) and cooler canopy genotypes are considered to be the heat-tolerant lines (Hütsch et al. 2019). Higher temperature indirectly induces drought stress. Plant will adopt with changing in root parameters to absorb more water from the deep layers of soil. Cooler canopy indicates higher water use efficiency and nutrient use efficiency with stable yield (Singh et al. 2017). Transpiration is inevitable process and crucial for plants for water and nutrient uptake, photosynthesis and maintaining canopy temperature. The rise in temperature causes decrease in stomatal conductivity thereby halting transpiration process. Tolerant lines maintain higher stomatal conductance compared to susceptible genotypes so that they maintain required transpiration rate and water balance (Pooja and Munjal 2019). High throughput, non-destructive instruments like infrared gun, green seeker, porometer, and chlorophyll meter can be used for evaluation of wheat genotypes under heat and drought condition. Canopy temperature is negatively correlated with transpiration and grain yield (Manu et al. 2020; Shashikumara et al. 2020). Normalized difference vegetation index (NDVI) recorded using green seeker measures greenness of plant canopy and thus chlorophyll content, this can be estimated at different growth stages of crop (Harikrishna et al. 2016). Similarly, chlorophyll meter is used to estimate chlorophyll content thus genotypes having higher NDVI and chlorophyll content index under heat stress will have more chlorophyll content resulting in higher photosynthetic efficiency (Puttamadanayaka et al. 2020). Many studies have reported QTLs for physiological traits like NDVI, chlorophyll reading, and CT under heat and drought stress in wheat (Sunil et al. 2020; Puttamadanayaka et al. 2020).

Stay green is the key trait associated with heat tolerance in wheat. Majorly, the exposure to heat stress during anthesis stage and grain filling stage reduces pollen viability, seed set, starch and protein accumulation (Barnab’as et al. 2008; Zahedi and Jenner 2003). Under such circumstances, heat-tolerant wheat genotypes have increased availability of stem water-soluble carbohydrates for the developing grains. This increases grain filling producing higher number of grains per spike than heat susceptible lines. The delayed senescence, i.e., stay greenness, is important key indicator of adaptation to heat stress, which is an indicative of higher leaf chlorophyll contents at anthesis stage (Fokar et al. 1998). The stay-green genotype is able to maintain better grain filling under higher temperatures. This modification allows wheat genotypes to have high photosynthetic rate, reduced canopy temperature, and remobilization of assimilates to grain developing under heat stress (Rehman et al. 2021). Genotypes showing delayed senescence remain greener during later stages of crop growth and can divert photosynthesis to developing grains maintaining stable yield under terminal heat stress (Thomas and Ougham 2014). There is significant variability available in wheat for stay greenness, hence this trait can be exploited to develop heat-tolerant genotypes. Accumulation of osmolytes such as sugars and sugar alcohols (polyols), proline, tertiary and quaternary ammonium compounds, and tertiary sulfonium compounds under various abiotic stresses including heat stress is a key adaptive mechanism in plants. For example, accumulation of glycine-betaine and prolines will buffer redox potential of cells under heat and other abiotic stresses (Sakamoto and Murata 2002). Similarly, other osmolytes like c-4-aminobutyric acid (GABA) accumulates in the stressed tissue, which triggers various timely physiological responses in plants for adaptation to stress conditions (Kinnersley and Turano 2000). Overall wheat heat-tolerant genotype will show physiological adaptation through increasing transpiration, efficient photosynthesis during post anthesis stages, and higher stem reserve mobilization.

1.3.3 Molecular Adaptation Strategies

Plants exposed to heat stress, initially it causes plasma membrane disruption, ionic effects, and osmotic changes. This initiates a downstream signaling and transcriptional cascade that activate stress-responsive mechanism for repair damaged membranes and proteins for reestablishment of cellular homeostasis. Rise in temperature beyond critical limit causes changes in the plasma membrane fluidity which in turn leads to formation of phosphatidic acid (PA) and phosphatidylinositol 4, 5-bisphosphate (PIP2) and triggers Ca^{2+} influx and cytoskeletal reorganization resulting in the activation of some mitogen-activated protein kinases (MAPK) and calcium-dependent protein kinases (CDPK). These molecules play role as signal for G-protein-coupled receptors and these molecules activate secondary messenger proteins which travel over nuclear membrane of cells and activate genes associated with heat tolerance (Mishkind et al. 2009; Singh Kuldeep et al. 2012). The heat stress causes dissociation of chaperons from heat shock transcription factors (HSFs) and then these factors will bind to heat response elements (HREs). Now these HREs activate heat response genes (HRGs) (Zhu 2016). In general, under normal condition chaperon BIP bound by a common transcription factor bZIP28, and prevents movement of bZIP28 into nucleus. When cell exposed to heat stress, it causes destabilization in the interaction between bZIP28 and BIP, which leads to dissociation of molecules. Then bZIP28 moves to nucleus and activates various genes related to heat tolerance (Abhinandan et al. 2018). HSP 70 and HSP 90 are present in low amount and bound to transcription factor (TF), HsfA1 under normal condition. The rise in temperature causes production of ROS in the plant cell, which destabilizes interaction between HSP 70 and HSP 90 with *TF HsfA1,* which is free until now will enter the nucleus and activates heat shock protein coding genes (Ohama et al. 2017).

Induction of HSPs is a mechanism of acquiring heat tolerance as they play chaperone-like activity and also involved in signal transduction, gene activation, maintaining cellular redox state and in protecting photosynthetic electron transport (Nollen and Morimoto 2002). The transcriptome analysis of heat susceptible (Chinese Spring) and tolerant wheat (TAM107) genotypes identified putative heat stress-responsive genes encoding HSFs, HSP, transcription factors, and proteins involved in phytohormones biosynthesis/signaling, calcium and sugar signal pathways, RNA metabolism, ribosomal proteins, and primary and secondary metabolisms (Qin et al. 2008). The expression level of HSP90 and HSP101 under heat stress was observed in heat-tolerant genotype C306 (Vishwakarma et al. 2018; Almeselmani et al. 2012). Chauhan et al. (2011) identified many genes such as HSPs, transporters, lipid transfer protein, L-myo-inositol-1-phosphate synthase, protein modifiers, calcium binding proteins, signaling molecules, helicase-like protein, membrane binding proteins, alanine amino transferase, activator of HSP90, peptidyl prolyl isomerase, stress-induced protein Sti-1, and heat shock factor, which were highly inducible by high temperature and remained stable at both temperature regimes. *TaHsfA6f*, a member of the A6 subclass of heat shock transcription factors, which is upregulated during heat stress, regulates TaGAAP, TaHSP, and TaRof1 genes in wheat and has a positive impact on thermotolerance (Xue et al. 2015).

1.3.4 Biochemical Adaptation Strategies

The adaptive responses that plants have developed to reduce heat-induced damage to cells at biochemical level is to cease the efficiency of ribulose-1,5-bisphosphate (RuBP) carboxylase/oxygenase (Rubisco) in the Calvin–Benson–Bassham cycle (Weis 1981; Degen et al. 2021). The render inactive is mainly because of an ineffectual Rubisco activity regulation by the molecular chaperone called as Rubisco activase (Rca) (Crafts-Brandner and Salvucci 2000; Salvucci et al. 2001) as its regulation is sensitive to temperature (Salvucci et al. 2001; Carmo-Silva and Salvucci 2011, Carmo-Silva et al. 2012). Degen et al. (2021) studied the activity of three isoforms of Rca (Rca1β, Rca2β, and Rca2α) in wheat, which differ in their thermostability. The transcript of Rca1β is increased by 40-fold in 4 h at higher temperature and again comes to its natural level in controlled temperature after 4 h. The rapid change in the Rca isoform abundance at higher temperature improved the knowledge of the regulation and insight mechanism into the carbon assimilation to improve wheat productivity.

Phytohormones play an important role in coordinating the response to heat stress in wheat crop and act as naturally occurring heat dissipating barrier in plant by maintaining antioxidants level (Ahammed et al. 2016). Phytohormones such as abscisic acid (ABA), salicylic acid (SA), and cytokinins have vital role and crucial function in the response of HS to plant (Mittler et al. 2012; Peleg and Blumwald 2011; Xia et al. 2015; Zhou et al. 2014). ABA hormone plays a major role in root to shoot stress signaling (Kudoyarova et al. 2011), stomatal closure and prevents leaf expansion. Artificial application of ABA enhances the filling rate and sink capacity in grain by adjusting endogenous hormone molecule to stimulate endosperm cell division and aggregation of photosynthate products (Yang et al. 2014). Salicylic acid (SA) regulates important plant physiological processes including photosynthesis and proline metabolism under stress conditions, thereby providing protection to plant (Khan et al. 2013; Miura and Tada 2014).

SA is an important component of the signaling pathway that counters to hypersensitive response and systemic acquired resistance (Kawano et al. 1998). It helps to balance the heat shock transcription factors and encourage to stick up with the heat shock element along with the promoter of heat shock-related genes, thus controlling the signaling pathway in high temperature stress condition and advocating the growth of the plant (Wang and Li 2006). The role of cytokinin is no doubt so important to mitigate heat tress by changing grain cytokinin content under HS conditions. It is the general coordinator between the stay-green trait and senescence. Exogenous cytokinins increase grain yield of winter wheat cultivars by improving stay-green characteristics under heat stress (Yang et al. 2016). Banowetz et al. (1999) found the correlation with the cytokinin content and the reduction in grain filling and grain weight during HS.

The production of reactive oxygen species (ROS) in the cell organelle (PS I and PS II of chloroplasts, mitochondria, peroxisomes, and endoplasmic reticulum) causes oxidative damage during the initial stage of heat stress (Wahid et al. 2007; Liu and Huang 2000), if continues for the long time then proteolysis occurs in the cell membrane and triggers the cell death program (Qi et al. 2011; Mittler et al. 2011; Marutani et al. 2012; Suzuki et al. 2012). To overcome the production of ROS, the enzymatic and nonenzymatic defense mechanism exists in wheat. In the enzymatic defense mechanism, superoxide dismutase (SOD) has the property to convert superoxide radical (O^{2-}) into hydrogen peroxide (H_2O_2) and singlet oxygen (O_2), which is evacuated by ascorbate peroxidase (APX) using ascorbate as substrate and catalase (CAT) by glutathione reductase to convert glutathione disulfide to the sulfhydryl from glutathione (Suzuki et al. 2011). The nonenzymatic defense system involves reduction in glutathione (scavengers of singlet oxygen, hydrogen peroxide and hydroxyl radical), tocopherols (scavengers of H_2O_2; upregulation of APX and GR), ascorbic acid (donate electron in various enzymatic and nonenzymatic reaction), and carotenoids (inhibit singlet oxygen formation) (Sharma et al. 2012; Kumar et al. 2013; Puthur 2016; Tiwari et al. 2018). Thus, to overcome the negative effect of ROS, the enrichment of antioxidant in cell is a better approach (Sharma and Dubey 2005). Reassurance against oxidative stress is a major key to determine the endurance of a crop under HS. To get more information about the expression aggregation and development pathway of antioxidants under heat stress condition will help to plan and make a significant step in the development of heat-tolerant lines.

1.3.5 Epigenetic Mechanism for Stress Response and Adaptation

Environmental stresses such as heat, drought, salinity, and freezing are the main abiotic stress threat to wheat. The recent study confirms that the plants engage with sophisticated epigenetic mechanisms to overcome the loss and damage by the environmental stresses (Kong et al. 2020). Among abiotic stress epigenetic regulation to HS responses has attracted increasing interest in researchers. Epigenetics is defined as the change in gene expression without alteration in the DNA sequences, which is crucial for the plant to environmental stresses (McCormick 2018; Zhao et al. 2021). The regulatory mechanism of epigenetics in response to HS (also other abiotic stress) involves DNA methylation, modification in histone chromatin remodeling, lncRNAs, sRNAs, change in the gene expression pattern and/or epigenetic memory of plants under HS (Liu et al. 2015; Ueda and Seki 2020).

DNA Methylation

DNA Methylation is one of the main epigenetic mechanisms that controls and regulates the genome stability, growth and cures different stress in plant (Gahlaut et al. 2020). In DNA methylation process, the methyl group (CH_3) occupies the cytosine position of DNA to form 5-methylcytosine, to make CG, CHG, and CHH (H represents A, T, or C) (Cokus et al. 2008; Law and Jacobsen 2010) in which CG is the most abundant and widespread methylation site (Park et al. 2016). Several reports have been studied so far and confirm the involvement of DNA methylation in the regulation of genes implicated in the plant response to HS (Popova et al. 2013, Liu et al. 2015, 2017a, b). Cytosine-5 DNA methyltransferase (C5-MTases) gene family plays a crucial role in plant abiotic stress responses via DNA methylation. Fifty-two cytosine-5 DNA methyltransferases (C5-MTases) genes were identified through genome-wide identification and expression profiling of cytosine-5 DNA methyltransferases in wheat genome under heat and drought stress, the expression pattern of C5-MTase genes was differ, and it is specific for the developmental stage and particular tissue in plant (Gahlaut et al. 2020). In *Arabidopsis,* the histone acetyltransferase GENERAL CONTROL OF NONREPRESSED PROTEIN5 (GCN5) has the main role to mitigate the irreversible effect due to HS by promoting H3K9 and H3K14 acetylation of heat shock factor A3 (HSFA3) and UV-HYPERSENSITIVE6 (UVH6). The same function result was confirmed by the Ni et al. (2018) in wheat, where the histone acetyltransferase TaGCN5 gene is upregulated under heat stress as in *Arabidopsis*.

Histone Modification

Histone is the basic structural unit of chromatin having a pair of copies of H2A, H2B, H3, and H4, encased in ≈147 bp DNA in the nucleosome. Methylation of DNA, histone acetylation and methylation intervene the plant HS response through activation or repression of gene expression. The histone methylation mostly takes place on the histone H3 lysine residue, viz., H3K4me3, H3K36me3, H3K79me3, H3K9me2, and H3K27me3, out of which H3K4me3 and H3K27me3 are more potent to conserve epigenetic marks for activation or repression of gene (Shi et al. 2004; Yaish et al. 2009; Thorstensen et al. 2011; Black et al. 2012; Yuan et al. 2013; Wang et al. 2020). The lysine-specific histone demethylase 1 (LSD1) is the main enzyme that specifically demethylates histone H3 lysine 4 (H3K4) me1/2 (Shi et al. 2004). In response to HS, plant will start the cascade process mediated through epigenetic regulators viz., acetyltransferases, methyltransferases, deacetylases, and demethylases-mediated methylation and acetylation which are initiated by heat response-associated recruiters (e.g., TFs, lncRNAs) to specific histones in chromatin to regulate gene expression (Deng et al. 2018; Ueda and Seki 2020). Wang et al. (2016) studied the transgenerational memory of the plant where winding of histone demethylation and the global methylation of DNA are assured. The gene for LSD1, putative nucleic acid methyl transferases and binding proteins RNA methyltransferase, and ribosomal RNA FtsJ-like methyltransferase has showed greater level in the offspring of primed plants than in those of the non-primed plants under HS at post anthesis stage.

Chromatin Remodeling

Chromatin is a complex architecture of highly condensed and tightly coiled DNA and histone protein (Cedar and Bergman 2009). The condensed and tightly coiled chromatin (default state) restrains the access of RNA polymerase and other transcription factors to genes. To facilitate transcription, the tightly coiled structure of chromatin must be opened; this process is known as chromatin remodeling (Bannister and Kouzarides 2011). There are several chromatin remodelers available viz., SWI/SNF, ISWI, INO80, and CHD that responded chromatin on different organisms, under varied stresses, which leads to change in chromatin from transcriptionally inactive to a transcriptionally active state. The structure of chromatin at a given promoter is decisive to know the transcriptional readout (Bhadouriya et al. 2021).

Epigenetic Memory

To deal with the environmental stress plants elaborate their mechanism via epigenetic memory. If the memory of the stress is present only in the first stress-free generation, then it is called intergenerational memory, while if the memory is detected in a minimum of two stress-free generations, it is known as transgenerational memory (Tardieu et al. 2018; Bhadouriya et al. 2021). In transgenerational memory, the phenotypic traits possessed by the progeny are a result of environmental stress stimulus in an earlier generation but not in the parent or progeny (Bhadouriya et al. 2021). This transgenerational epigenetic memory can be maintained for at least three generations (Suter and Widmer 2013; Zhong et al. 2013). As these changes in the epigenome are stably inherited and passed to further generations, knowledge about these changes is crucial for stress management in plants (Bhadouriya et al. 2021).

1.4 Heat Stress Priming in Wheat

Plants adopt mechanism to withstand harsh environmental conditions. Priming is one among them where tolerance is developed in plant by prior exposure to the stress in early stage of life. Initial study on priming was concentrated to understand pathogen defense mechanism (Conrath et al. 2002). Priming enhances the tolerance by prior exposure to sublethal stress to reprogramming and to activate the molecular machinery (Lämke and Bäurle 2017). Priming can be an excellent stress tolerance strategy in vast majority of stresses in many crops in less time to any variety if tolerant cultivar is not available.

Mechanism of heat stress priming is relatively conserved well among many organisms (Mittler et al. 2012), exposer to sublethal heat stress induces reprograming of cellular mechanism which led to thermotolerance in later stages of plant growth. Overall mechanism of priming and tolerance can be divided into three major steps as heat priming by exposure to sublethal heat stress, memory establishment, and adaptive response on second episode of lethal heat stress (Sanyal et al. 2018). Heat stress memory state induced by initial priming will be in activated state for several days by heat stress memory genes which enables relevant transcript to be expressed in high quantity to enhance the stability of proteins or enzymes upon occurrence of further heat stress (Charng et al. 2006, 2007; Nishizawa et al. 2006; Meiri and Breiman 2009; Lämke and Bäurle 2017).

Heat stress priming during early vegetative stage like stem elongation has positive impact on grain quality, yield, photosynthesis, etc. in wheat. However, phenological stage of crop during priming treatment influences the efficiency of priming (Fan et al. 2018). An early priming treatment at three and five leaf stage are not showing any significant difference over control (Mendanha et al. 2018), whereas priming at early booting stage has positive impact on tolerance (Fan et al. 2018) emphasizing importance of stage of crop during priming treatment. And it is true that priming response differs from cultivar to cultivar (Mendanha et al. 2018) and number of priming treatments. Multiple heat priming at seedling stage found to enhance antioxidant activity at later stage in winter wheat (Wang et al. 2014). There are many examples of transgenerational effect of heat priming, where progenies of heat primed parents performed well under heat stress and produced healthy phenotype and comparatively higher yield over control (Wang et al. 2016). This acquired thermotolerance in next generation plants may be a result of heritable epigenetic modification or signal transduction to trigger stress response factors in the progenies (Wang et al. 2016). Heat stress tolerance in the progenies of drought primed plant (Zhang et al. 2016) and increased nitrogen use efficiency of early-stage drought priming (Liu et al. 2017a, b) show interrelation among priming phenomenon of different abiotic stresses. Genome-wide expression analysis, transcriptome and proteome analysis revealed higher rate of photosynthesis, activity of antioxidants, lower cell membrane oxidative damage by the upregulation of genes responsible for heat stress response like HSPs, redox homeostasis, sensing, and signaling. However, metabolism-related genes were downregulated (Xin et al. 2016), which might have protective role by conserving energy and diverting it to plant survival. Heat priming acts as an adaptive mechanism to heat stress at cellular level and ensures the survival of plant under further heat stress condition. All the adaptive mechanisms discussed above along with their attributing traits are presented in Fig. 1.1.

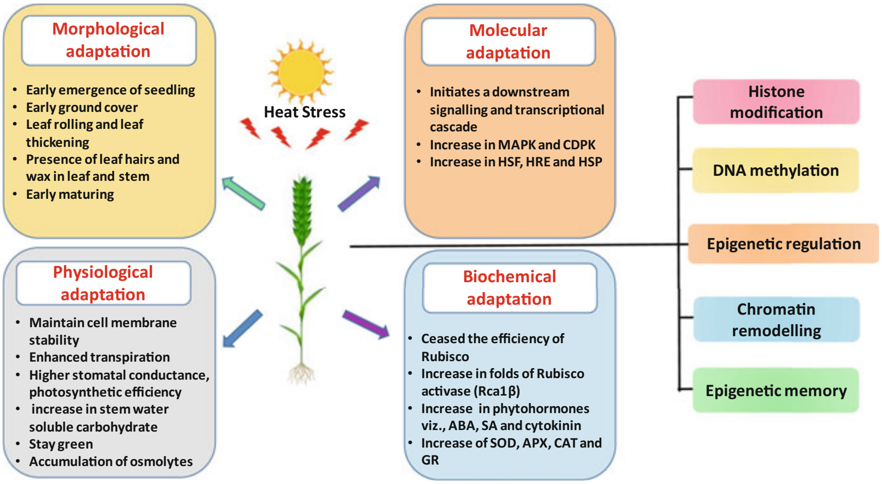

Fig. 1.1 Different heat stress adaptation mechanism in wheat

1.5 Summary

Wheat crop is challenged by heat stress affecting productivity in major wheat growing areas. In order to avoid effects of heat stress, plant adapted various strategies at morphological, physiological, molecular, epigenetic, and biochemical levels. Understanding about different mechanism by which wheat crop tolerate under heat stress situation will help us in selection and in improving the heat-tolerant varieties in wheat. Wheat being cold loving crop, the study on its response to heat stress is critical to have better insight into metabolisms, several pathways and cascades involved during the heat stress. Further, this knowledge will help us to screen the genotypes for heat tolerance and identifying number of genotypes having different desirable traits so that they can be recombined to develop tailor-made wheat with better heat tolerance. The various novel techniques like proteomics, genomics, and metabolomics can be applied to dissect genetics behind the heat stress response. This enables identification of molecular markers linked to heat stress tolerance genes facilitating the breeding for heat stress tolerance in wheat.

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Molecular Markers Mediated Heat Stress Tolerance in Crop Plants

2

Md. Shamim, Mahesh Kumar, and Deepti Srivastava

Abstract

Plants' developmental and growth rates can be influenced by temperature from seedling to maturity stage. Among the abiotic stress, heat stress has an impact on agronomic qualities at all phases of development; however, pre-flowering and anthesis stages are more vulnerable to high temperatures than post-flowering stages. As evident by massive yield losses in various food crops, the escalating adverse impacts of heat stress (HS) are putting the global food as well as nutritional security at great risk. In most of cereal crops, heat tolerance is a quantitative attribute that is influenced by a variety of genes and QTLs (quantitative trait loci). Attempts have been made over the last three decades to determine whether the condition under heat stress has been reviewed. Advances in molecular markers and quantitative genetics have made it possible to discover QTL that influence heat tolerance in cereal crop. Using various characteristics as indications of heat tolerance, many important QTL with significant effects on heat tolerance were discovered. There has been an increase in interest in using functional marker tools and technologies based on transcriptomics, proteomics, and metabolomics data to find and understand the molecular components of heat stress tolerance and the underlying mechanisms in recent years.

Keywords

Heat stress · Molecular markers · Quantitative trait loci

M. Shamim (✉) · M. Kumar
Department of Molecular Biology and Genetic Engineering, Dr. Kalam Agricultural College, Bihar Agricultural University, Bhagalpur, Bihar, India

D. Srivastava
Department of Agriculture, Integral Institute of Agricultural Science and Technology, Integral University, Lucknow, Uttar Pradesh, India

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2.1 Introduction

Heat stress severely limits the production of cereal crops in many areas of the world. The development of high yielding, heat stress tolerant cultivars will be aided by knowledge of the degree of genetic diversity within different grains, as well as their selection features (Sharma et al. 2016). Heat resistance is a multi-genetic trait, with different components of resistance controlled by different sets of genes in different tissues or at different stages of development. Plants react to extreme temperature stress by activating a series of events and turning on a slew of stress-responsive genes. However, the complex and poorly known mechanism of heat tolerance (HT), restricted access to precise phenotyping tools, and, most importantly, significant G X E effects all pose major roadblocks to breeding for improved HT (Jha et al. 2014).

DNA markers have a wide range of uses for strengthening a plant's genetic structure, including genetic identification of parents, genetic variation assessment, and the identification, genetic confirmation and establishment of high-resolution genetic linkage groups. For crop genetic study, a wide range of molecular markers is available. These markers are categorised according to how they are used, such as PCR (Polymerase chain reaction)-based vs. non-PCR-based. RFLP (Restriction fragment length polymorphism) markers are DNA markers that are based on hybridisation procedures. During the twentieth century, these markers were widely utilised in the field of molecular biology for gene mapping and other genetic analysis methods. Mullis and Faloona were the first to use PCR (1987).

PCR-based genetic markers dramatically lowered the time and cost required for genetic mapping utilising probe hybridisation. PCR is a technique for amplifying DNA sequences for a gene or locus in vitro. Primers are oligonucleotides that are tiny in size. Primers in a target sequence complement neighbouring gene sequences at both ends of the sequence. From a little quantity of a single pattern, the continual cycling of DNA replication and melting yields huge amounts of sequences of interest (Ullah 2009). PCR-based molecular markers such as single nucleotide polymorphisms (SNP), sequence-characterised amplified regions (SCAR) and simple sequence repeats (SSR) require gene sequence information from the sample to be used (Younis et al. 2020). Such markers are especially helpful in the mapping of stress-related genes' QTLs (quantitative trait loci) (Younis et al. 2020). When dealing with quantitative features like HT, molecular markers that allow for the exact and time-saving recovery of desired genotypes become essential (Shirasawa et al. 2013).

Because of the quantitative nature of heat, traditional breeding tactics have only made limited progress in generating heat-tolerant genotypes. Heat stress tolerance and unpredictability in the phenotyping and genotyping data of high quality are required for the discovery of quantitative trait loci (QTLs)/genes, as well as the development of markers for marker-assisted breeding, particularly for features that are difficult to breed. Heat tolerance, for example, is difficult to breed in the field with the help of traditional breeding approach.

2.2 Impacts of the Heat Stress on Major Cereal Plants

2.2.1 Effect of Heat Stress in Rice

The finding of genes/QTLs that increase tolerance to high temperatures has agricultural ramifications. Cao et al. (2003) were the first to map QTLs for heat tolerance in rice, based on percent spikelet fertility in a doubled haploid population produced from an IR64/Azucena hybrid. Following that, numerous research groups have used F2, backcross inbred lines (BIL) and recombinant inbred lines (RIL) populations to map QTLs for heat stress resistance, which were assessed at the time of heading in controlled environment circumstances (Shanmugavadivel et al. 2017). Rather than mapping QTLs based on phenotypic performance in a stress environment alone, the relative performance of genotypes under stress and control conditions can be utilised as an indicator to locate and map QTLs, which can then be employed in breeding crop varieties for stress tolerance (Raman et al. 2012). This has practical implications since genotypes with low production potential under control conditions generally demonstrate greater stress tolerance than genotypes with high yield potential.

For the heat stress breeding in rice, when cultivated rice var. of *O. sativa* ssp. Japonica and wild rice accession (*Oryza meridionalis*) grown under heat stress condition, wild rice grew faster and had less effect on photosynthesis at 45 °C (Scafaro et al. 2010). HS tolerance was higher in two rice genotypes, namely 'Dular' and 'Todorokiw-ase', at the booting stage at 39 °C, whereas 'Milyang 23' demonstrated tolerance at the flowering stage at 38 °C (Tonorio et al. 2013). Ginzberg et al., on the other hand, demonstrated tolerance at both the above-mentioned stages (2011). Under both controlled and HS circumstances, Jagadish et al. (2008) found that the genotype CG14 (*O. glaberrima*) achieved peak anthesis stage earlier than *O. sativa*.

Among the rice genotypes evaluated against heat stress, rice cultivar 'N22' demonstrated the highest level of HS tolerance (Jagadish et al. 2010a, b; Madan et al. 2012), with 64–86% spikelet fertility at 38 °C compared to the susceptible cultivars, namely 'Azucena' and 'Moroberekan', which had low fertility (up to 8%) (Jagadish et al. 2008). Higher pollen viability and spike fertility in rice genotypes 'N22' and 'NH219' were later confirmed under HS (Poli et al. 2013). Introgression breeding in rice has recently eased the transfer of HT from the 'N22' line to the 'Xieqingzao B' line by producing BC1F8 lines (Jiang-lin et al. 2011). Furthermore, an advanced line produced from the Gayabyeo/N22 cross has demonstrated HS tolerance as well as excellent yield (Manigbas et al. 2014). Given the relevance of anther dehiscence in conferring HS resistance, a rice study was conducted to assess anther characteristics, particularly the closure of locules under high temperatures (Matsui and Omasa 2002). As a result, the japonica rice cultivars 'Nipponbare' and 'Akitakomachi' have shown better fertility during flowering at 37.5 °C/26 °C (Matsui and Omasa 2002). Several QTLs for heat stress tolerance have been identified in rice (Table 2.1).

Molecular Marker and QTLs for Heat Stress Tolerance in Rice

With the development of molecular markers, several heat stress gene and responsive QTLs were discovered and transferred in the elite rice cultivars (Table 2.1). Three QTLs for HT were discovered on chromosomes 1, 4 and 7 using 245 restriction fragment length polymorphism (RFLP) markers in 98 backcross inbred lines (BILs) produced from the cross (Nipponbare 9 Kasalath) 9 Nipponbare (Zhu et al. 2005). During the grain-filling stage, these three QTLs explained 8.94%, 17.25% and 13.50% of the total PV, respectively (Zhu et al. 2005). In addition to QTL analysis, BSA utilising a set of SSR markers in 279 F2 (996 94 628) individuals resulted in the discovery of two loci linked to HT, namely RM3735 (chromosome 4) and RM3586 (chromosome 3), which controlled 17% and 3% of the total PV, respectively (Gui-lian et al. 2009).

Similarly, eight QTLs influencing spike fertility under high temperatures were found on chromosomes 1, 2, 3, 8, 10 and 11, among others (Jagadish et al. 2010a). From the genotype HT54, a large dominant locus OsHTAS (Oryza sativa heat tolerance at seedling stage) was recently found, which contributed strong temperature tolerance at 48 °C, particularly during the seedling and grain-filling stages (Wei et al. 2013). Similarly, in BC1F1 and F2 progeny generated from the cross IR64 9 N22, two significant QTLs for HT, qHTSF1.1 (R2 = 12.6%) and qHTSF4.1 (R2 = 17.6%), were found on chromosomes 1 and 4, respectively (Ye et al. 2012). QTL research comprising 90 introgression lines recently revealed five QTLs that explained PVs ranging from 6.83 to 14.63 percent (Lei et al. 2013). A QTL research comprising 90 introgression lines recently revealed five QTLs that explained PVs ranging from 6.83 to 14.63% (Lei et al. 2013). While transferring genes from wild rice (*O. rufipogon* Griff.) to the introgression line Y106, two QTLs for HS tolerance (qHTS1–1 and qHTS3) were discovered (Lei et al. 2013). In rice, sophisticated backcross was employed to create introgression lines in the background of ‘Teqing’, and later screening of these lines revealed one heat-sensitive line, ‘YIL106’ (Lei et al. 2013).

Heat-tolerant (XN0437T) and heat-sensitive (XN0437S) introgression lines were also recovered from another backcross inbred population derived from the cross (Xieqingzao B 9 N22) 9 (Jiang-lin et al. 2011). Heat-induced injuries such as white-back kernels were significantly reduced in nearisogenic lines (NILs) created by introducing the qWB6-allele from ‘Hana-echizen’ into the background of ‘Niigata-wase’ (Kobayashi et al. 2013). A 1.5-Mb chromosomal area bounded by markers ktIndel001 and RFT1 was transferred from ‘Kokoromachi’ to ‘Tohoku 168’ utilising marker-assisted backcrossing in a recent rice study. The grain quality of the NILs was much better than the susceptible parent ‘Tohoku 168’. The chromosomal region in question had a strong QTL that influenced more than 20% of the PV and was responsible for higher grain quality under HS (Shirasawa et al. 2013). Table 2.1 contains a non-exhaustive list of DNA markers related to various HT/component features found in various crops. With such strong markers/candidate gene(s)/QTLs in place, early generation marker-based selection combined with a selective mating strategy would maximise genetic gains while breeding for HT.

Table 2.1 Important QTLs and linked marker in major cereal crops (Rice, barley, maize and wheat)

Crop	Markers linked to the QTLs	Name/No. of the QTL/loci	Chromosomal location/Linkage group (LG)	Mapping population	Range of PV (%)	References
Rice	–	*qhr1, qhr3-1, qhr4-3, qhr8-1, qhr11-1* and *qhr11-2*	1, 3, 4, 8 and 11	DH (IR64 9 Azucena)	1.3–22.8	Cao et al. (2003)
	RFLP	3 QTLs	1, 4 and 7	BIL Nipponbare/ Kasalath//Nipponbare	8.94–17.25	Zhu et al. (2005)
	–	9 QTLs thermotolerance for amylose content and gel	6 and 8	Nipponbare/Kasalath// Nipponbare	–	Zhu et al. (2006)
	SSR	WBK–*qWK1-1*, *qWK1-2*, *qWK2* and *qWK8*	1, 2 and 8	RIL (Chiyonishiki 9 Koshijiwase)	8.8–15	Tabata et al. (2007)
	SSR	2 putative QTLs associated with white-back kernels	4, 6	(Hana-echizen 9 Niigata-wase)	15.2–59.6	Kobayashi et al. (2007)
	SSR	*qHt3, qHt9a*	3 and 9	RIL (T219 9 T226)	7.6–11.4	Chen et al. (2008)
	SSR	3 QTLs (*qhts-2, qhts-3* and *qhts-5)*	LG 2, 3 and 5	RIL (Zhongyouzao No. 8 9 Fengjin)	6.59–10.72	Zhang et al. (2008)
	SSR	*RM3735* and *RM3586* loci	4 and 3	F_2 (996 94628)	3–17	Gui-lian et al. (2009)
	–	*qtl_2.3,qtl_4.1, qtl_1.1, qtl_2.2,qtl_8.2,qtl_1.1, qtl_8.1, qtl_10.1,qtl_1.1, qtl_3.4,qtl_8.3,qtl_10.1, qtl_1.1,qtl_11.1,qtl_10.1, qtl_1.1,qtl_10.1 and qtl_11.1*	1, 2, 3, 4, 8, 10, 11	(Bala 9 Azucena)	7–17.6	Jagadish et al. (2010a)

(continued)

Table 2.1 (continued)

Crop	Markers linked to the QTLs	Name/No. of the QTL/loci	Chromosomal location/Linkage group (LG)	Mapping population	Range of PV (%)	References
	SSR	2 QTLs	4 and 10	RIL (996 94628)	21.3–25.8 11.5–11.6	Xiao et al. (2011)
	SSR	*qPF4*, *qPF6*	4 and 6	RIL (996 9 4628)	15.1–9.31	Ying-hui et al. (2011)
	SNP	*qHTSF1.1*	1	BC_1F_1, BC_2F_2 and F_2 (IR64 9 N22)	12.6–17.6	Ye et al. (2012)
		qHTSF4.1	4			
	SNP	*OsHTAS* locus	9	F_1 and F_2 (HT54 9 HT13)	–	Wei et al. (2013)
	SSR	*qHTS1-1, qHTS1-2, qHTS2, qHTS3 and qHTS8*	1, 2, 3 and 8	Introgressed line YIL106 (Teqing 9 *O. rufipogon*)	6.83–14.63	Lei et al. (2013)
	SSR	*(qWB3*, *qWB4*, *qWB6* and *qWB9)* QTLs for WBK *(qKW3-1, qKW3-2, qKW6, qKW7* and *KW10)* QTLs for KW *(qDH1, qDH3 and qDH6)* QTLs for DTH	3, 4, 6 and 9 for WBK 3, 3, 6, 7 and 10 for KW 1, 3 and 6 for DTH	(Hana-echizen 9 Niigata-wase)	(31.5–36.8) WBK (8.4–12.1) DTH	Kobayashi et al. (2013)
	SSR SNP	QTL for white-back grains	6	RIL (Tohoku 168 9 Kokoromachi)	–	Shirasawa et al. (2013)
	SSR	9 QTLs	3, 4, 6, 8, 10 and 11	BC_2F_2 (OM5930x N22)	17.1–36.2	Buu et al. (2014)
	SSR	11QTLs	*1,2,3,4,5,7,8,10,11*	33 chromosome segment substitution lines [CSSLs (SL401–SL433)] and their parents Sasanishiki and Habataki,	–	Zhao et al. (2016)

	SNP	5QTLs	3,5,9,12	RIL derived from Nagina22, and IR64	–	Shanmugavadivel et al. (2017)
	SNP	6 QTLs	*1,4,6,7*	F8 recombinant inbred lines (RILs) obtained by crossing heat-tolerant ‘N22’ and heat-susceptible ‘IR64’	–	Kilasi et al. (2018)
	SSR	1 QTLs	*4*	F3 Uma × N22,	–	Waghmare et al. (2020)
	Bulked-segregant analysis (BSA)-seqmethod	1	*8*	F2: 3 population derived from a cross between Huanghuazhan (HHZ), a heat-tolerant cultivar, and 9311, a heat-sensitive variety	–	Chen et al. (2021)
Barley	SSR	34 putative QTLs	–	BC$_2$DH (Scarlett 9 ISR42–8)	–	Mohammed (2004)
	DArT markers	6 QTLs	4H, 5H and 6H	ND24260 x flagship doubled haploid population	2.6–5.9	Gous et al. (2016)
Maize	RFLP	6 QTLs (cellular membrane stability)	–	RIL (T232 9 CM37)	–	Ottaviano et al. (1991)
	RFLP	3–8 QTLs, heat-shock protein (HSP) expression	–	RIL (T232 9 CM37)	–	Frova and Sari-Gorla (1993)
	RFLP	5 QTLs for IPGG and six QTLs for IPTG	–	RIL (T232 9 CM37)	–	Frova and Sari-Gorla (1994)
	SNP	Fifteen QTL for leaf firing, leaf blotching, tassel blasting, reduction in spikelet size, plant death at early vegetative, middle vegetative and late vegetative stage	1,5,8, 9,10	B73 × NC350	–	McNellie et al. (2018)
			1,2,3	B73 × CML103	–	

(continued)

Table 2.1 (continued)

Crop	Markers linked to the QTLs	Name/No. of the QTL/loci	Chromosomal location/Linkage group (LG)	Mapping population	Range of PV (%)	References
	SNP	6 QTLs for heat susceptibility index (HSI) of five traits (leaf length: LL, plant height: PH, leaf scorching: SC, leaf greenness: SD, leaf growth rate: LR)	2,5,9,10	Segregating populations derived from pairwise crosses of four Dent (S067 = *D*1, P040 = *D*2, S058 = *D*3, S070 = *D*4) and four Flint (L012 = *F*1, L017 = *F*2, L043 = *F*3, L023 = *F*4) maize inbred lines	–	Inghelandt et al. (2019)
Wheat	SSR	1.4 genes, 2 QTLs	–	(Ventnor 9 Karl 92)	11–12.	Yang et al. (2002)
	AFLP and SSR	3 QTLs	LG1B, 5B and 7B	RIL (Kauz 9 MTRWA116)	27.3–44.3	Mohammadi et al. (2008)
	SSR	Common QTL for drought and heat stress traits	1B-a, 2B-a, 3B-b, 4A-a, 4B-b and 7A-a	RIL (Seri 9 Babax)	17 (yield QTL)	Pinto et al. (2010)
	AFLP, SSR	*(Q75%Gh.ksu-2A, Q75% Gh.ksu-2A,* Q75%Gh. ksu-3B) 75%G, *(Q25%Gh.ksu-2A, Q25% Gh.ksu-2A*) 25%G, (Q50%Gh.ksu-2A, Q50% Gh.ksu-6A) 50%G, (QMrsh.ksu-2A) (*QTmrsh.ksu-2A, QTmrsh.ksu-6A,QTmrsh. ksu-6B*) TMRS, *(QPgmsh.ksu-3A, QPgmsh.ksu-6B*) PGMS, (QFv/Fmh.ksu-7A) Fv/Fm	2A, 6B, 3A and 7A	RILs (Ventnor 9 Karl 92)	53 (75%Q), 28 (25%G), 63 (50%G), 40 (MRS), 55 (TMRS), 36.4 (PGMS), 11.2 (Fv/Fm)	Vijayalakshmi et al. (2010)

	SSR	5 QTLs	1A, 2A, 2B and 3B associated with HSI	RIL (Halberd 9 Cutter)	–	Mason et al. (2010)
	SSR	3 QTLs (*QSg. bhu-1A*, *QSg.bhu-3B* and *QSg. bhu-7D*)	1AS, 3BS and 7DS	RIL (Chirya3 x Sonalika)	38.7	Kumar et al. (2010)
	SSR	14 QTLs for heat susceptibility index (HSI), 7 QTLs co-localised for HSI and TD trait	1B, 3B, 4A, 5A, 5B and 6D	$F_{2:6}$ RIL (Halberd 9 Karl 92)	Individual QTL (4.5–19.3)	Mason et al. (2011)
	SSR	*Xgwm132-linked QTL*, *Xgwm577-linked QTL* and Xgwm617-linked QTL	6B, 7B and 6A	F_1, F_2 (Debra 9 Yecora Rojo)	3–25.	Barakat et al. (2011)
	SSR	12 QTLs	1A, 7A, (3B, 3A, 5B), 2D, 1D, (2A, 2B, 2D)	F_2 (Ksu106 9 Yecora Rojo)	22–64	Barakat et al. (2012)
	SSR	*QHthsitgw.bhu-2B*, *QHthsitgw.bhu-7B*, *QHthsitgw.bhu- 7D*, *(TGW)*, *QHthsiYLD.bhu-7B*, *QlsYLD.bhu-7B*, *(YLD)*, *QHthsigfd.bhu-2B (GFD)*, QHtctd.bhu-7B (CTD), Qls-dm.bhu-7D (DM)	1B, 7D, (4A, 5D), (7A, 7B, 7D), 5A and 3B, BL, 7BL, 7DS (TGW), 7BL (YLD) 2B1 (GFD), 7BL (CTD) and 7DS (DM)	RIL (NW1014 9 HUW468)	9.78–20.34 (TGW), 13.21 (YLD), 20.15 (GFD), 19.81 (CTD) and 7.42 (DM)	Paliwal et al. (2012)
	DArT and SSR	2 QTLs, (*Q.Yld.aww-3B-2* and *Q.Yld.aww-3D)*	3B	DH, RIL (RAC875 9 Kukri)	22	Bennett et al. (2012)

(continued)

Table 2.1 (continued)

Crop	Markers linked to the QTLs	Name/No. of the QTL/loci	Chromosomal location/Linkage group (LG)	Mapping population	Range of PV (%)	References
	SSR	4 QTLs (*Qsdscon.tam-1B, Qsdsheat.tam-1D, Qsdscon.tam-4A* and *Qsdssta.tam-7A)*	LG 1B, 1D, 4A and 7A	RILs (Halberd 9 Cutter)	18–30	Beecher et al. (2012)
	SSR	Marker locus *gwm299*	3BL	DH, RIL (RAC875 9 Kukri)	–	Bonneau et al. (2013)
	AFLP, SSR, DArT	TKW QTLs linked or pleiotropic to DH and DM	4B and 7D-b	RIL (Barbax 9 Seri)	39 (TKW)	Lopes et al. (2013)
	AFLP, SSR, DArT	Few QTLs associated with ECG	7D-a and 7D-b	RIL (Barbax 9 Seri)	17.4 (ECG)	Lopes et al. (2013)
	AFLP, SSR, DArT	Consistent QTLs were detected for CTvg and CTgf	4A	RIL (Barbax 9 Seri)	16 (Ctgf)	Lopes et al. (2013)
	SSR	QChlc.tamu-1B (chlorophyll content), QFlt.tamu-2B	–	19 families consisting of 384 individuals developed from 3-way cross	–	Ali et al. (2013)
	SSR	7 QTLs associated with HSI traits, GFD, TGW, GY and CT 9 QTLs associated with GFD, TGW, GY and CT under HS	1D, 6B, 2D and 7A	DH (Berkut 9 Krichauff)	–	Tiwari et al. (2013)
	SSR	QTL for leaf and spike temperature depression and leaf wax	1B and 5A	RIL (Halberd 9 Karl 92)	8–12	Mondal et al. (2014)

	SNP	QTL for grain weight (SGW or GWS)	3B and 6B	DH (Drysdale and Waagan)	–	Shirdelmoghanloo et al. (2016)
	SSR	QTLs for various heat tolerance component traits	1B, 2B,3A,3B,5B, 7A AND 7B	Heat-tolerant accession of *Ae. Speltoides* pau3809 was crossed with Triticum durum cv. PDW274, and BC2F4-6 backcross introgression lines (BILs)	11.1–28.7	Awlachew et al. (2016)

2.2.2 Effect of Heat Stress in Wheat

Because of early senescence and acceleration of grain-filling activities in wheat (Paulsen 1994) and shortening grain filling duration (GFD) together with restriction of carbon absorption, terminal heat stress during crop growth is a major environmental factor that affects grain yield (GY). When the temperature was raised from 15 to 20 °C (day/night) to 40 to 15 °C (day/night) on the third day after anthesis, kernel weight loss was estimated to be up to 23% (Stone and Nicolas 1994). By 2050, approximately 51% of the Indo-Gangetic plain (IGP) may be classed as a heat-stressed, short-season producing mega-environment (Ortiz et al. 2008).

When comparing the wild progenitor *Aegilops tauschii* Coss. to the tolerant cultivar 'C273' for critical HT-related properties including cell membrane stability and 'TTC' (2, 3, 5-triphenyl tetrazolium chloride)-based cell viability, wide variation was found in the wild progenitor *Aegilops tauschii* Coss (Gupta et al. 2010). *A. tauschii* was recently employed as a donor for backcrossing cultivar 'PBW550' to incorporate HT-relevant constituent features like cell membrane integrity and chlorophyll retention (Sehgal et al. 2011). Similarly, the discovery of *A. speltoides* Tausch and *A. geniculata* Roth species with HS tolerance in the reproductive stage brings up new possibilities for HT gene inclusion in hexaploid wheat in the near future (Pradhan et al. 2012a).

By screening wheat genotypes for drought and HS stress, the ALTAR 84/AO′S′ and ALTAR 84/A. tauschii genotypes were shown to be the least impacted, particularly at two important stages: (1) emergence to anthesis and (2) emergence to post-anthesis (additional 21 days after anthesis) (Pradhan et al. 2012b). More recently, efforts have been done in wheat to introduce the wheat-Leymus racemosus chromosome into the 'Chinese spring' cultivar in order to improve HT and better adapt to HS (Mohammed et al. 2014). Hede et al. (1999) investigated the potential of wheat landraces in search of new sources of HT, and as a result, three Mexican landraces with superior canopy temperature depression characteristic were identified. Furthermore, two genotypes, 'Moomal-2000' and 'Mehran-89', performed better at 20–30 °C in terms of germination-related characteristics (Buriro et al. 2011). While researching photosynthetic activity in flag leaves during the grain-filling stage recently, Feng et al. (2014), reported that the cultivar 'Jimai22' has a 6% lower drop in grain yield under HS. Furthermore, this cultivar provided benefits such as PSII stability and carboxylation activity under HS. On the basis of maximum grain development and higher survival under challenged conditions, evaluation in the terminal growth stage focusing on the stay green trait resulted in the discovery of three potential genotypes (Rehman et al. 2009). In wheat, promising genotypes 'WH1021' and 'WH730' with increased yield under HS have just been developed (Dhanda and Munjal 2012). Similarly, using cluster analysis of morphological features and ISSR markers, three synthetic wheat lines, SYN 11, SYN36 and SYN44, were pronounced to be very heat resistant (Sharma et al. 2014).

Molecular Markers for Heat Stress in Wheat and Important QTLs

Many genes are implicated in stress tolerance because abiotic stimuli cause many physiological and biochemical changes. The identification of functional markers and, as a result, enhanced selection efficiency for improved heat stress tolerance will be aided by understanding the molecular and genetic underpinnings of stress tolerance (Table 2.1).

Senescence-related traits were evaluated in wheat and nine QTLs were discovered across distinct chromosomes (2A, 6A, 6B, 3A, 3B and 7A) (Vijayalakshmi et al. 2010). Using metrics such as spike yield and temperature depression (TD) of spike, a heat susceptibility index (HSI) was created, and a total of 14 QTLs related with HSI were found (Mason et al. 2011). Four QTLs for increased baking quality under HS circumstances were discovered on wheat chromosomes 1B, 1D, 4A and 7A after a sodium dodecyl sulphate sedimentation (SDSS) test (Beecher et al. 2012).

In 148 RILs (NW1014 9 HUW468), three significant QTLs related with HT were found on chromosomes 2B, 7B and 7D, accounting for up to 20% PV (Paliwal et al. 2012). BSA was used in an F2 population of Ksu106 9 Yecora Rojo employing SSR markers, with grain-filling rate (GFR) as a critical feature governing grain production in wheat (Barakat et al. 2012). As a result, 12 SSR markers in wheat were discovered to have a strong relationship with GFR. Kirigwi et al. (2007) also found QTLs for GFR on the 4A chromosome. In wheat, a large QTL with a 17 percent yield variation was discovered under HS on chromosome 4A (Pinto et al. 2010). Because single nucleotide polymorphism (SNP) markers are amenable to automated genotyping tests, one possible SNP marker was recently revealed in wheat that separated heat-tolerant (K7903) from heat-sensitive (RAJ4014) cultivars (Garg et al. 2012).

Fufa et al. (2005) used 51 SSR markers and ten morphological features in 30 bread wheat cultivars to investigate diversity analyses. Because morphological features continue to be an efficient technique of regularly evaluating multiple wheat elite lines developed in a breeding programme for breeders, the positive correlation from the outcome has crucial implications. Sharma et al. (2016) also looked at genetic diversity using 41 polymorphic simple sequence repeat (SSR) markers and 15 phenotypic variables averaged across stress and non-stress field conditions. It is critical to perform rapid and precise introgression of HT-related gene(s)/QTLs into heat-susceptible cultivars in order to restore genotypes with improved heat stress tolerance.

2.2.3 Effect of Heat Stress and Molecular Markers in Maize for Heat Stress

The current pace of maize yield growth, when combined with population expansion and projected climate change consequences, will not be enough to meet future food demand. The current tendency of growing maize in the winter, particularly in South Asia’s Indo-Gangetic Plains, has increased the chances of maize crops being exposed to suboptimal temperatures. In the winter, the average minimum temperature is below 5 °C, notably in the Indo-Gangetic Plains' North-West Plains. Under growing climate variability, improving crop yield and livelihoods for smallholders will necessitate a multi-disciplinary approach to crop genetic modification (Hansen et al. 2019; Prasanna et al. 2021). When it comes to resource allocation, temperate maize has gotten a lot more over time than tropical maize (Andorf et al. 2019).

In tropical regions, however, the potential to directly benefit smallholder farmers' lives through better maize yields is greater. According to a recent study, improvements in maize breeding have benefited an estimated 53 million individuals in Sub-Saharan Africa (Cairns and Prasanna 2018). In order to provide farmers with a consistent stream of improved varieties, increasing genetic gain, including a reduction in breeding cycle time, is critical (Atlin et al. 2017; Andorf et al. 2019; Bailey-Serres et al. 2019). Heat stress is becoming a serious limitation to maize output on its own and in combination with drought (Cairns et al. 2013). Depending on the emissions scenario, temperatures are expected to rise by at least 1 °C (Zhai et al. 2020). A 2 °C increase in temperature would result in a 20% drop in maize yields, while a 20% decrease in precipitation would result in a 20% reduction (Lobell and Burke 2010).

In most tropical semi-arid maize-growing locations, notably in South and Southeast Asia, frequent spells of high temperatures (typically above 35 °C) combined with moisture stress are a common occurrence, impacting maize reproductive growth in particular.

Aside from early development, the blooming stage of the maize crop is especially vulnerable to cold temperatures, as it causes male sterility (Heslop-Harrison 1961) or weak anthesis, and hence poor grain set (Thakur et al. 2010; Enders et al. 2019). Maize crops in northern India and Nepal's Tarai region were subjected to extreme cold stress during the winter seasons of 2002–2003, 2009–2010 and 2017–2018, resulting in significant production losses (Enders et al. 2019). Waterlogging affects more than 18% of the entire maize production area in South and Southeast Asia, resulting in yearly production losses of 25–30% (Zaidi et al. 2010; Cairns et al. 2012). Soil waterlogging is a common occurrence in areas with unpredictable and strong rainfall and low soil drainage ability. Because maize is a tropical non-wetland crop, it is particularly sensitive to waterlogging at practically all phases of development, especially before tassel emergence (Zaidi et al. 2004; Kuang et al. 2012). Salinity stress has been shown to impact maize seed germination, vegetative growth and reproductive capacity (Munns and James 2003; Abdullah et al. 2001; Kaya et al. 2013).

The molecular and physiological consequences of heat stress in maize seedlings were studied (Table 2.1) in controlled surroundings and then in field circumstances using managed heat stress phenotyping (Cairns et al. 2013; Rattalino-Edreira and Otegu 2013). Under HS, five QTLs influencing pollen germination and six QTLs controlling pollen tube growth were discovered using RFLP markers in a RIL population (Frova and Sari-Gorla 1994). Previously, six QTLs in maize that influenced cellular membrane stability under HS were found using the same mapping population (Ottaviano et al. 1991). Frey et al. (2016) created a heat susceptibility index to characterise segregating families of temperate maize populations developed for heat stress tolerance characterisation and discovered two QTL hotspots on chromosomes 2 and 3 for a variety of heat stress-related characteristics. The area previously discovered for pollen viability under heat stress was shown to be co-localised with QTLs identified on chromosome 3 (Frova and Sari-Gorla 1994). On chromosome 9, QTL hotspots for heat susceptibility index calculated for leaf blistering and plant height under heat stress were also discovered (Inghelandt et al. 2019).

2.2.4 Effect of Heat Stress and Molecular Markers in Barley for Heat Stress Adaptation

Wild barley, *Hordeum vulgare* ssp. spontaneum L. (hereinafter referred to as *H. spontaneum*), is thought to be a drought and heat-tolerant progenitor of barley (*Hordeum vulgare* ssp. vulgare L.) cultivars (Hubner et al. 2009; Bahrami et al. 2019; Arzani and Ashraf 2016). The main selective factors underpinning the evolution of wild barley (*H. spontaneum*) over the Fertile Crescent have been proposed to be high temperatures and aridity (Hubner et al. 2009). Due to a lack of genetic variety in modern barley cultivars, the wild spontaneum subspecies' genetic variability must be extensively exploited in order to generate barley cultivars with greater heat tolerance (Table 2.1). A breeding effort disucussed above could provide the different benefits for heat adaptation of wild subspecies (Bahrami et al. 2019).

It's also been suggested that *H. spontaneum*, which has evolved effective high-temperature tolerance techniques to adapt to the hot climate in south-west Iran, could be used as a rich genetic resource for enhancing the heat stress tolerance of farmed barley. The physiological basis of *H. spontaneum* resistance to both heat (Bahrami et al. 2019) and salinity (Ebrahim et al. 2020) conditions has recently been proven using this rich genetic resource of *H. spontaneum* germplasm from West-Iran. Drought tolerance in barley can be improved by identifying genomic areas associated with drought-related characteristics. On a collection of 107 barley accessions tested under well-watered and drought-stressed conditions, association mapping was used to study the correlations between 76 SSR markers and six drought-related characteristics.

All examined traits showed highly significant differences between well-watered and drought-stressed environments. With SSR markers, there was a significant level of polymorphism. For drought-related phenotypes, a total of 36 significant marker–trait correlations were discovered. Between markers on separate chromosomes, there was a high degree of significant LD ($> 61\%$), implying epistatic interaction. Several molecular markers are significantly related with multiple phenotypic traits, implying that pleiotropic or indirect effects may exist. Individual marker–trait relationships explained 6.33% to 35.78% of the phenotypic variation (Abou-Elwafa 2016).

Comadran et al. (2009) found significant marker–trait associations in a barley population that represents a historical survey of barley diversity in Mediterranean environments, with a large proportion of genetic variation underlying the different mechanisms for adaptation to drought-prone environments. The role of physiological characteristics such as reserved metabolites, as well as the genetic control of heat stress in barley, is little understood in comparison to other abiotic stresses like drought. There have been few research on the mapping of quantitative trait loci (QTL)/genes that govern heat tolerance in barley during flowering and seed set. The six heat stress QTLs on chromosomes 3H, 4H, 5H and 6H in barley are an exception. The QTL on chromosome 5H was found to be highly similar to a previously discovered QTL for root length and root–shoot ratio (Gous et al. 2016).

The transcriptome alterations of genes in barley during heat stress were the subject of two investigations. Mangelsen et al. (2011) discovered that heat stress enhanced the expression of 958 genes, whereas 1122 genes were downregulated in growing barley grains, using the Affymetrix 22 K Barley1 GeneChip microarray. The downregulation of genes tailors the manufacture of storage chemicals as well as cell growth, demonstrating that heat stress hampered grain formation quickly. Templer et al. (2017) discovered 25 metabolic QTL in barley under heat and/or drought stress, three of which matched with critical player genes in the production pathways of these metabolites. Weichert et al. (2017) looked at how the barley sucrose transport gene (HvSUT) affected seed quality and yield in wheat during heat stress.

They discovered that increasing the expression of this gene in a winter wheat cultivar (cv. Certo) boosted grain production, grain size and above-ground biomass by exposing plants to heatwaves. The heatwave reduced grain production, above-ground biomass, grain size, starch and water content in the wild-type, but increased grain sucrose content. Dawood et al. (2020) investigated a group of 60 Egyptian spring barley genotypes that had been subjected to heat stress testing in the field. Several features were scored to assess changes in yield-related traits and grain-reserve metrics as markers of heat tolerance. Using 16,966 single nucleotide polymorphisms, single-marker analysis was used to identify the causal genes that regulate the variation of all attributes of interest (SNP). The BC6 lines produced in this study can be used to unravel the involvement of the LTP2 gene in the response to various abiotic stressors, such as drought, salinity and heat. The BC6 lines established in this work can be used as a one-of-a-kind plant material to investigate the role of the LTP2 gene. The LTP2 may be important in lipidome change in response to abiotic stress because of its role in lipid transfer.

2.3 Summary

Heat stress is a major cause of yield loss, and heat events are predicted to become more often and longer in the future. Heat stress has become a major limiting factor in agricultural productivity since numerous crops are vulnerable to it, especially during the reproductive and early grain-filling stages. Because it is a polygenic regulating feature, heat tolerance is difficult to define. Until recently, there was no direct method for choosing heat-tolerant plants, but certain characteristics, such as canopy temperature depression and membrane thermo-stability, appear to be good indicators of plant heat tolerance and can be used in traditional breeding. It's vital to understand how heat stress affects wheat production and quality, as well as to provide useful indicators and genes for genetic improvement. Various mapping approaches and genetic research have greatly contributed in gaining a better understanding of the genetic roots of heat stress resistance in wheat. These studies discovered molecular markers connected to heat tolerance that could be utilised to diagnose MAS. However, there are few reports of molecular markers being utilised in cereal crop breeding. Increased knowledge of the molecular mechanisms of heat tolerance, on the other hand, is expected to pave the way for the development of heat-tolerant plants with acceptable commercial yields. Although several wheat genes have been successfully changed to improve heat stress tolerance, their function in different genetic backgrounds and under different heat stress settings remains unknown.

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Physiology of Crop Yield Under Heat Stress

3

Prakshi Aneja, Aditi Dwivedi, and Aashish Ranjan

Abstract

With the rising climatological extremities, heat stress is a major concern towards sustainable crop yield and productivity as it adversely affects the normal growth and physiology of the crop plants. Continuously increasing global temperature due to climate change leads to a significant loss in crop yield due to negative effects on major plant physiological processes. In order to devise the strategies to minimize the yield penalty due to high-temperature stress, it is imperative to understand the crop developmental and physiological responses to heat stress. In this chapter, we outline how plants sense the fluctuations in the temperature, focusing on the thermosensors and the sensing mechanisms that have been discovered till now. We discuss the effects of heat stress at different developmental stages impairing the normal growth of plants. We assess the impact of elevated temperatures on major physiological processes affecting net carbon assimilation, membrane stability, and water balance. Towards the end, we also highlight the alterations in the levels of phytohormones and their influence on plants for acquiring thermotolerance.

Keywords

Heat stress · Climate change · Crop yield · Thermosensing · Growth and development · Crop physiology · Phytohormones · Thermotolerance

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Prakshi Aneja and Aditi Dwivedi contributed equally with all other contributors.

P. Aneja · A. Dwivedi · A. Ranjan (✉)
National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi, India
e-mail: aranjan@nipgr.ac.in

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3.1 Introduction

The gradual rise in atmospheric temperature due to global warming and heatwaves due to climate change affect crop performance and are detrimental to the yield of staple crops worldwide (Lobell et al. 2011; Lobell and Gourdji 2012). Each degree Celsius increase in temperature is projected to decrease crop yield by 6% in wheat and 10–12% in rice, further threatening global food security (Asseng et al. 2015; Nelson et al. 2010). If we look back in last 136 years, most of the warmest years have been recorded since 2000 (Zhu et al. 2020) and a further increase in global temperature is expected in coming decades, leading to a 4.8 °C increase by 2100 (IPPC 2021; IPCC 2007). Optimum temperature is one of the primary requirements for plants to maintain their physiology along with growth and development. In natural environments, plants being sessile are exposed to a wide range of temperature conditions and thus, need to constantly adjust their growth, development, and physiology during their life cycle, both on a daily and seasonal basis. The extent of the impact of heat varies with the range, rate, and duration of high temperature and also the developmental age of the plants. Numerous physiological and developmental responses to temperature rise are reported. Plants' responses to temperature have been divided into three categories: (a) thermomorphogenesis, (b) heat acclimation, and (c) heat stress. The temperatures that induce these responses have a degree of overlap. Correspondingly, there are numerous instances where signaling routes between these phases overlap.

At temperatures slightly above the normal growth temperature, but still within the physiological range (i.e., 5–10 degrees rise), plants exhibit extensive alteration in their morphology and development to avoid their exposure to harmful temperatures in a process termed thermomorphogenesis (Casal and Balasubramanian 2019). At 10–15 degrees higher temperatures than the ambient temperature, plants start experiencing heat stress leading to pronounced negative effects on growth and physiology, and thus numerous detrimental consequences on overall plant performance. Hampered growth rates of root and shoots along with damaging effects on photosynthesis, reproductive development, and other processes become obvious. Plants use a range of acclimatization strategies to ameliorate the heat-stress tolerance at high temperatures, such as activating molecular chaperones, changing the constitution of cell membranes, hormonal changes, etc. (Krishnan et al. 2011). When temperatures rise beyond 10–15 degrees increase, cells are subjected to extreme heat stress causing global cellular damage including compromised membrane permeability and function, damaged photosynthetic apparatus, metabolic flux activities malfunction, and eventually cell death (Krishnan et al. 2011).

Heat stress, thus, is described as a rise in temperature beyond a critical threshold for a time duration long enough to cause irreparable harm to plant growth and development, and sometimes death occurring during extreme hot seasons. Warm temperature conditions affect the morphology and physiology of plants that help in adjusting growth to acclimatize high-temperature conditions detrimental to plants (Quint et al. 2016; Vu et al. 2019). In contrast to warm temperature acclimatization of vegetative development, early reproductive development is more susceptible to higher temperatures that directly affect grain output (Wu et al. 2021). The impact of temporary or constant high temperatures on morphological, physiological, molecular, biochemical, and developmental features depends upon the age of the plant and the duration of the heatwave. The major physiological impacts of elevated temperature on crops include increased fluidity and permeability of membranes, denaturation, degradation and aggregation of proteins, inhibition of protein synthesis, chlorophyll degradation, and reduction in net photosynthesis rate (Akter and Islam 2017; Hasanuzzaman et al. 2013). In addition, the high temperature reduces the duration of growth and development resulting in early and fast organ growth, early flowering, early seed setting, hastened leaf senescence, and decreased chlorophyll content, thereby ultimately affecting final crop yield (Bokszczanin et al. 2013; Fahad et al. 2019).

In this chapter, we provide the recent advances in temperature sensing mechanisms along with comprehensive insights on the effects of heat stress on crop developmental and physiological responses.

3.2 Temperature Sensing in Plants

Sensing external environmental cues and triggering downstream diverse pathways is of utmost importance to achieve cellular homeostasis in plants. Proper sensing of temperature fluctuations and accordingly initiation of timely adaptive mechanisms for resisting the detrimental effects of high temperature and maintaining cell function and viability are essential for plants. A number of thermosensors and sensing mechanisms have been discovered in recent years for both heat stress and high ambient temperature.

3.2.1 Heat Stress Perception Through Plasma Membrane Channels

Heat stress response (HSR) in plants is substantially conserved and at least four ostensible sensors have been suggested to activate it (Mittler et al. 2012). These sensors include a plasma membrane channel that initiates an inward Ca^{2+} flux, a nucleus histone sensor, and two unfolded protein sensors—one each in the ER and cytosol (Che et al. 2010; Kumar and Wigge 2010; Saidi et al. 2009; Sugio et al. 2009). Each of these sensors is intended to trigger a relatable set of HSR genes, but the link between the various routes and the hierarchical order remains ambiguous.

Plasma membranes, being thermally sensitive macromolecular structures, detect even slight changes in temperatures. Upon heat stress, membrane fluidity increases, and therefore membrane proteins detect physical phase transition ultimately resulting in conformational changes and phosphorylation/dephosphorylation events (Balogh et al. 2013; Niu and Xiang 2018). When an increase in temperature is sensed by the plasma membrane, it opens up particular calcium ion channels causing a rapid influx of calcium ions into the cell and activating HSR. These calcium channels are triggered probably by the increased fluidity of the plasma membrane due to high temperature. The rapid influx of Ca^{2+} into the cytosol was found to be essential for the induction of HSPs via Ca^{2+}/calmodulin dependent kinases as well as to attain thermal tolerance. Consequently, it can be speculated that Ca^{2+} channels might act as thermosensors. In *Physcomitrella patens* and Arabidopsis, heat stimulated Ca^{2+} rise in cytosol seemed to be mediated by cyclic nucleotide-gated channels, CNGCs (Finka et al. 2012; Saidi et al. 2009). The proposed function of heat-induced cAMP in activating the Ca^{2+}-mediated heat response led to the notion that instead of Ca^{2+}, adenylyl cyclase activity may function as a membrane-associated thermosensor (Winkelmüller et al. 2021). Recently in maize, two plasma membrane adenyl cyclases have been identified which are important for heat-induced cAMP production and induction of HSP expression (Hao Yang et al. 2021). However, their mechanism of action has remained unknown.

3.2.2 Phytochrome B: A Thermosensor for Warm Temperature

Phytochrome B (PhyB), in addition to its role in light perception, is recently identified and established as a major temperature sensor. PhyB is a red/far-red light receptor that exists in two alternative forms (Burgie et al. 2014; Rockwell et al. 2006). Pr, the inactive form of PhyB, gets converted into the active form Pfr upon exposure to red light. Reversion of Pfr to the inactive Pr form can occur either by far-red light absorption or by light-independent thermal relaxation process termed as thermal or dark reversion (Mancinelli 1994; Rockwell et al. 2006). Two independent research efforts discovered that phyB participates as a temperature sensor via temperature-dependent reversion of phyB. Legris et al. (2016) showed that increased rates of thermal reversion upon exposing Arabidopsis seedlings to high temperature lowers the quantity of the physiologically active Pfr-Pfr dimer pool of PhyB as well as the size of associated nuclear bodies. Jung et al. (2016) showed that null mutants of phyB show a constitutive warm temperature response and phyB binds to the promoters of key target genes in a temperature-dependent manner. Together, these two studies proved that phyB, known to perceive the ratio of red to far-red light for decades, also functions as a temperature sensor (Jung et al. 2016; Legris et al. 2016).

3.2.3 A Prion-Like Domain in ELF3 Acts as a Thermosensor

The evening complex functions as a temperature-sensitive transcriptional repressor, allowing growth to be rhythmic and temperature-responsive. It consists of a large scaffold protein, EARLY FLOWERING 3 (ELF3); a small alpha-helical protein, ELF4; and a DNA binding protein LUX ARRYTHMO (LUX) (Ezer et al. 2017; H. Huang et al. 2016; Nusinow et al. 2011). ELF3, the key component in temperature perception possesses a polyQ repeat embedded in the prion-like domain (Jung et al. 2020). Jung et al. showed that the length of this polyQ repeat is correlated to temperature sensitivity. Plants, that are inhabitant to tropical hot locations, have no active prion-like domain at high temperatures and lack of thermal responsiveness.

They also show that the temperature sensitivity in plants is controlled by a particular phase transition behavior of ELF3 protein. ELF3 is soluble and active at low temperatures; however, it changes phase into an inactive liquid droplet form at high temperatures. Therefore, this thermosensory process demonstrates the capacity of temperature to rapidly change ELF3 between an active soluble form to an inactive droplet form through a phase transition.

To summarize, thermosensing appears to be a highly dispersed capacity depending on a variety of processes that are just now beginning to be disclosed. The variety of processes involved in temperature sensing further underscores the importance of the need for rapid adjustments of plants to temperature changes.

3.3 Heat Stress Effects on Growth and Development of Plants

Temperature changes have remarkable effects on plant growth and development at both vegetative and reproductive stages including phase transition. An overview of the developmental changes in plants under heat stress has been depicted in Fig. 3.1. Temperature alterations affect growth and development from cellular levels affecting cell division, growth, and maturation to organismal levels reflected in morphological changes. While excessive high temperatures (e.g., heat shock exceeding 40 °C) are damaging to plants, a slight increase in temperature from the optimum temperature affects plant growth and development in two ways: (1) mild increase in temperature enhances the pace of growth of a particular organ, thereby decreasing its growth duration without affecting final plant body; (2) warm temperatures hasten growth and development by influencing plant body to promote evaporative cooling, to increase convection, and to directly prevent heat flux from the sun.

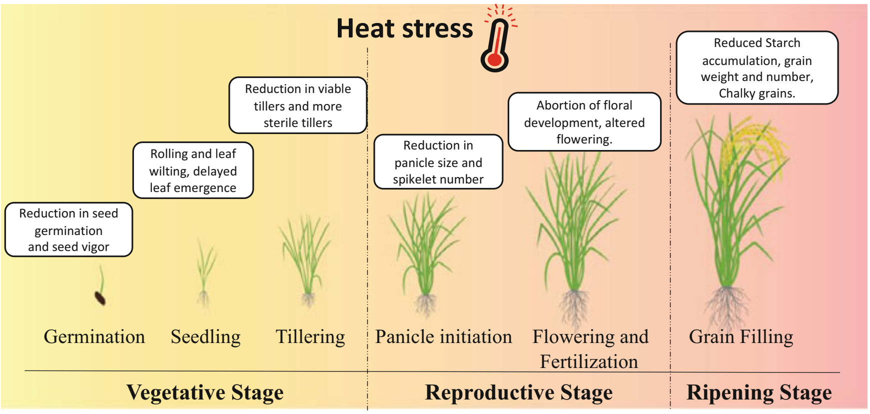

Fig. 3.1 Impacts of heat stress on different growth and developmental stages of crop plants. Heat stress adversely affects almost all the stages of plant development from seed germination and vegetative stage to reproductive and grain filling

3.3.1 Seed Germination

Seed germination is the process by which a seed turns into a seedling that further grows into a plant. Seed germination and seedling establishment are pivotal stages of the plant life cycle. Both these processes are very critical for a good crop stand and thus, it impacts the crop life cycle ultimately affecting the quality of the crop (Ellis 1992). Seed dormancy is among the most critical factors preventing seed germination and impeding crop output in agriculture. It enables seeds to survive at times that are unsuitable for seedling establishment and is hence essential for agriculture and plant ecology (Bentsink and Koornneef 2008). Even when dormant, seeds continue to degrade and are vulnerable to a variety of environmental changes like temperature, oxygen pressure, and moisture content that eventually affect seed viability (Nonogaki 2019). Rice and wheat seeds show decreased longevity when exposed to high temperatures. For example, rice seeds exposed to 40 °C for 40 days and wheat seeds for 37–50 °C for a year show strong negative effects on seed viability (Ellis et al. 1993). The ability to stimulate seed germination at the appropriate time is critical in agronomy and is correlated to the extent of seed dormancy (Benech-Arnold et al. 2013). Water, temperature, and oxygen are basic requirements for a seed to germinate. Among the three, the temperature is an important factor that affects the time and efficiency of seed germination. Albeit heat stress adversely impacts the metabolism of plants at different levels of growth from germination to the mature stage, the extent of stress produced is diverse or variable at different stages of plant growth. Seed germination and reproductive stage of a plant are considered to be more vulnerable to heat stress in comparison to other growth stages (Devasirvatham et al. 2012; Nakagawa et al. 2020). Various reports stipulate that heat stress strikingly damages seed germination of most crops, thereupon catastrophically influencing the overall growth of the crops (Begcy et al. 2018; Bolton et al. 2019; Grass and Burris 1995; Tlig et al. 2008). Delayed germination and loss of vigor are common problems of growing seeds under heat stress, which ultimately results in substandard emergence and seedling establishment (Weaich et al. 1996). Previous investigations have shown a noticeable reduction in seed germination of rice and spinach as a result of heat stress. Reduction in seed germination and seed vigor under heat stress was primarily attributed to reduced thermal stability and fluidity of plasma membranes (Bolton et al. 2019). These proved to be the probable components for delayed activation of kinases, Ca^{2+} signaling, and heat-shock proteins (Begcy et al. 2018).

Delayed seed germination under increased temperature is usually associated with the induction of ABA (Essemine et al. 2010). Very high temperature (45 °C) drastically inhibits the seed germination rate of wheat and resulted in the death of cells and embryo leading to a decline in seedling establishment rate (Cheng et al. 2009a, b).

3.3.2 Seedling Growth

In the early stages following germination, the seedlings are extremely vulnerable to temperature stress. Right after germination, growth is majorly supported by enzymatic degradation of seed reserves. At the seedling stage, the optimal growing temperature for rice is 25–28 °C. Beyond 35 °C, growth slows and seedlings start showing damaging effects of high temperature on growth and physiology (Fahad et al. 2019). These damaging effects include loss of water from seedlings, wilting and yellowing of leaves, impaired root growth, and eventually seedlings even die (Kilasi et al. 2018). Considerable natural variation in heat stress resistance has been observed at the seedling stage in rice, as the extent of resistance varies with genetic background. Seedlings of an *indica* cultivar can survive 48 °C for 79 h, but Nipponbare, a *japonica* cultivar, seedlings are wilted in 72 h at 45 °C (Wei et al. 2013). In another study on two wheat cultivars, a common response to heat stress during germination and early seedling development was found. They showed that an increase of 10°C to 15°C in the optimum temperature prevents the establishment of wheat seedlings. Additionally, heat stress impairs mobilization of reserved material by affecting starch breakdown enzymes and preventing nutrient transport during seedling establishment (Essemine et al. 2010).

Along with the growth, high-temperature stress considerably affects leaf emergence at the seedling stage as well as plant height. Although a moderate increase in temperature accelerates leaf emergence (Fahad et al. 2019), heat stress directly impacts plant meristems and slows down the growth and development of plants by increasing leaf senescence and abscission and decreasing photosynthetic rate (Akter and Islam 2017). Considerable variation has also been observed for heat stress response across different crop species. For example, a study showed that maize is comparatively more susceptible to heat stress in comparison to pearl millet. High temperature causes a substantial loss in shoot dry mass in maize during the vegetative stage, but the dry mass of pearl millet remained nearly unaltered. The negative effect of heat stress on plant height is primarily due to reduced relative growth rate (RGR). The effect of heat stress on the net assimilation rate (NAR) is the prime contributing factor for reducing RGR under high temperatures in crops (Ashraf and Hafeez 2004).

3.3.3 Tillering

Axillary meristems, from the primary stem of rice plants, develop into independent growth units known as "tillers" during the vegetative stage of plant development. The number of tillers grows steadily until plants attain a maximum number of tillers at the time of panicle initiation. Ideally, each tiller has the ability to form a panicle; however, a few tillers may degenerate. Subsequently, their reserves will be reallocated to still surviving tillers for supplemental panicle development (Fageria 2007; Li et al. 2003; Xu et al. 2020a). A positive correlation between the number of tillers and panicles has been observed in both favorable and stressful environments

(Prasanth et al. 2017). A comprehensive comparison of various vegetative traits, such as plant height and tiller number, was performed for wheat under optimal environmental conditions and heat stress. Under heat stress, all varieties performed poorly for all the vegetative traits. Heat stress reduced the number of viable tillers while increasing the number of sterile tillers (Ahamed et al. 2010). Previous reports in rice suggest that both temperature regime and genotype determine the effects of high-temperature stress on panicle and tiller development (Xu et al. 2020a). Exposure of rice to HDT (high day temperature) and HNT (high night temperature) led to fewer tillers and panicles than at the normal temperature range. However, the effect of HDT and HNT varied among genotypes. Interestingly, exposure to HDNT (high day and night temperature) during the formation of tillers and panicle development reported a significant drop in tiller, and thus panicle, counts in both *indica* as well as *japonica* rice, with japonica rice being more susceptible to temperature effects on tillering (Chaturvedi et al. 2017; Cheng et al. 2009b; Mohammed and Tarpley 2009; Soda et al. 2018; Wang et al. 2016a; Zhang et al. 2013). Genotypes that are able to maintain the optimal tiller numbers are observed to have less yield penalty under heat stress. Therefore, tiller number is frequently employed as a morphological marker in the selection of rice cultivars for thermotolerance (Prasanth et al. 2017).

3.3.4 Reproductive Stage and Grain Filling

Although almost every developmental stage and plant tissues are sensitive to heat stress, the reproductive stage and tissues are particularly more susceptible to heat stress as only a few degree rise in temperature during flowering may lead to drastic loss of grain yield (Hatfield et al. 2011). While analyzing the effects of increased temperature on the output of maize grain, a reduction of about 80–90% grain yield was observed in the plants that experienced increased temperature specifically during the reproductive stage than those plants which were under normal temperature (Hatfield and Prueger 2015). Hence, the adverse effects of high temperature could be less on vegetative growth and higher on grain yield. Depending on the species, genotype, and other factors like duration and intensity of heat, temperature stress can cause early or late flowering. The flowering stage is the most sensitive to high temperatures, with pollen viability being extremely susceptible to small increases in temperature (Jagadish et al. 2007). Heat stress for a short period during the reproductive stage can cause considerable reduction in floral buds and abortion of flowers, resulting in no flower or abnormal and sterile flowers resulting in crop sterility. However, the extent of heat stress effects on flowering and reproductive development varies greatly between plant species and different genotypes. The major effects of heat stress at reproductive development leading to crop sterility include impaired meiosis, compromised pollen development and pollen tube growth, less number of pollen on the stigma reduced germination of pollen grains on the stigma and inhibited elongation of pollen tubes (Endo et al. 2009; Xu et al. 2020b). Overall, these heat stress effects adversely hinder the pollination and fertilization process, ultimately lowering the spikelet fertility (Shi et al. 2018).

Grain filling is the final stage of crop growth that encompasses the phase in the crop life cycle from seed setting to physiological maturity and determines the crop yield and productivity. Grain filling involves a complex process of synthesis and transport of protein, carbohydrates, and lipids in grains (Sreenivasulu et al. 2015). Grain filling largely depends upon the duration and rate of grain filling along with the efficiency of underlying metabolic processes. Both the duration of grain filling and metabolic processes are sensitive to temperature fluctuations. Heat stress adversely affects both the quality and quantity of yield by impacting the timing as well as the biochemistry of grain filling. Heat stress during the growth and development of grains improves dry matter accumulation but reduces grain filling time. With every degree increase in temperature than the ambient temperature, the duration of grain development and growth shortens that ultimately reduces grain weight and number of mature grains.

Starch is the major constituent of most of the crop grains. Heat stress limits grain filling by disrupting starch accumulation in seeds, reducing the grain weight by 60–70%. This could be due to the perturbed ratio of amylose to amylopectin under heat stress at the time of grain filling, for example in rice endosperm (Ahmed et al. 2008; Umemoto et al. 1995). This disturbed ratio of amylose to amylopectin under heat stress reflects that key enzymes required for starch biosynthesis are sensitive to temperature and affect grain filling by altering the functions of starch biosynthetic enzymes (Oh et al. 2018). Heat stress at the ripening stage results in round-shaped and loosely packed damaged chalky grains containing aberrant starch granules (Mitsui et al. 2013). A rise in temperature during the grain filling impedes starch synthesis resulting in reduced starch accumulation in grains along with irregular starch granules. Taken together, heat stress considerably lowers crop yield due to impaired grain filling that results due to effects on duration and rate of grain development as well as altered functions of enzymes related to starch biosynthesis. In addition, heat stress effects on synthesis and transport of other biochemical constituents of seeds would also impede the grain filling and crop yield.

3.4 Heat Stress Effects on the Physiology of Plants

The immobile nature of plants limits the range of their behavioral responses and strongly affects plant growth and development. It impairs the function of cells, tissues, and whole plant leading to a huge loss in crop yield (Kumar and Rai 2014). Even minor increases in temperature (1.5 °C) have a notable negative impact on yield (Warland et al. 2006). The heat stress effects on plant growth and development and eventually on crop yield is strongly associated with physiological responses at cellular and organismal level. The response of plants to elevated temperature varies remarkably depending upon the extent and duration of the temperature, and the developmental stage at which heat stress is encountered (Ruelland and Zachowski 2010). Heat stress induces changes in major physiological processes affecting net carbon assimilation including photosynthesis, respiration, transpiration, and photoassimilate partitioning (Fig. 3.2). In addition, it causes injury

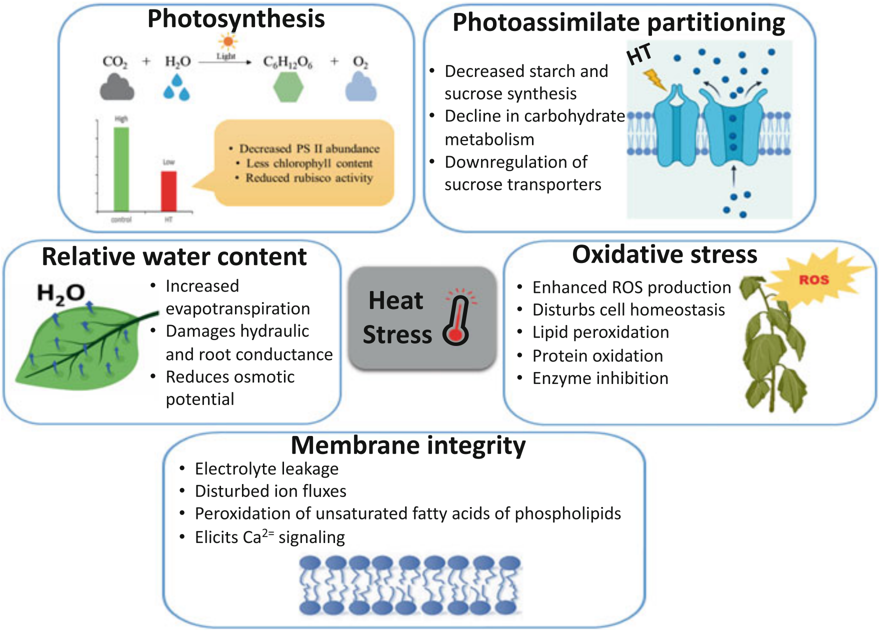

Fig. 3.2 The effects of heat stress on major plant physiological process, including photosynthesis, photoassimilate partitioning, membrane integrity, water relations, and oxidative stress

to membranes due to increased oxidative stress under high temperatures. Plants exposed to high temperatures also experience water-deficient conditions leading to osmotic imbalance. The effect of heat stress on these physiological processes has been discussed in detail in the subsequent sections of this book chapter.

3.4.1 Photosynthesis

Heat stress triggers the changes in physiological effects that are tightly coupled to crop yield and productivity. Of all, photosynthesis is the most heat-sensitive process and it is inhibited before the other physiological functions of the cell get impaired. Photosynthesis is a multifaceted biochemical reaction that converts the sunlight into usable chemical energy, i.e., ATP, and in the dark reactions, CO_2 is fixed into carbohydrates using the chemical energy generated in the light-dependent reactions. It is regarded as the global sensor of environmental stresses, which is evinced by the alterations in various metabolic and redox reactions of the thylakoid membrane (Berry and Bjorkman 1980). The extent to which photosynthetic rate plummets depend on the duration of heat stress exposure to plants. Studies have reported that photosynthesis declines significantly within three hours of heat stress. The detrimental effects on photosynthesis could be completely reversed if temperature returns to normal within six hours of heat stress. However, photosynthesis can be recovered only up to 70% and 45% of normal photosynthesis after twelve and twenty-four hours of heat stress, respectively (Song et al. 2014).

Heat stress remarkably affects the leaf water status, stomatal conductance, and intercellular CO_2 (Greer and Weedon 2012). At elevated temperatures, transpiration rate increases that influences the water use efficiency (Topbjerg et al. 2015), which leads to low leaf water potential resulting in decreased stomatal conductance. Intercellular CO_2 concentration also falls as a consequence of stomatal closure due to heat stress. Therefore, stomatal conductance and intercellular CO_2 are important determinants through which the rate of photosynthesis is affected during heat stress (Farquhar and Sharkey 1982). Heat stress also stimulates excessive production of ROS leading to cellular energy imbalance via a reduction in synthesis of NADPH and ATP (Schrader et al. 2004). Overproduction of ROS destabilizes the thylakoid membrane resulting in loss of chlorophyll, eventually leading to a decline in leaf photosynthetic rate (Krause and Weis 1984; Ristic et al. 2008).

Photosynthetic machinery in a leaf comprises photosynthetic pigments, electron transport chain, and components of light-independent reactions. The photosynthetic efficiency of plants declines as a number of components of photosynthetic machinery is affected due to heat shock. Among all, Photosystem II (PSII) is one of the key targets which has been elucidated as thermally labile (Berry and Bjorkman 1980). The biosynthesis of chlorophyll is also impaired beyond the optimum temperature range, attributing to the reduction in photosynthesis (Dutta et al. 2009). Another major target of heat stress is the Rubisco, the rate-limiting enzyme CO_2-fixing enzyme, whose enzymatic activity decreases gradually as temperature rises (Weis 1981). The effects of heat stress on these photosynthetic components have been discussed in the following section.

3.4.1.1 Photosystem

Photosystems are the functional and structural units of protein complexes that harness and transfer light energy and electrons. Of the two photosystems, PSII is more thermosensitive than PSI. Two primary reasons account for the heat-sensitive nature of the PSII. First, the heat-labile nature of the thylakoid membrane, due to which PSII lodges off from the thylakoid membrane. Second, the integrity of PSII is dependent on the dynamics of the electron transport chain. Hence, any metabolic process impaired owing to heat stress, that either donates or accepts the electron from PSII, would lead to dislodging of the PSII from the membrane (Prasad et al. 2008). Physical separation of light-harvesting pigments from the PSII complex as well as disruption of the oxygen-evolving complex along with the functional manganese ion takes place under heat stress (Havaux and Tardy 1996). Studies suggest that these denaturation events alter the interaction between lipids and proteins in the thylakoid membrane, leading to increased fluidity, and hence disintegration of the molecular organization of the PSII (Berry and Bjorkman 1980). Photosynthetic electron transport chain and ATP synthesis are markedly affected if PSII suffers acute disruptions due to high temperature (Wang et al. 2018). Ferreira et al. (2004) showed the increase in photosynthetic linear (or cyclic) electron flow due to decreased abundance of PSII. It was also suggested that enhanced linear electron flow may act against thylakoid membrane leakiness and also offer protection against irreversible damage (Tozzi et al. 2013).

3.4.1.2 Chlorophyll

Chlorophyll is the main photosynthetic pigment that harvests light energy and drives the electron transfer during photosynthesis. Plants under normal conditions maintain an equilibrium between chlorophyll biosynthesis and its degradation, which gets perturbed due to environmental changes, including heat stress. High temperatures lower chlorophyll levels due to reduced chlorophyll synthesis or enhanced degradation or a combination of both. Downregulation of the gene expression and protein abundance of enzymes involved in the tetrapyrrole metabolism leads to impaired chlorophyll biosynthesis (Dutta et al. 2009). Additionally, the activity of chlorophyllases and chlorophyll degrading peroxidases accelerates drastically (Wang et al. 2018), resulting in significantly decreased levels of chlorophyll. It is crucial to maintain the balance between chlorophyll biosynthesis and degradation as it profoundly impacts the photosynthetic efficiency, ultimately affecting crop development and yield (Hu et al. 2020).

3.4.1.3 Rubisco

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a key enzyme that catalyzes the first reaction of CO_2 assimilation, that is carboxylation of 5-carbon sugar ribulose-1,5-bisphosphate (RuBP). It is incapable of discriminating between CO_2 and O_2, subjecting to competitive inhibition by O_2. Under heat stress, the affinity of Rubisco decreases for CO_2 and catalyzes oxygenase reaction, resulting in photorespiration and reduction in the efficiency of photosynthesis. This process consumes ATP, releases fixed CO_2, and produces photorespiratory products (glyoxylate), that further utilize NADH2 and eventually reduce the photosynthetic efficiency and crop yield (Walker et al. 2016). Moreover, Rubisco activation requires another enzyme, Rubisco activase, that rapidly forms carbamate in the Rubisco active site (Portis 2003). It removes sugar-phosphate inhibitors, namely XuBP (D-xylulose-1,5-bisphosphate), RuBP (Ribulose-1,5-bisphosphate), CA1P (2-Carboxy-D-arabinitol-1-phosphate), and CTBP (2-Carboxytetritol-1,4-bisphosphate) from the active site and catalyzes the carboxylation reaction. It also prevents the aggregation of the nascent protein, but it is heat-labile. The activity of Rubisco activase is inhibited as temperature rises above the optimal range, thereby leading to thermal inactivation of the Rubisco (Sage et al. 2008). Thus Rubisco activase is a limiting factor for efficient photosynthesis under heat stress (Wang et al. 2018).

3.4.2 Photoassimilate Partitioning

Photoassimilate partitioning is one of the crucial determinants that significantly affect the overall crop performance, as it regulates the distribution of photosynthates from source tissues to different sink tissues. The central role of photosynthesis is to provide energy to the plants, not only to the photosynthetic tissues but also to the non-photosynthetic tissues. Carbohydrates assimilated by photosynthesis are translocated from photosynthetically active “source” to non-photosynthetic “sink” tissues through phloem via SWEETs and SUTs sucrose transporters. Phloem translocation is an imperative process that critically affects both vegetative and reproductive development, and ultimately crop yield (Moore et al. 2021). Sucrose is the primary sugar that forms the building blocks of storage carbohydrates and an energy source for the production and maintenance of biomass (Osorio et al. 2014). Plants undergo a substantial number of changes in sucrose metabolism and transport in both source and sink tissues, along with the extent of competition among numerous sinks for a common pool of sugars, during its lifetime. Alterations in sucrose transport and metabolism affect the source-sink dynamics that strongly influence the biomass accumulation and grain yield of the crop (Mathan et al. 2021). Partitioning of photosynthates strongly depends on the source and sink strength. The metabolic status of the source tissue is primarily determined by the activity of major carbohydrate metabolic enzymes, such as ADP-glucose pyrophosphorylase (AGPase), sucrose phosphate synthase (SPS), sucrose phosphate phosphatase (SPP), and sucrose synthase (SUS) (Osorio et al. 2014; Ruan 2014), while the strength of sink is dictated by its ability to drive the photosynthates towards storage and maintenance (Smith et al. 2018; White et al. 2016).

Under heat stress, the dynamics of source and sink tissues are highly affected due to considerable alterations in the carbon allocation and partitioning patterns. At high temperatures, the synthesis of starch and sucrose is adversely affected. The activity of key enzymes involved in carbohydrate metabolism also declines (Yang et al. 2018). Decreased transport of sucrose has been reported under heat stress due to blocking of sucrose transporters (Ribeiro et al. 2014; Zhang et al. 2018). This decreased transport led to reduced root: shoot biomass under heat stress, which suggests that the level of carbon translocation is alleviated at high temperatures. Microarray analysis showed downregulation of 19 out of 22 sugar transporters when plants were exposed to heat stress (Qin et al. 2008). Suwa et al. (2010) showed a reduced export rate of ^{13}C under heat stress, which suggests constrained sucrose loading into the phloem. Further, heat stress altered the amount of starch in the mesophyll cells of tomato leaves. Together, these studies suggest that the import and export of sugars in phloem via transporters decreases under prolonged exposure to heat stress, which eventually leads to the accumulation of starch (Julius et al. 2017).

At the structural level, altered protein conformations and callose depositions in form of collars around the plasmodesmata and sieve pores block the phloem due to heat stress in broad bean, ultimately impeding the phloem translocation (Furch et al. 2007). Callose deposition in response to heat stress was also observed in rice leaf and sheath plasmodesmata of heat-sensitive mutants that showed reduced carbon translocation, which could possibly be due to blocking of phloem loading and unloading (Zhang et al. 2018). The underlying mechanism of callose depositions is still not clear and needs to be investigated further. Moreover, anatomical features of phloem, such as the number and cross-sectional area of phloem cell, strongly influence photosynthetic capacity (Muller et al. 2014). Stewart et al. (2016) demonstrated a decreased number of phloem cells and cross-sectional area in an Arabidopsis ecotype at high temperature as compared to the growth at optimal temperature.

At high temperatures, the photosynthetic efficiency of plants decreases, while respiration rate and photorespiration increase that can affect reproductive development. Under heat stress, grain filling is critically affected in crops, such as wheat, rice, and maize (Hurkman et al. 2003; Yamakawa and Hakata 2010; Huan Yang et al. 2018). Due to reduced photosynthesis, stem reserves are utilized as a carbon source for grain filling during the pre-anthesis stage. Altered source-sink dynamics also affect seed set and seed filling (Akter and Islam 2017). Additionally, reduced levels of non-structural carbohydrates have been observed in many crop species under short-term heat stress. Under long-term heat stress, non-structural carbohydrates accumulate and negatively impact root:shoot biomass and carbon export rates (Moore et al. 2021). Conclusively, all these studies suggest that heat stress impairs the process of carbon partitioning, primarily towards the reproductive sinks. Therefore, it is imperative to ameliorate these negative effects of heat stress on source-sink dynamics to improve the yield of crop cultivars.

3.4.3 Membrane Integrity

Cell membranes are flexible bilayer structures typically made up of lipids and proteins. This dynamic structure surrounds the cell and regulates the essential biophysical and biochemical activities by regulating the movement of ions in and out of the cell. It acts as an interface for the flow of material between the cells and the environment (Krishnan et al. 2011). Therefore, membranes are the first structure to be associated with the perception and further downstream signaling of the external signals (Krishnan et al. 2011). Heat stress is one of the major factors known to cause rapid damage to membrane integrity. Elevated temperature affects the organization of microtubules, cell elongation, expansion, and differentiation (Djanaguiraman et al. 2018). This leads to an increase in the kinetic energy of the hydrogen bonds between adjacent fatty acids, thereby reducing the strength of the bonds causing increased fluidity of phospholipid bilayer at temperatures above the optimal level (Bita and Gerats 2013; Niu and Xiang 2018). The increased membrane fluidity results in electrolyte leakage, disturbed ion flux, changes in relative water content, and disruption of homeostasis that reduces cell viability.

Increased fluidity activates the calcium channels, which elicits an influx of Ca^{2+} ions into the cell. More than 40 calcium channels are encoded by the *Arabidopsis thaliana* genome and most of them are known to be localized in plasma membranes (Mathur and Jajoo 2014). Ca^{2+} ions are considered a notable channel responsible for sensing heat (Mathur and Jajoo 2014). Apart from calcium and lipid signaling, elevated temperature triggers the conformational changes in protein structure, leading to protein unfolding. It has been suggested that most of the heat sensing occurs through protein unfolding (Hemantaranjan 2014). Yamada et al. (2007) indicated that protein unfolding induced by heat could stimulate some heat stress transcription factors (HSFs). In eukaryotes, HSFs play a central role in transcriptional dynamics induced by heat stress (von Koskull-Döring et al. 2007).

Functional cell membrane plays a central role in sustaining physiological processes, including photosynthesis, respiration, and transpiration, that are related to synthesis and translocating the carbohydrates into the grain (Khan et al. 2021). Therefore, membrane integrity has a direct impact on the yield and productivity of the crops. Alterations in the composition and structure of membranes play a critical role in the adaptation of the plants to heat stress tolerance. Measurement of the level of electrolyte leakage and degree of peroxidation of unsaturated fatty acids in phospholipids is regarded as a reliable indicator of assessing the thermostability of membranes. Cell membrane thermostability is often used as an important criterion while selecting heat stress-tolerant genotypes (J. Z. Wang et al. 2009). It has been suggested that cell membrane thermostability should be implemented as a significant criterion, with some cautions, in crop breeding programs aiming to enhance the heat tolerance of crops (Blum et al. 2001).

3.4.4 Oxidative Stress

One of the major consequences of heat stress is oxidative stress, which is a common phenomenon in most abiotic stresses (Apel and Hirt 2004). Heat stress uncouples enzymes and the metabolic pathways, which elicits the production of unwanted and deleterious by-products, namely ROS (Reactive oxygen species). ROS has dual roles in plants. It acts as a signal transduction molecule as well as a toxic compound when produced excessively (Zhou et al. 2019). Therefore, disturbance in the production and scavenging of ROS leads to detrimental effects on plant health. A total reduction of oxygen leads to the formation of water, while partial reductions of oxygen result in the development of ROS, such as superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH\bullet$). The primary sites of ROS production are chloroplast, mitochondria, and peroxisomes. In chloroplast, reaction centers of PSI and PSII are the principal sites for ROS production. The maximal efficiency of PSII is linearly correlated with ROS accumulation (Hasanuzzaman et al. 2013). Additionally, auto-oxidation of ubi-semiquinone complex I and complex III leads to the production of superoxide radicals (Khan et al. 2020). Diverse kinds of ROS are produced by different reactions during different cellular oxygen-consuming redox processes. Superoxide radical ions ($O_2\bullet^-$) are produced via photooxidative reactions, Mehler reaction in the chloroplast, as well as during mitochondrial electron transport chain and glyoxysomal photorespiration, and through NADPH oxidase in the plasma membrane. Hydroxyl ion (OH^-) is generated by the reaction of H_2O_2 with $O_2\bullet^-$ (Haber–Weiss reaction), the reaction of H_2O_2 with Fe^{2+} (Fenton reaction), and the breakdown of O_3 in the apoplastic region (Møller et al. 2007). Photoinhibition of PSII produces singlet oxygen (Huang and Xu 2008).

Oxidative stress intensifies the production of these toxic molecules and perturbs cell homeostasis. It vitiates protein, nucleic acid, lipids, and carbohydrates, eventually increasing membrane peroxidation and decreasing membrane thermostability

(Gill and Tuteja 2010). ROS accumulation causes lipid peroxidation, protein oxidation, and enzyme inhibition (Sharma et al. 2012). It has been reported that even the short heat pulses lead to oxidative burst of O_2^- or H_2O_2 (Vallelian-Bindschedler et al. 1998). Continuous heat stress leads to accumulation of ROS in the plasma membrane, which results in depolarization of cell membrane, activation of NADPH oxidases (also known as respiratory burst oxidase homologous D, RbohD enzyme), and stimulates programmed cell death (Mittler et al. 2011). As a consequence of elevated temperature, the activity of antioxidants decreases and the amount of malondialdehyde (MDA) increases, a product of peroxidation of unsaturated fatty acids, which is regarded as an indicator of oxidative stress (Møller et al. 2007).

Plants have defensive mechanisms to counter the effects of these toxicants. The antioxidant system encompasses enzymatic and non-enzymatic detoxification mechanisms. The enzymatic system is usually the most effective (Farooq et al. 2008), which includes superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), glutathione reductase (GR), and ascorbate peroxidase (APX) while carotenoids, glutathione, and ascorbate form the non-enzymatic component of the antioxidant system (Hasanuzzaman et al. 2013; Sharma et al. 2012). Many studies have revealed that enhanced antioxidant activity of crop plants is strongly correlated with heat tolerance. Therefore, understanding the regulatory mechanism underlying antioxidants expression would be an imperative measure to ameliorate redox state as well as heat tolerance in crop plants.

3.4.5 Water Relation

Under heat stress, the water status of the plant is found to be the most fluctuant parameter and it has a direct impact on the growth, development, and physiology of plants. Temperatures above the optimal growth temperature lead to dehydration that deteriorates the plant's health (Akter and Islam 2017). The increasing temperature severely affects a number of physiological processes due to perturbance in relative water content. Relative water content is regarded as a cue for water status in a cell and is also correlated with biotic and abiotic stress, including heat stress (Sattar et al. 2020). A decrease in relative water content is a result of increased transpiration and osmotic potential (Haworth et al. 2018). Transpiration is the process by which plants lose water in the form of vapor for maintaining the optimal temperature of the plant, which is controlled by the regulation of stomatal conductance. At high temperatures, the rate of transpiration increases leading to low water potential that results in reducing the stomatal conductance (Mathur et al. 2014). As stomatal conductance also regulates the passage for CO_2, therefore it is strongly correlated with photosynthetic efficiency and crop yield (Hasanuzzaman et al. 2013). Heat stress also damages hydraulic and root conductance, which becomes more lethal to plants owing to increased transpiration (Sattar et al. 2020).

Heat stress causes evapotranspiration resulting in drought stress (Lamaoui et al. 2018), which might severely affect the crop yield due to the combined effects of heat and drought stress. As the amount of water in soil plummets, the level of ABA shoots up in leaf that triggers the closure of stomata, reducing stomatal conductance and water loss by transpiration (Haworth et al. 2018). Sattar et al. (2020) reported that combined effects of heat and drought stress are more fatal than individual stresses, and the synergistic effect of both are hypo-additive in nature. Altogether, the alterations in the plant physiological processes, including gas exchange traits and water relation status, under heat stress could be detrimental to the yield and growth of the crop plants.

3.5 Hormonal Changes Under Heat Stress

The dynamic and complementary actions of the major phytohormones regulate a plethora of growth, developmental and physiological processes. The crosstalk ability of these phytohormones and their dose-dependent response make them a suitable candidate to act as moderators for environmental stress responses (Sreenivasulu et al. 2012). Studies discussed below have shown that exogenous application of phytohormones remarkably mitigates heat-stress-induced damages and boosts up plant heat stress tolerance. This clearly indicates the involvement of phytohormones in plants' response to heat stress and heat stress tolerance mechanism (Fig. 3.3). Quantification of the endogenous level of phytohormones following heat stress is performed in a number of studies that are confined to the investigation of biochemical and physiological parameters, along with the assessment of various physiological growth metrics such as photosynthesis, respiration, biomass production, etc. Hormonal studies in response to heat stress are frequently accompanied by the application of exogenous hormones, primarily on the foliar part. Another approach to decipher the complex hormone signaling pathway under heat stress is to perform the functional analysis by generating mutants and overexpression plants for the candidate genes involved in the hormone biosynthesis and signaling. All the major phytohormones viz. abscisic acid (ABA), auxin, cytokinin (CTK), gibberellic acids

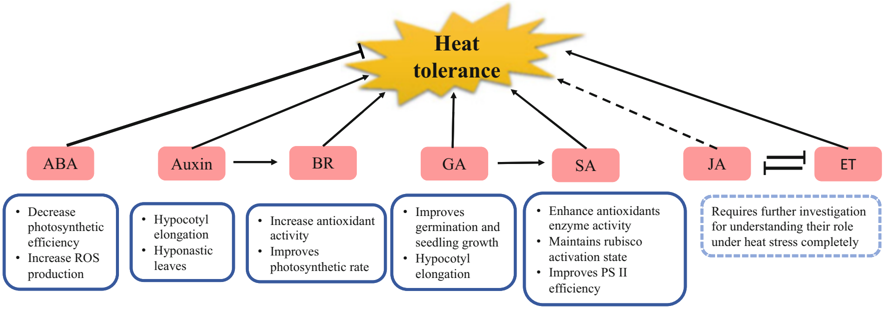

Fig. 3.3 Graphical representation of different phytohormones heat-stress-induced developmental and physiological changes in plants. The detailed mechanistic understanding of involvement of phytohormones in heat stress effects would be instrumental in developing heat-tolerant crop plants via optimizing hormone biosynthesis and/or signaling

(GAs), jasmonic acid (JA), brassinosteroids (BRs), ethylene, and salicylic acid (SA) have been shown to be involved in plants' response to heat stress (Li et al. 2021).

3.5.1 Abscisic Acid (ABA)

Abscisic acid, also known as the stress hormone, regulates the physiology, growth, and development of plants to acclimatize for abiotic stress conditions (Sah et al. 2016). ABA levels are raised in plants under various environmental stresses including high temperatures (Suzuki et al. 2016). When ambient temperatures surpass specific threshold levels for a particular plant species, oxidative stress and membrane damage are caused that bring down the plant photosynthetic and transpiration efficiencies (Bita and Gerats 2013; Hasanuzzaman et al. 2013). Endogenous high ABA levels under heat stress raise ROS levels, which ameliorate antioxidant capacity and provide heat tolerance to the plants. In *Pisum sativum*, ABA levels are increased transiently and rapidly in just 10 min of heat stress indicating that ABA might also be involved in heat perception and adaptation (Liu et al. 2006). Studies have shown that ABA is biosynthetic and signaling mutants show impaired heat tolerance due to less accumulation of ROS, thus resulting in increased sensitivity to heat (Larkindale et al. 2005; Larkindale and Knight 2002). Hence, ABA deficient mutants show extreme sensitivity to heat-induced damage (Wang et al. 2014; Wu et al. 2017). An ABA deficient mutant genotype in tomato, *notabilis* (*not*), is susceptible to heat stress as indicated by lower photochemical efficiency (Fv/Fm) and enhanced lipid peroxidation when compared to wild-type tomato genotype (Li et al. 2015). Exogenous application of ABA promotes heat stress tolerance by counteracting the negative effects of heat stress on growth and physiological parameters. For example, ABA when applied externally reduces pollen sterility caused by heat stress and thus impeding the negative effects of heat stress on reproductive development (Rezaul et al. 2019). Interestingly, ABA regulates the carbohydrates level by accelerating sucrose transport and metabolism to preserve carbon balance and energy homeostasis to strengthen thermal tolerance. This could be accomplished through a number of means, i.e., increased content of non-structural carbohydrates, soluble sugar, and starch under heat stress on exogenous supply of ABA as well as significantly higher expression of genes related to sugar metabolism and transport, such as sucrose transporters (*SUT*), sucrose synthase (*SUS*), and invertase (*INV*) genes in spikelet contribute to ABA regulation of carbohydrate levels under heat stress (Rezaul et al. 2019).

3.5.2 Auxin

Auxin is one of the most important phytohormones that control various aspects of the plant's development and physiology. Previous studies have also shown that auxin is involved in regulating the plant response to environmental stress. Auxin plays a significant role in thermomorphogenesis that involves hypocotyl elongation and leaf hyponasty (Küpers et al. 2020). To cope with the high temperature, hypocotyl elongates to distance their meristematic as well as photosynthetic tissue away from the heat-absorbing soil to enable the plants to take better advantage of the cooling effect of moving air (Gray et al. 1998). The auxin mutants or transgenic plants with lower levels of auxin biosynthesis show a restrained temperature-induced hypocotyl elongation (Gray et al. 1998). However, it has also been suggested that higher temperature induces auxin production, which in turn stimulates the biosynthesis of brassinosteroids that ultimately regulates the high-temperature-mediated hypocotyl elongation (Maharjan and Choe 2011). Apart from this, Phytochrome-Interacting Factor 4 (PIF4) is regarded as the key molecular player integrating environmental cues, such as temperature stimuli, and endogenous cues, such as auxin, to manifest the developmental changes under high ambient temperature (Ahammed et al. 2016). Recently, Wang et al. (2016b) also evinced that HSP90 plays a central role in auxin-mediated growth responses in *Arabidopsis* under heat stress.

All plant parts in *Arabidopsis* are capable of producing auxin, and the regulatory functions of auxin are controlled by its polar transport. Under high ambient temperature, cotyledons show high levels of transcripts of auxin biosynthetic genes, *YUCCA8* and *YUCCA9*, as compared to hypocotyl (Ahammed et al. 2016). This suggests that cotyledons act as a source of auxin, which is then transported to hypocotyl, resulting in hypocotyl elongation. Additionally, when auxin transport was inhibited blocking the polar auxin transport, the temperature-induced hypocotyl elongation was not observed (Stavang et al. 2009; de Wit et al. 2014). These findings suggest that auxin synthesis, transport, and signaling pathways are involved in high-temperature-induced hypocotyl elongation.

In addition to hypocotyl elongation, *Arabidopsis* plants grown at high temperatures show hyponastic leaves with elongated petiole and small lamina as an auxin-mediated response. High-temperature grown Arabidopsis seedlings also show decreased number of stomata, but they acquire better cooling capacity. This is probably due to compact shoot structure and hyponastic leaves that likely improve transpiration (Ahammed et al. 2016). Moreover, exposure to high temperature inhibits DNA proliferation in the chloroplast, mitochondria, and nuclei of developing panicles in barley, likely due to a remarkably lower amount of auxin in developing panicles (Oshino et al. 2011). A lower level of auxin was also detected in the developing anthers of Arabidopsis and barley, along with decreased expression of auxin biosynthetic gene, which causes male sterility. The exogenous application of auxin reverses back the temperature-induced male sterility (Sakata et al. 2010). These findings clearly indicate that the reduced level of auxin is a major cause of the temperature-induced negative effects on plant growth and development.

3.5.3 Gibberellin

Gibberellins (GAs) belong to the class of diterpenoids and are known to be involved in a number of physiological processes during plant development that includes seed germination, starch metabolism, cell elongation, flowering induction, and fruit development (Sun and Gubler 2004). The role of GA in abiotic stress is becoming growingly evident, and it has also been implicated in plants' response to high temperatures (Ahammed et al. 2016). Exogenous treatment of GA_3 relieved the heat-stress-induced inhibition of germination and seedling growth (Alonso-Ramírez et al. 2009). In addition, overexpression GASA (Gibberellic Acid Stimulated in Arabidopsis) gene family from *Fagus sylvatica* improved thermotolerance in *Arabidopsis*. This enhanced thermotolerance was associated with the upregulation of ISOCHORISMATE SYNTHASE1 and NONEXPRESSOR OF PATHOGENESIS RELATED GENES 1 (NPR1) along with an enhanced accumulation of salicylic acid (Alonso-Ramírez et al. 2009). This suggests that GA improves the seed germination and seedling growth caused by heat stress by regulating the biosynthesis and signaling of salicylic acid (Alonso-Ramírez et al. 2009).

The hypocotyl elongation response was absent in *ga-1* mutants, which were deficient in GA biosynthesis (Stavang et al. 2009). Moreover, the expression of GA biosynthesis gene *GA20ox1* and *GA3ox1* elevated in hypocotyl when seedlings were exposed to high temperatures. A subsequent decrease in expression of GA inactivating enzyme was also observed in seedlings under heat stress (Stavang et al. 2009). This suggests that enhanced accumulation of GA in hypocotyl affects the process of thermomorphogenesis at high temperatures. GA affects thermomorphogenesis by negatively regulating the stability of DELLA proteins (Casal and Balasubramanian 2019). GA stimulates the activity of PIF4, the central regulator of thermomorphogenesis, post-translationally at elevated temperatures (Stavang et al. 2009). Studies have also revealed direct molecular links between auxin, GA, PIF4, and growth elongation response suggesting that PIF4 acts as a node integrating environmental and endogenous cues. Taken together, GA, in addition to auxin, plays a significant role in temperature-induced plant developmental changes.

3.5.4 Cytokinin

Cytokinins are major phytohormones controlling various aspects of growth and development. The significance of cytokinins in different developmental stages has been well established. However, our understanding of its impact on plant stress tolerance mechanism remains fragmentary. Studies have shown that temperature affects the cytokinin response and that cytokinin levels contribute to plant adaptive mechanisms to high-temperature stress (Dobrá et al. 2015; Skalák et al. 2016).

As discussed earlier, heat stress affects the reproductive stage of crops limiting grain yield. It has been shown that heat stress aborts flower development during the pre-anthesis stage in many diverse species. In passion fruit (*Passiflora edulis Sims*), high temperature during summers aborts the floral primordia. However, passion fruit flowers showed enhanced resistance towards heat stress on exogenous application of cytokinin at a specific stage of flower development (Sobol et al. 2014). Further, Arabidopsis plants with manipulated cytokinin content using a transgenic approach underpinned a defensive role for cytokinin on reproductive development under heat stress (Sobol et al. 2014). Exogenous application of cytokinin to wild-type plants supplemented the role of cytokinin in ameliorating flower developmental defects under heat stress. Consistent with this, a positive correlation between cytokinin levels in floral meristems and the number of spikelets in a panicle was observed in rice. Increased degradation of cytokinin in rice floral meristems results in a reduced number of spikelets (Ashikari et al. 2005), while induction in cytokinin biosynthesis leads to more number of spikelets per panicle (Ding et al. 2014). Thus, heat stress alters cytokinin levels that affect the development of branches and florets resulting in a reduced number and size of spikelets per panicle.

3.5.5 Salicylic Acid

Salicylic acid (SA), a phenolic compound, is now recognized as a phytohormone as it performs numerous physiological and metabolic functions that directly influence the growth and development of plants. Many studies have reported salicylic acid as a central signaling molecule that induces plant tolerance to various biotic and abiotic stresses (Klessig et al. 2018). The role of SA has been well established in basal thermotolerance in a variety of crop plants including potato, tomato, bean, mustard, and *Arabidopsis thaliana* (Horváth et al. 2007; Wang et al. 2010). Arabidopsis SA signaling *npr-1* mutant showed reduced thermotolerance, whereas Arabidopsis transgenics with elevated SA levels showed higher thermotolerance, suggesting the key importance of SA in heat stress signaling and tolerance (Ahammed et al. 2015).

Plants treated with SA prior to heat exposure showed improved plant growth and development, including plant height, biomass, and photosynthetic capacity (Wassie et al. 2020). SA maintains a higher Rubisco activation state and increases the efficiency of PSII to alleviate the heat-induced photosynthesis inhibition (Wang et al. 2010). Apart from this, SA also triggers the activity of antioxidant enzymes, including catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX), and superoxide dismutase (SOD), at higher temperatures (Khanna et al. 2016). This enhances the ROS scavenging mechanism and thereby protects photosynthetic machinery enabling the plants to survive in stressful conditions (Janda et al. 2020). Additionally, levels of chloroplast HSP21 were found to be higher in SA-treated leaves as compared to the control leaves during the recovery period. This suggests that SA not only improves photosynthetic efficiency under heat stress but also recovers photosynthesis after the stress period (Li et al. 2021).

SA regulates the levels of proline, which acts as an antioxidant, under abiotic stress via inducing the activity of proline biosynthetic enzymes and inhibiting proline metabolizing enzymes (Lv et al. 2011). Exogenous application of SA elevates the proline content and improves the osmotic potential that directly impacts the water uptake, thereby affecting stomatal aperture along with photosynthesis (Jahan et al. 2019). Thus, SA-induced proline synthesis enhances thermotolerance in plants. Hence, SA plays a major role in alleviating the heat-induced damage to plants and could be considered as a potential target that can be manipulated to improve crop yield under high stress.

3.5.6 Brassinosteroids

Brassinosteroids (BR) are polyhydroxy steroid plant hormones that regulate plant growth and development like cell growth, cell division, gene expression, nucleic acid, and protein biosynthesis (Vriet et al. 2013). BR regulates the physiology of plants like seed germination, photosynthesis, source/sink relationships, flowering, spikelet differentiation, senescence, etc. (Zhang et al. 2019; Zhao et al. 2013). Thus, BR influences tolerance to various abiotic and biotic stresses including salt stress, drought stress, heat stress, cold stress, heavy metal toxicity, and various pathogenic stresses (Anwar et al. 2018). It has been shown that BR biosynthetic and signaling mutants are sensitive to heat stress compared to wild-type plants in Arabidopsis (Setsungnern et al. 2020). Thus, refining BR action and signaling could be used to enhance plant tolerance and adaptation to heat stress and to maintain yield. Heat stress adversely affects the growth and development of crops; however, studies have shown that the exogenous application of BR partially mitigates the harmful effects of high temperatures. Foliar application of BR to *Leymus chinensis* induced antioxidant activity and reduced malondialdehyde activity resulting in improved thermal tolerance. The exogenous application of BR also boosted the growth and development of *L. chinensis* by increasing the biosynthesis of photosynthetic pigments, and levels of osmolytes and antioxidants under high temperatures (Niu et al. 2016). Likewise, BR improved plant performance of banana, *Ficus concinna*, *Brassica* sp., and Arabidopsis under heat stress by improving physiological functions and antioxidant defense systems (Hassan Nassar 2004; Kagale et al. 2006; Jin et al. 2015). Application of BR mimics at the reproductive stage of rice ameliorates the impact of heat stress by enhancing the net photosynthetic rate, transpiration rate, stomatal conductance, and carbohydrate content in the straw and seed under normal and high-temperature conditions (Sonjaroon et al. 2018). Recently, it has been shown that BRs regulate the impact of heat stress on pistil fertilization ability during anthesis and attenuate the deleterious effects of heat stress on pistil fertility in photo-thermosensitive genetic male-sterile (PTSGMS) rice (*Oryza sativa* L.) lines (Chen et al. 2021). Studies on tomato seedlings with modified BR homeostasis for seedling survival, lipid peroxidation, and ion leakage showed that the heat tolerance mechanism is independent of BR homeostasis in tomatoes; however, heat stress-mediated oxidative response is dependent on BR levels (Mazorra et al. 2011). Overall, BRs are potential regulators to safeguard plants from heat stress injuries.

3.5.7 Jasmonic Acid

Jasmonic Acid (JA) belongs to a group of oxylipin compounds that plays a significant role in responses to various developmental and environmental cues. Recent studies by Clarke et al. (2009) established the involvement of JA in basal thermotolerance. Several jasmonates, including 12-oxophytodienoic acid (OPDA), Methyl Jasmonate (MeJa), JA, and Jasmonoyl-isoleucine (JA-Ile), accumulate in *Arabidopsis* on exposure to heat stress. Moreover, exogenous application of MeJa maintained cell viability in plants under heat stress, which was evident through the electrolyte leakage test (Sharma and Laxmi 2016). Functional studies of Arabidopsis JA-related mutants confirmed the role of JA in conferring thermotolerance. *Arabidopsis cpr-5* (constitutive expressor of PR1) mutant, having constitutively active JA, SA, and ethylene signaling pathways, show higher thermotolerance. Crossing of *cpr5* mutant with mutants that are deficient in either JA biosynthesis or JA signaling led to compromised thermotolerance ability of plants (Clarke et al. 2009).

Studies have shown that JA controls the response of plants to heat stress via a subset of JA-inducible transcription factors of the WRKY superfamily (Li et al. 2010; Dang et al. 2013). WRKY40 is involved in the regulation of heat stress in the *Capsicum annum*, with the induction of *WRKY40* expression on exogenous JA application (Dang et al. 2013). This suggests that JA regulates the expression of *WRKY40* that further controls the downstream thermotolerance-related genes. However, the expression of genes encoding heat-shock proteins, key molecular players of thermotolerance, was not induced by exogenous JA treatment or in transgenic lines with higher levels of JA (Li et al. 2021). Though the role of JA in inducing basal thermotolerance in plants has been observed, the underlying molecular mechanism needs to be investigated in detail to optimize the heat-stress tolerance via JA.

3.5.8 Ethylene

Ethylene (ET) is a gaseous hormone and a pivotal regulator of many abiotic and biotic stress signaling. Variation in ET levels across a range of concentrations in plants is accompanied by changes in plant growth and developmental behavior (Khan et al. 2020; Thao et al. 2015). ET has been recognized as a negative regulator of heat stress in Arabidopsis (Li et al. 2021). ET insensitive mutant in Arabidopsis, *ethylene insensitive 2-1* (*ein2-1*), which is deficient in primary regulation of ET signaling, exhibited increased thermotolerance (Clarke et al. 2009). In addition, Munné-Bosch et al. (2004) demonstrated that air-borne ET decreases thermotolerance in *Quercus ilex* at high temperatures by impeding antioxidant defense mechanisms. However, enhanced synthesis of ET in plants at higher temperatures leads to reduced grain yield, indicating the concentration-dependent function of ET (Kaur et al. 2021). It has also been reported that elevated levels of ET results in yield penalty by negative effects on the reproductive features, including grain weight and spikelet fertility, and by speeding up the senescence. At higher temperatures, ET is produced mainly in the reproductive tissues, such as pedicel, floral, and fruit tissues (Savada et al. 2017).

In contrast, some studies have also reported that ET is involved in alleviating the heat-stress-induced harmful effects. Wu and Yang (2019) reported that ET mediates basal thermotolerance in rice. The extent of cell membrane oxidation and electrolyte leakage was reduced in rice seedlings when treated with ET. In addition, higher expression of heat-shock factors and ET signaling-related genes was also observed after ET treatment of rice seedlings (Wu and Yang 2019). Exogenous application of ET stimulated abundant stress-related proteins which conferred enhanced thermotolerance by maintaining the cellular redox state (Jegadeesan et al. 2018). The expression of ethylene response factor (ERF), the central player in ET signaling and response pathway, increases when plants were exposed to temperatures above optima. It has been suggested that ERFs bind to dehydration-response elements (DRE), which induces the activation of downstream stress-responsive genes that regulates the thermotolerance in plants (Müller and Munné-Bosch 2015). Thus, the physiological, biochemical, and molecular roles of ET vary depending upon concentrations, in different tissues of a plant, and also in different species. Nonetheless, ET could be instrumental in mediating thermotolerance in plants that needs further detailed studies.

Phytohormones act as the endogenous messengers that regulate the flow of information and control the plant growth, development, and responses to various stress encountered by plants. The involvement of phytohormones in regulating thermotolerance is well expected and documented. An overview of phytohormones contributing to heat stress tolerance is shown in Fig. 3.3. High temperature modulates the biosynthesis and signaling of different hormones that eventually link to heat stress responses in plants. The frequency and magnitude of temperature have increased tremendously attributed to global climatic change which is detrimental to agricultural productivity and food security. Thus, a comprehensive understanding of the mechanism involved in heat tolerance is vitally important. However, the effects of heat stress on different phytohormones differ along with the phytohormone-mediated regulation of physiological and developmental responses. Therefore, it is of paramount importance to thoroughly investigate heat-responsive hormonal changes and the sophisticated crosstalk between different phytohormones.

3.6 Conclusion and Future Perspectives

Global warming is a pressing worldwide issue of this century. Surging temperatures pose a serious threat to the growth, development, and productivity of crops plants. Heat stress is the second most detrimental stress, as it can hamper the development of crops at any stage and can lead to drastic declines in crop yield and productivity. Hence, efforts to investigate the deleterious effects of heat stress on crop development and physiology have been increased enormously due to the expected increase in the temperature in near future. Along with investigating the deleterious effects of heat stress, a thorough understanding of underlying genetic and molecular mechanisms is also warranted.

The extent of heat stress effects on plant development and physiology depends upon the sensitivity of the crop plant as well as the stage where plants encounter heat stress. Even a slight increase in ambient temperature starts impacting the crop features at different developmental stages, with extremely high temperatures resulting in extensive physiological and biochemical alterations. Compared to vegetative growth, the impact of heat stress is more pronounced and detrimental at the reproductive phase by causing infertility and abortion of flowers, leading to significant yield losses. During grain filling, elevated temperature retards the growth of the seed by disturbing the biochemical processes necessary for the seed development. Temperatures above the optima have detrimental effects on almost all the major physiological processes, including photosynthesis, photoassimilate partitioning, membrane integrity, and water balance in plants. These physiological processes are tightly coupled to the crop yield, and the negative effect of supra-optimal temperature ultimately results in yield penalty. High temperatures drastically alter the level of phytohormones in plants, and phytohormone biosynthesis and signaling could be integral to heat stress perception and signaling. Modulations in hormonal levels and the crosstalk between different hormonal signaling could be potentially used to alleviate heat stress and ameliorate thermotolerance. Identifying the molecular and genetic mechanisms underlying heat-stress signaling is necessary to devise suitable strategies to produce thermo-tolerant crops. With the recent progress in genetics, molecular biology, and omics technologies, we should be able to have a comprehensive understanding of the effects of both short-term and long-term impact of heat stress on various physiological processes. Increased understanding of the crop physiological responses to heat stress and underlying mechanisms would equip us better to improve and enhance the thermotolerance, and hence the grain yield and quality of crops, using genetic engineering or breeding approaches.

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Physiological Traits for Improving Heat Stress Tolerance in Plants

4

Sourabh Karwa, Akshay Sakhare, Chandrapal Vishwakarma,
Sunder Singh Arya, Madan Pal, and Sudhir Kumar

Abstract

Rice is stable and principle crop that feeds the maximum world population. Under the present climatic change scenario, there is a rise in demand for rice production for sustaining an increasing population as the climatic changes leads to yield reduction and quality deterioration of rice. In rice, anthesis and grain filling are considered as very crucial stages, and any changes in the temperature and water availability cause significant reduction in yield. In this climatic change scenario, the rise in temperature is the most potent factor, which is responsible for the reduction of plant growth and yield. It does not only reduce the yield, but also deteriorates the quality of grains in all agricultural crops. Most of the rice is currently cultivated in regions where temperatures are above the optimal for growth (28/22 °C) if there is an increasing temperature above optimal will result in a reduction in productivity and quality. As the temperature rise caused a reduction in precipitation, this will lead to drought stress. Because of these concerning reasons, the demand for tolerant varieties is increasing nowadays. Physiological and biochemical approaches are proving to be the most important screening tools for the identification/development of tolerant genotypes in any crops under high temperature and drought stresses. The chapter was to focus on identifying the major traits that help in developing the significant adaptive and resilient rice varieties which can sustain high temperature and drought stress. This can act as a potential donor or feeding variety to the current population.

S. Karwa · C. Vishwakarma · S. S. Arya · M. Pal · S. Kumar (✉)
Division of Plant Physiology, ICAR-Indian Agricultural Research Institute, New Delhi, India

A. Sakhare
Indian Rice Research Institute, Hyderabad, Telangana, India

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Heat stress · ROS · Membrane stability · Pollen viability · Spikelet fertility · Phytohormones

4.1 Introduction

The present climatic change scenario is a challenging act in agriculture that causes a catastrophic impact on food security to feed the rapidly increasing world population. The effects of these climatic changes have started showing their impact on the crops' productivity and nutritional quality of all the produce (Kadam et al. 2014). Cereals, mainly rice, wheat, and maize, are essential for present food security demands. In all of these, rice is an important staple crop and a primary source of nutrition to more than 3.7 billion people across the globe (International Rice Research Institute, 2016 http://ricestat.irri.org:8080/wrsv3/entrypoint.htm, accessed on April 10, 2021). Demand for food will increase, as the growing population may be reaching nine billion by 2050 (Godfray et al. 2010), mainly in rice-feeding people living in Africa and Asia (FAO 2014). Even with high production demand, less available freshwater and climate change are posing a severe risk to global rice production (Emerick and Ronald 2019). The projected global temperature will rise by 2.8–4.8 °C by 2100 (IPCC, 2018-19). Repeatedly episode of warmth waves has been reported at the regional scale in the last ten years and had a disastrous impact on agricultural crop production across the globe (Kadam et al. 2014). According to the A1B climate change scenario, approximately 121 million ha of irrigated global rice area is projected to be vulnerable to a high temperature by 2100 (Teixeira et al. 2013). Another critical factor that affects the production was the availability of freshwater crops development and growth. Under these high-temperature scenarios, precipitation levels are also affected, which will ultimately lead to low water. This climate change impacts the earth's crust resulting in infrequent and erratic precipitations, elevated temperatures, and expansion of affected land areas under flood or water deficit. These adverse conditions contribute to the development of the drought-prone regions and, consequently, plant growth and crop productivity.

This stress showed significant detrimental effects on the rice reproductive stage. Rice reproductive stage is considered the most sensitive stage to high stress (HS) (Wassmann et al. 2009; Jagadish et al. 2008; Yoshida 1981). High temperature coinciding at the reproductive stage could result in impaired and poor pollen development, and another dehiscence resulted in high spikelet sterility in rice (Powell et al. 2012; Jagadish et al. 2010a, b). Bahuguna and Krishna (2015) suggested that a high temperature beyond 33 °C (physiological optimum) could affect plant development via alternating molecular and physiological events. Generation of reactive oxygen species (ROS) content is one of the primary events under HS, resulting in the peroxidation of lipids and lower plasma membrane integrity. On the other hand, plants have a robust defense mechanism (antioxidant defense) in maintaining ROS levels under detrimental limits (Szymańska et al. 2017). High-temperature stress also affects the photosynthetic machinery by affecting photochemical reactions in thylakoid lamellae and carbon metabolism within the stroma of chloroplast (Wise et al. 2004; Wahid 2007). Bahuguna and Krishna (2015) suggested that the HS at the later stage could affect the reproductive stage, seed set, and grain filling in rice plants by changing carbon metabolism enzymes and hormonal regulation. Starch synthesis and accumulation during early to mid-grain filling stage result in poor grain filling in HS (Bahuguna et al. 2015).

During flowering, drought stress (DS) on rice has detrimental effects on rice, which resulted in a loss in viability of pollen, pollination failure, and increased flower abortion (Westgate and Ladisch 1993). The key reason for these failures was a reduction in the water potential of floral tissue, i.e., pollen and ovary. Under drought stress, higher production of ROS at the cellular level resulted in significant oxidative damage (Mittler et al. 2004). Short-term and prolonged drought exposure resulted in decreased photosynthesis, leaf development, transpiration, loss of tissue turgor pressure, and various other physiological processes (Tardieu et al. 2018; Jongdee et al. 2002). These changes ultimately affected the anthesis and grain filling stage and resulted in spikelet sterility, and poor grain yield under soil water deficit environment in rice (Jongdee et al. 2002).

Various researchers have studied the impact of these stresses individually, but no reports are available for combined stress effects considered necessary in climatic change. Under natural field conditions, drought and high-temperature stresses can occur concurrently and have more detrimental effects (Rang et al. 2011) than individual stress effects. In Asia, in rice-growing areas, the episode of drought and heat stress at two crucial stages, i.e., flowering and early grain filling, are much every day and responsible for the significant detrimental effect on rice growth and productivity (Wassmann et al. 2009). Other studies documented on various other cereals (rice, maize, wheat, and barley) suggested that combined stress of drought and high temperature showed more detrimental effects than individual ones (Zhang et al. 2013). Heat and drought stresses combined to cause more oxidative stress at the tissue level through overproduction of reactive oxygen species, which damaged protein functions and membrane integrity.

Agricultural crop production faces significant challenges and is considered vulnerable to climatic change like heat spikes, drought spells, increased CO_2, and changing rainfall patterns. Crops productivity was dwindled because of both abiotic and biotic factors. In the above stresses, high temperature and drought are the significant constraints that deteriorated productivity and affect the quality of agricultural food crops worldwide (Schleussner et al. 2018). The rising world population and more demands on agricultural productivity to sustain the world population is a challenging task. Therefore rise in demand for cultivated stress-tolerant crop production to uphold the world population is needed (FAO 2017; Godfray et al. 2010). Crop plants can sense stress responses; adapting and sustaining these responses was carried out by alteration in physiological and metabolisms processes. These advances facilitate us to understate the adaptive strategies of plants in these stress conditions (Bita and Gerats 2013; Basu et al. 2016). Crop productive and metabolite process under these stresses was regulated by using certain growth regulators. Due to these climatic changes variability, plants need to develop adaptive mechanisms, modify their metabolism, or alter specific metabolites to cope with these stresses.

4.2 Rice and Its Importance

Cereals play a significant role in human nourishment, contributing to many calories on a per-day basis. Maize, wheat, and rice contribute 10%, 17%, and 23% calories worldwide (Khush et al. 2001). Among all cereals, rice (*Oryza sativa* L.) is a significant and second most cultivated cereals crop. Rice is grown in flooded conditions and averagely consuming 24.0–30.0% of total available freshwater (Bouman et al. 2007) and requires an optimum air temperature of 32/28 °C day and night temperature. Rice is sensitive to high temperature and drought stress conditions, and the reproductive stage is considered as the most sensitive stage to these stresses (Yoshida 1981; Jagadish et al. 2007, 2008; Sarsu et al. 2018; Basu et al. 2016; Bahuguna et al. 2018). However, the present climate change prediction model suggests a rise of 2.5–4.8 °C temperature by 2100 (Alexander 2016; Masson-Delmotte et al. 2018). As the population demand for freshwater consumption increases and freshwater availability can limit and threaten global rice production (Kadam et al. 2015). In the rice-growing season, the primary concern of drought and HS was conceded at the flowering time, which resulted in S.F. and yield penalty under natural conditions (Matsui and Omasa 2002; Serraj et al. 2011; Liu et al. 2004; Teixeira et al. 2013; Kadam et al. 2015; Pandey and Shukla 2015). Moreover, accurate phenotyping and characterizing a diverse set of germplasms in ambient conditions (field) might give potential tolerant donors which can be utilized in the breeding programs. Although, very few genotypes in rice have been identified and characterized for HS-tolerance.

4.3 Effect of High-Temperature Stress on Rice

Rice's most susceptible stage to high temperature is flowering (anthesis and fertilization) and also some level at micROS porogenesis stage at booting (Yoshida 1981; Prasad et al. 2006; Jagadish et al. 2008; Farrell et al. 2006). The global climatic change in air surface temperature rises significantly by "0.15 ± 0.05 °C" in 10 years (Jones et al. 2016). The present climatic change model predicate that the average rise in air surface temperature will be of "1.4–5.8 °C by 2100" (Corbera et al. 2016).

The optimum temperature that's required for proper rice growth is 32/28 °C day/night temperature. It has been suggested that high temperature at the flowering and grain filling stage was more detrimental in rice (Yang et al. 2007). Peng et al. (2004) revealed the rise in average nighttime temperature by 1.13 °C over 25 years in these stages caused severe damage to growth and yield in rice. It resulted in the sterility of spikelet and decline in yield per plant (Nakagawa et al. 2003). The negative effect of short HS spells has been demonstrated at the flowering stage, resulting in a significantly reduced yield in past decades in China (Welch et al. 2010;

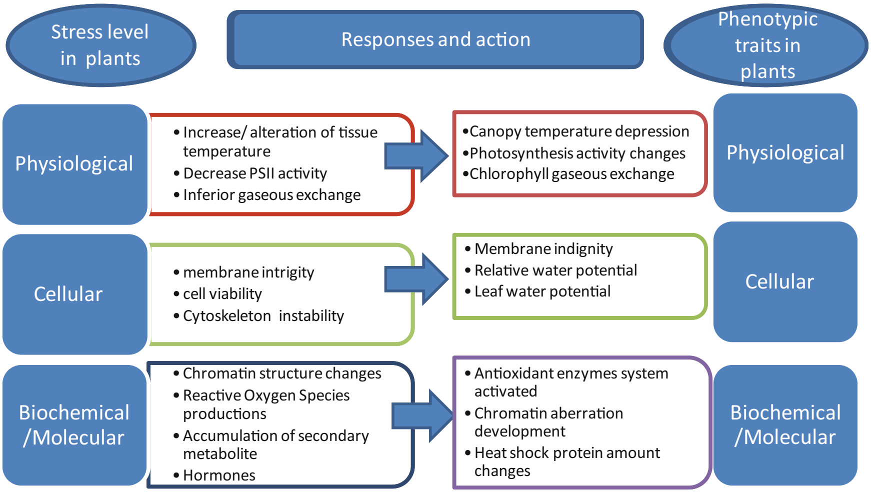

Fig. 4.1 Physiochemical markers to illustrate the effect of high-temperature stress in rice

Yang et al. 2004; Zou et al. 2009). Hasegawa et al. (2009) suggested that high temperatures at the flowering stage result in the reduction of rice productivity. Shi and Chan (2014) investigated popular rice cultivars grown in the high-temperature sensitive region and found much more prone to HS at the flowering stage. For this problem, there is a need to identify true tolerant and high-yielding varieties that did not only sustain but also get yield under HS conditions (Battisti and Naylor 2009; Lobell and Burke 2008). It was reported earlier that N-22 and NL-44 are high-temperature stress tolerance donors at the vegetative and flowering stages by maintaining higher biomass (Bahuguna et al. 2015; Jagadish et al. 2007). Moreover, precise high-temperature phenotyping is needed to characterize germplasms set under natural and field conditions to identify potential donors used in the future breeding program (Fig. 4.1).

4.4 Effect of Drought Stress on Rice

Another major constraint responsible for decreased agricultural productivity is drought or water deficit stress (Sharp et al. 2004; Saini and Westgate 2000). Rice is an irrigated crop and is highly vulnerable to drought (Cabuslay et al. 2002). Around ~23 million ha *Oryza Sativa* L. production is affected by water deficit across the globe (Serraj et al. 2011; Pandey and Shukla 2015). Moreover, its impact is more pronounced because of increased unnatural climatic conditions, which promote drought stress events (Pachauri et al. 2014). As per the climatic model, the frequency of drought spells shortly can be more coinciding with acute stages and results in losses to rice productivity (Kumar et al. 2014). O'toole (1982) reported that drought stress at the vegetative stage result in a lesser reduction in productivity, while the same treatment at meiosis and fertilization results in more considerable yield reduction. At flowering, water deficit resulted in the decline of the grain yield by 30–69% under natural conditions (Cabuslay et al. 2002; Garrity and O'Toole 1994; Sheoran and Saini 1996). Water deficit resulted in stomatal closure and limited carbon accumulation in plants. Leaf water potential was also affected, which diminished turgor pressure in leaf and inhibited stomatal activity and another metabolism (Jaleel et al. 2008). Under drought stress, various plant physiological and biochemical processes were hindered such as membrane integrity, plant water relation, gas exchange, osmolytes content, nutrient management, source-sink association, and growth (Farooq et al. 2008; Benjamin and Nielsen 2006; Jaleel et al. 2008; Razmjoo et al. 2008). Breeding efforts for developing drought resistance rice cultivar that will perform under these predicated climatic change scenarios, but the drought resistance mechanism is poorly understood at the flowering stage (Cabuslay et al. 2002; Condon et al. 2004).

4.5 Effect of High Temperature on Rice Grain Yield, Pollen Viability, and Spikelet Fertility

The flowering stage in rice is most prone to HS and D.S. concerning the vegetative stage (Yoshida 1981). At flowering or anthesis, pollen containing anthers further followed to micro-gametogenesis is the most sensitive tissue to HS and drought stress (Satake and Yoshida 1978; Sheoran and Saini 1996; Nakagawa et al. 2003). The previous report from Yoshida (1981) suggested that manual pollen shaded from non-stress plants to stress stigma showed an ability to fertilize at 41 °C in rice. Therefore pollen grains in the reproductive organ are responsible for lesser S.F. and grain yield in *Oryza Sativa* L. (Wassmann et al. 2009). A study performed in rice at 35 °C for 5 days during panicle development to anthesis suggested a 35% decline of spikelet fertility (Yoshida 1981). A similar response has been conformed in wheat under high temperatures (Saini and Aspinall 1982).

High-temperature tolerance is mainly associated with three primary mechanisms, i.e., avoiding, escaping, and withstand adverse weather conditions. In avoiding high temperature, stress plant adjusts its tissue temperature by maintaining transpiration rate (Weerakoon et al. 2008). Escaping was associated with early morning flowering and avoiding during peak hours (Ishimaru et al. 2010). Another process is to withstand the adverse climatic conditions with resilient developing reproductive physiology (Jagadish et al. 2010a, b). Based on a field study, Prasad et al. (2006) demonstrated that a rise in 5 °C above ambient temperature significantly reduces spikelet fertility, grain yield, and reduction in harvest index, in various rice cultivars was mainly genotype-dependent. They have also shown a strong positive correlation between S.F. and pollen receptivity and pollen production. A similar response is reported in Japanese cultivars by Matsui et al. (2001) and attributed mainly because of the lower pollen count and poor anther dehiscence on the stigma surface. Jagadish et al. (2007) conducted a controlled environment study in rice and reported that at flowering <1 h, high-temperature exposure was sufficient to decline spikelet fertility. It is related to the fertilization and anther dehiscence in a genotypically dependent manner. Other reports suggested that flowering patterns or early flowering attributes under high temperature can be an essential adaptive trait associated with HS (Jones et al. 1997; Ishimaru et al. 2010).

A comparative study by Fu et al. (2008) reported that HS under field and greenhouse recommended that rise in daily mean temperature was responsible for the reduction of spikelet fertility (6–16%) and grain yield and one thousand grain weight in sensitive genotype more as compared to a tolerant hybrid. Jagadish et al. (2008) reported that 6-h exposure to high temperatures at 33.7 °C and 36.2 °C showed a reduction in spikelet fertility and affected the flowering pattern of rice genotypes. Similarly, Cao et al. (2009) suggested that high temperature from the heading resulted in a more considerable yield reduction in temperature-sensitive genotypes due to poor fertilization and low-speed setting. In contrast, the minimal effect was observed on female reproductive organs in rice. Spikelet fertility reduced significantly in sensitive genotypes, and this reduction was closely associated with germinating pollen on stigma and pollen tube length to the ovary (Jagadish et al. 2010a, b). A study performed by Nagarajan et al. (2010) in rice (basmati and non-basmati genotypes) under high temperature recorded a reduction of S.F. and yield/plant. Bahuguna et al. (2015) identified Nerica L44 (NL-44) for reproductive resilience, with better ROS managing ability, robust antioxidant enzyme system, and high spikelet fertility and grain yield in HS at flowering and vegetative stages.

4.6 Effect of Stress on Rice Grain Yield, Spikelet Fertility, and Pollen Viability

The HS stress is not the only factor responsible for reducing S.F., P.V., and grain yield; drought stress may be responsible for the decline. Mild drought at flowering resulted in a 70% reduction in secondary branching and decreased by 40% in spikelet fertility and panicle number (Kato et al. 2008). In maize, prolonged drought results in reduced sink size and grains number (Bahuguna et al. 2018; Barnabás et al. 2008; Aslam et al. 2013). Drought stress at anthesis results in increased improper anther dehiscence, ovary and pollen abortion, panicle exertion, lower spikelet fertility in rice maize and wheat (Rang et al. 2011; Powell et al. 2012; Praba et al. 2009; Aslam et al. 2013). Drought at pre-anthesis results in asynchronous flowering, or delayed flowering was recorded in wheat (Cattivelli et al. 2008; Foulkes et al. 2007).

The drought stress can affect panicle exertion and cause a reduction in spikelet fertility and grain yield in rice. O'Toole and Namuco (1983) reported that decline in spikelet fertility related to failure in panicle exertion from leaf sheath and matrix water potential of the flag leaf. Selote and Khanna-Chopra (2004) suggested that water stress can decrease spikelet fertility in sensitive rice high-yielding genotypes compared to N22 (tolerant). Various studies indicate that the reproductive stage comprises the microsporangium process for developing functional and viable pollen grains for fertilization and is most critical to stress. Under drought stress, structural and functional disparities create and lead to improper fertilization and seed setting (Saini and Westgate 2000). Pollen development processes like anther dehiscence and pollen shedding are also affected by drought stress (Ekanayake et al. 1990; Satake and Yoshida 1978). It suggested that in DS, sucrose transport to pollen represses, which results in lower P.V. in rice and other cereals (Powell et al. 2012). A similar drought result suggested that drought affects pollen viability during the pollen development stage (Kato et al. 2008; Dolferus et al. 2011). Lilley et al. (1996) and Ekanayake et al. (1993) also suggested that upland rice was more adapted to the water conserved mechanisms than others during drought spells. A greenhouse study performed by Rang et al. (2011) tested five genotypes to drought, found a significant decline in pollen count (52 to 70%), pollen on stigma (59%), and spikelet fertility by 81% in sensitivity genotypes, whereas tolerant (N22) have a lesser reduction in all above traits.

Sharma et al. (2018) performed a pot study in rice and phenotyped 15 rice cultivars by withholding irrigation at anthesis and drought were maintained at ~70 kPa and reported that P.V. (50%), S.F. (48%), and grain yield per plant and 1000 grain weight significantly affected under drought as compared to well-watered conditions. Bahuguna et al. (2018) reported that drought at flowering reduces spikelet fertility and grain yield/plant. They also recommended that priming at the critical stage helps in kick-start stress signaling and helps in developing acclimatize response against stress. The genetic diversity analysis performed on 296 Indica rice panels under drought stress also significantly reduced grain yield, spikelet fertility, 1000 grain weight, and spikelet number per panicle at 64 kPa and 45 kPa, respectively (Kadam et al. 2018).

4.7 Effect of High Temperature on Rice Grain Yield, Spikelet Fertility, and Pollen Viability

Some studies have reported combined effects of drought and high-temperatures stress and suggested that combined stress was much more detrimental than individual ones. Rang et al. (2011) indicated a decline in S.F. concerning particular stress and normal conditions. Lawas et al. (2019) reported a reduction of 73% yield due to combined high temperature and drought stress in sensitive genotypes compared to control ones.

4.8 Heat Susceptibility Index and Cumulative Stress Response Index

Fischer and Maurer (1978) categorized wheat genotypes in water deficit based on yield; they performed under drought stress and identified them according to their susceptibility index. In brief, the rate of change in yield between two environments relatively means a shift in overall genotypes. Later, Dai et al. (1994) and Koti et al. (2007) suggested the cumulative stress response index, all the sum of individual relative components response of treatment was the best way to identify the actual response of cultivars in any stress conditions.

4.9 Effect of High Temperature on Rice Seed Quality

Studying the HS effect on rice quality is essential for rice productivity and marketability. Rice's appearance, cooking, and texture are the main quality traits that are major concerns of consumers (Okabe 1979; Rousset et al. 1999). Various studies have suggested rice grain quality can be a more concerning issue in HS (Madan et al. 2012; Lyman et al. 2013). Most reports are available on control conditions studies (Fitzgerald and Resurreccion 2009; Lisle et al. 2000), and very few studies carried in field conditions. Heat stress at the grain filling and flowering stage can make an abnormal change in color and grain texture due to insufficient starch accumulation (Tsukaguchi and Iida 2008). The grain chalkiness was the major component in determining the rice price and quality.

Yoshioka et al. (2007) characterized conventionally chalky grains using image-based techniques in the white core, milky white, white back, and white belly rice. High temperature deteriorated quality traits like grain appearance hulling, milling yield, high chalkiness percentage, head rice recovery, amylose content which can affect market values of rice (Inatsu 1979; Cock et al. 1976). It may result from changes in the activity starch biosynthesis pathway and alteration in granules packing in rice grains (Tetlow and Emes 2014; Dai et al. 2005).

Head rice recovery is an essential trait for rice selection in West Africa (Sakurai et al. 2006). The study suggested that head recovery is affected by high air temperature and relative humidity (Cnossen et al. 2003; Schluterman and Siebenmorgen 2007). Cooper et al. (2008) suggested that high night temperature was also responsible for significantly reducing head rice yields. Nagata et al. (2004) indicated under high temperature at early grain filling and post-flowering was primarily accountable for rice grain fissuring, which resulted in the reduction of head rice yield. Under high-temperature stress, sink capacity is altered, resulting in lower starch synthesis activity and 1000 grain weight (Jeng et al. 2003; Oh-e et al. 2007). Grain chalkiness is another essential trait that determines rice quality and price in the international market. The high temperature at early grain filling and post-flowering affects the packing of starch granules and created air spaces. As a result, in grains chalky regions developed at maturity (Tashiro and Wardlaw 1991; Zakaria et al. 2002). These loose packing of amyloplasts in kernels results in the formation of chalky grain and differs from translucent grains (Lisle et al. 2000).

Rice grain mainly consists of two types of starch, i.e., amylopectin and amylose. Under normal conditions, amylose concerning amylopectin is responsible for the firmness of cooking grain (Juliano 1992; Blakeney et al. 1994). Under high-temperature reduction of amylose content increases amylopectin in rice grains (Umemoto et al. 2002; Asaoka et al. 1989; Umemoto and Terashima 2002). These changes in amylose content led to sticky rice after cooking (Sakurai et al. 2006) and are not favorable for consumption (Sakurai et al. 2006).

4.10 High Temperature Effect on Gaseous Exchange and Tissue Temperature

Photosynthesis rate (i.e., CO_2 uptake) and transpiration rate (i.e., H_2O loss) are regulated through the stomatal behavior of the plants. This stomatal pore opening and closing maintain the tissue temperature and moment of metabolites or signaling compounds in plants (Brownlee 2001; Lake et al. 2001).

Pn "(rate of photosynthesis)" is affected under high temperature and drought (Hassan 2006; Yordanov et al. 1999). The limitation in the rate of photosynthesis in DS and HS can occur either due to nonstomatal or stomatal attributes (Shangguan et al. 1999; Ort et al. 1994). Whichever limitation in gas exchange might limit plant growth and development in various stresses. Various researchers recommended that in high temperature and drought individually or in combination rate of photosynthesis and transpiration decline (Zandalinas et al. 2016; Arbona et al. 2013). In high temperatures, the reduction in yield in cereals is associated with carbon assimilation (photosynthesis and transpiration) (Stone 2001). High temperature resulted in a lower rate of photosynthesis because of (1) inactivation of Rubisco enzyme activity (Prasad et al. 2004) and (2) destruction of Photosystem II (PSII) (3) by the decreased rate of consumption of CO_2/O_2 and/or increased photorespiration in mesophyll cells (Leegood and Lea 1999). PSII is the most prominent high-temperature sensitive component (Gombos et al. 1994). Wise et al. (2004) recorded that HS stress carbon metabolism in the stroma and photochemical reaction in thylakoid lamella of the chloroplast are affected.

Various studies have suggested that high-temperature stress can cause degradation of chlorophyll a and b in the leaf of different crop species (Karim et al. 1999). In *Solanum Lycopersicum* and *Saccharum officinarum*, HS increased the chlorophyll a: b ratio and decreased chlorophyll:carotenoids ratio in the least susceptible genotypes (Camejo et al. 2005; Wahid and Ghazanfar 2006). Such chlorophyll degradation or photosynthesis apparatus has been associated with ROS production (Guo et al. 2006). The rate of photosynthesis is affected under high temperatures due to disruption of the thylakoid membrane or inhibiting membrane-associated electron carriers and enzymes (Rexroth et al. 2011). More than 35 °C decreased the activity of Rubisco and can limit the photosynthesis system in various plants (Griffin et al. 2004). Zhang et al. (2018) reported lower photosynthetic rate ($P_{.N.}$) insensitive rice genotypes as compared to N22 under heat stress. Similarly, reduction in Rubisco activity in rice and wheat study has been suggested as a cause of lower $P_{.N.}$ in HS (Perdomo et al. 2017)

In drought stress, stomatal conductance decreases and ultimately reduces CO_2 flow in mesophyll cells. Similarly, reduction in the activity of RuBP (Ribulose biphosphate) (Parry et al. 2002) or Rubisco content (Bota et al. 2004) can affect the rate of photosynthesis under drought stress. Speer et al. (1988) reported that drought stress caused instability to the photosynthesis membrane via dehydration in later stages on the plasma membrane. Chaves et al. (2011) suggested that under severe DS, de novo synthesis of photosynthesis proteins slows down, which adversely affects the photosynthesis capacity and enhanced leaf senescence.

4.11 Effect of High Temperature on Relative Water Content (R.W.C.)

Relative water content (R.W.C.) is a critical trait that showed the degree of hydration in tissue and cell and is an essential feature in better growth and physiological functioning (Silva et al. 2007). Blokhina et al. (2003) suggested that plasma membrane permeability and suitability decreased due to dehydration under drought stress. Vendruscolo et al. (2007) indicated that tolerant varieties maintain R.W.C. concerning sensitive ones under water deficit environments. Those varieties keep relative water content-maintained protoplasm under water stress and ensure yield in rice (Sikuku et al. 2012).

4.12 Effect of High Temperature on Membrane Stability Index (MSI)

The membrane integrity defines the permeability or integrity of the plasma membrane (Blokhina et al. 2003). Under stress conditions, plasma membrane permeability increases and increases "electrolytes leakage" from the stress-imposed tissue (Chaturvedi et al. 2012). This electrolytic leakage can be addressed as electrolytic conductance (Agarie et al. 1995). Swapna and Shylaraj (2017) suggested that tolerant varieties have better MSI under drought stress conditions as compared to sensitive ones.

4.13 Effect of High Temperature on Reactive Oxygen Species and Antioxidant System

Under adverse environmental stress conditions, plants activate various types of defense mechanisms in genes, proteins, and signaling cascades to confer tolerance. These stress conditions are liable for the generation of ROS in the organism. ROS act as double-edged sword nature molecules in lower concentration by acting as signaling compounds, whereas in higher it was malevolent nature (Noctor et al. 2014; Baxter et al. 2014). A report summarized ROS as harmful molecules which were highly reactive to nucleic acid, proteins, lipids, and other metabolites (Gill and Tuteja 2010a, b). These ROS molecules comprise various molecules, namely H_2O_2 oxide radicals ($O•_2-$, OH•, singlet oxygen, and many others). Many reports have suggested that ROS overproduction caused toxicity to nucleic acid, protein, and other metabolites, resulting in cell death and damage (Gill and Tuteja 2010a, b). Under natural conditions balance of ROS and the scavenging mechanism is there, but under stress conditions, this balance gets disturbed and caused cellular damage (Das and Roychoudhury 2014). Szymańska et al. (2017) proposed a robust defense mechanism (antioxidant defense) in maintaining ROS levels under detrimental limits. As ROS activity increased, during HS and D.S., which will possibly affect in peroxidation of lipids and disturbing the integrity of the plasma membrane.

The ROS scavenging components are presented in both enzymatic and non-enzymatic forms. Researchers reported that under drought stress conditions, the expression of enzymatic antioxidants increased in rice (Fahramand et al. 2014) which include S.O.D. (superoxide dismutase), C.A.T. (catalase), A.P.X. (ascorbate peroxidase), G.P.X. (guaiacol peroxidase), G.R. (glutathione reductase), and others (Sahebi et al. 2018), while non-enzymatic are A.A. (ascorbic Acid), G.S.H. (reduced glutathione), α-tocopherol, carotenoids, flavonoids, and proline (Boukar et al. 2019). Abiko et al. (2005) suggested that tolerant plants protect themselves from the harmful effect of ROS by synthesizing antioxidants components. These antioxidants are located at various organs of cells and collaborate to detoxify reactive oxygen species. You and Chan (2015) suggested that in all the enzymatic antioxidants, S.O.D. are the primary defense line of defense which convert O_2•- into H_2O_2 then C.A.T., G.P.X., and A.P.X. play key role in detoxification of H_2O_2 (You and Chan 2015). It has been shown that ascorbic acid is required for A.P.X. activity while glycol for G.P.X. (Noctor et al. 2014; Dietz et al. 2006; Meyer et al. 2012). Several studies reported that in the rice genome, eight S.O.D. genes encoded viz. one Mn-SOD (manganese S.O.D.), CuZn-SOD (plastidial S.O.D.), CuZn-SOD-L (putative CuZnSOD-like) and 2 copper-zinc S.O.D, cytosolic (cCuZn-SOD1 and cCuZn-SOD2) and iron S.O.D.s (Fe-SOD2 and Fe-SOD3) (Nath et al. 2014). Sharma and Dubey (2005) demonstrated an upregulated activity of S.O.D. in *Oryza Sativa* L. in water deficit stress. Bahuguna et al. (2015) demonstrated a rise in activity S.O.D., A.P.X., and G.P.X. of tolerant genotypes under high temperature in rice in both vegetative and spikelets and helped minimize high-temperature stress levels. The various transgenic approaches also justified that under-stress overexpression of Mn-SOD1 reduces stress induction (Li et al. 2013). Teixeira et al. (2004, 2006) reported 8 APX genes in rice. Two mitochondrial A.P.X.s (OsAPX5 and OsAPX6), two peroxisomal A.P.X.s (OsAPX3 and OsAPX4), two cytosolic A.P.X.s (OsAPX1 and OsAPX2), and two chloroplastic A.P.X.s (OsAPX7 and OsAPX8) and two cytosolic A.P.X. genes have a crucial role in abiotic stress tolerance (Zhang et al. 2013; Sato et al. 2011) rice. Several researchers proposed under high-temperature stress condition ROS like H_2O_2, hydroxyl radicals produces at PSII system was scavenge by antioxidant including S.O.D. In signal transduction, the involvement of ROS implies the intensity of stress and to maintain ROS at nontoxic levels, there must be a synchronized function of the regulatory network in balancing ROS production.

Hussain et al. (2019) suggested that in combined HS and DS conditions, metabolites accumulation and enzymatic and non-enzymatic antioxidants increased to prevent oxidative damage in maize. Zhang et al. (2018) suggested similar in rice anthers under high temperature.

4.14 Osmolytes Accumulations in High Temperature and Drought

The critical adaptation for many kinds of cereal under abiotic stress, i.e., heat and drought, is the accumulation of specific low molecular mass organic molecules called osmolytes. Under stress conditions, these osmolytes are accumulated in plants to regulate stress tolerance. Common examples of these osmolytes are sugar, proline, glycine betaine, tertiary and quaternary ammonium compounds, and tertiary sulfonium compounds.

Amphoteric quaternary amine, glycine betaine (G.B.), has a vital role as compatible solutes in various plant abiotic stresses, especially in high temperatures and drought. The glycine betaine content varied from species to species under stress conditions. In maize, it has been reported that more accumulation of G.B. enhances the stress levels under high temperature and drought stress conditions. It was previously reported that it helps improve photosynthesis rate in individual heat and drought and combine stress in wheat. Under water scarcity, it has been reported rise in the level of G.B. in maize and sugarcane (Wahid 2007). It has also been suggested that a rise in temperature by 4–5 °C PSII efficiency inhibited and leads to the accumulation of G.B., which was reported to protect the PSII system from photoinhibition in high-temperature stress. In high-temperature stress, CO2 fixation was affected, resulting in the generation of ROS, G.B. stabilized the Rubisco, resulting in a reduction in ROS generation in crops.

Like glycine, proline is also a critical osmolyte, which accumulates in large quantities under environmental stress conditions. Proline is synthesized from glutamate by the action of two enzymes, Δ1-pyrroline-5-carboxylate synthetase and Δ1-pyrroline-5-carboxylate reductase. It was known that proline also regulates the cellular redox potential under stress environments, exceptionally high temperature, and drought stresses. It also has various functions, such as signaling molecule, a metal chelator, and antioxidant defense. High temperature showed its first presence at the cellular level, especially at the plasma membrane, affecting biochemical events. Kumar et al. (2016) suggested that under high temperature rise in proline content was noted. Under high temperatures, fruit set in tomato plants failed due to disrupting sugar metabolism and proline transport during the narrow window of male reproductive development. Hexose sensing in transgenic plants engineered to produce trehalose, fructans, or mannitol may significantly contribute to the stress-tolerant phenotypes.

Trehalose is a non-reducing disaccharide of glucose that functions as a compatible solute in stabilizing biological structures under abiotic stress in bacteria, fungi, and invertebrates. Trehalose also acts as a storage carbohydrate, and it possesses the unique feature of reversible water absorption capacity to protect biological structures from damage during drought and high temperatures. When water dissipates from the shell of macromolecules (such as protein) during severe dehydration, trehalose can act as a water substitute on the surface of the dried protein. Thus, proteins' native folding and biological activity are maintained, and denaturation and aggregation are prevented. A study revealed that upregulation of trehalose biosynthetic genes in rice has considerable potential for improving abiotic stress tolerance and, at the same time, augmenting productivity under both stress and non-stress conditions. It suggested that from overexpression of trehalose in a higher capacity for photosynthesis and a concomitant decrease in the extent of photo-oxidative damage during stress. In addition, trehalose must be interacting with other physiological processes to account for changes in ion uptake and partitioning during salt stress. Because other cereal crops, like rice, are also sensitive to abiotic stresses, likely, overexpression of trehalose biosynthetic genes in maize and wheat may also confer high levels of abiotic stress tolerance.

4.15 Hormone Metabolism in High-Temperature Stress

Crops are very susceptible to these changing environmental conditions. To overcome these harmful and adverse conditions, phytohormone, plays a key role, whose signaling helps the plants to adapt and survive under these stress conditions. These are part of key metabolic pathways that help sustain plants and control the yield components (Peleg and Blumwald 2011). Some basic phytohormones are classified into five standard groups viz. ethylene (E.T.), Abscisic acid (A.B.A.), auxin (I.A.A.), gibberellins (G.A.), and cytokinins (C.K.) were mainly present.

Under abiotic stress, crosstalk between hormones develops synergetic or antagonistic relations, which plays a pivotal role in plant development processes. Previous research suggested that auxin (I.A.A.), cytokinin (C.K.), and gibberellic acid (G.A.) are involved in plant development and regulate the response to stress environments (Eyidogan et al. 2012). Zhang et al. (2009) suggested that auxin regulation in modulating the drought response. Likewise, A.B.A. is involved in regulating stomatal closure to prevent water loss to minimize transpiration and decrease growth under drought stress (Schroeder et al. 2001). Xue-Xuan et al. (2010) reported that A.B.A. signaling plays an essential role in developing plants' drought and cold stress tolerance. E.T. is a gaseous hormone regulating process involving various stages of growth phases, viz. leaf, petals abscission, flower senescence, and fruit ripening and developed in abiotic stress response (Abeles et al. 2012).

Another newly identified group of chemical groups are nitric oxide (NO), salicylic acid (S.A.), strigolactone (S.L.), brassinosteroids (B.R.), jasmonate (J.A.), and polyamines (PAs). In all above, PAs (polyamine) are newly class molecules that play a crucial role in plant development and response under abiotic stresses conditions (Minocha et al. 2014). In various observations, PAs different concentrations were noted during "plant growth and development" and were correlated to "different stresses at cellular levels." The polyamines are reported for various adaptation and acclimation mechanisms to cope with different stress conditions (Pál et al. 2015; Karwa et al. 2020). Liu et al. (2007) suggested that polyamines act as a secondary messenger in a common signal transduction pathway in *Arabidopsis thaliana*. Various early and recent work told that PAs have crosstalk with various other hormones viz. gibberellins, auxin, and ethylene for maintaining different processes, but their pronounced relation is not establishing the interaction and mechanism of such crosstalks (Kusano et al. 2008; Alcázar et al. 2010; Bitrián et al. 2012; Xu et al. 2014).

4.16 Conclusion

As the rice is the source of nutrition to the large population of the world, the increasing high-temperature spikes and drought spell the demand for resilient rice varieties rises. Lack of knowledge, how plants can sense and cope up these harsh environments, physiological and biochemical traits which at par correlate with yield and quality traits that can also help us to identify the most important one under these stresses. Under the stress condition, plant either showed tolerant, escape, or avoiding the mechanism for which either it regulates the tissue temperature or by early flowering traits it avoids the high temperature and drought spells. There are various noticeable effects of high temperature and drought is observed on pollen viability, affected grain filling, structural changes of cell organelles, oxidative stress, lipid peroxidation of cell membranes, disruption of leaf water relations, and reduction in photosynthesis. All these effects regulate the water potential, membrane permeability, and osmolytes regulation, which can help in restricting the water losses under stress conditions. Hormone regulation helps in generating the systemic signaling pathway which regulates the H_2O_2 content and HSPs that help in proper regulation of photosynthesis and transpiration process under stress conditions.

In the future, efforts are needed to phenotype large genotypic variability on the basis of early flowers opening traits and by maintaining spikelet fertility, cell membrane thermostability, photosynthesis, transpiration, HSPs, and maintaining a greater non-structural carbohydrate pool under high temperature and drought stress. A challenge for the future is to achieve the robust tolerant mechanism that can help the plant to sustain high temperature and drought stress environments in climatic change scenarios.

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5

Understanding the Mechanism of High-Temperature Stress Effect and Tolerance in Wheat

Ranjan Das, Soibam Helena Devi, Sangita Das, Merentoshi Mollier, and Sujata Pachoni

Abstract

Notable global mean temperature rise due to climate change is affecting crop performance worldwide now-a-days. Intense heating effect of our globe has brought substantial yield losses as plants are undergoing adaptive modifications at numerous organizational levels, with hastening phenology to reduce biomasses well as reproductive yield. Heat stress has direct impact on the productivity of agricultural cereal crops as increasing temperature and consequent changes in climate adversely affect plant growth and development by disrupting the plant's vital physiological and biochemical processes and thereby reducing grain number and size, lowering photosynthetic activity and chlorophyll and starch content in the endosperm. Wheat is a major cereal crop that provides basic calories and protein to more than 80% of the world's population. Among various kinds of stresses heat stress is one that have a severe impact on wheat cultivation resulting in catastrophic loss of its productivity. Under stressful conditions excessive reactive oxygen species accumulates in the tissues, which causes significant progressive oxidative damage to the crop. Thus impact of rising temperatures on wheat production is becoming a global issue. Since the industrial revolution, global temperatures have been steadily rising at a rate of 0.15–0.17 °C every decade. Several studies have found that average maximum temperatures above 32 °C during the reproductive phase had a negative impact on wheat grain yield, with average yield losses of up to 30% reported. Thus, knowledge of high-temperature stress effect and tolerance at physiological, biochemical, and morphological levels is required in order to develop new upgraded crop varieties that can cope with future climate in order to have agricultural yield sustainability under higher temperatures.

R. Das (✉) · S. H. Devi · S. Das · M. Mollier · S. Pachoni
Department of Crop Physiology, Assam Agricultural University, Jorhat, Assam, India

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Keywords

Climate change · Food security · Global warming · High-temperature stress · Thermotolerance

5.1 Introduction

Wheat (Triticum spp.) is an important cereal crop of Poaceae family that shares approximately 30% of global grain yield and ½ of the global grain trade (Akter and Islam 2017). It serves as a chief source of energy at reasonable price contributing about 8–20% protein and 70–75% of our daily energy requirement (Day 2013). Among environmental stresses, elevated temperature stress is an important aspect which can interfere with the normal crop functioning and it is the main barrier preventing wheat crops from reaching their full genetic potential. Wheat is much sensitive to heat stress. Nevertheless, a mild heat stress may negatively affect crop yield and it is anticipated that for every 1 °C rise in temperature may reduce the world wheat yield by 6% (Asseng et al. 2011). At the time of reproductive phase an elevation of 1 °C over mean temperature will possibly lead to significant reduction in wheat production (Bennett et al. 2012; Yu et al. 2014). The optimum temperature for wheat during reproductive stages is between 15 and 20 °C (Shewry 2009). However, high daytime temperatures (>34 °C) are expected to become more common in wheat-growing regions around the world (Asseng et al. 2011). The reduction in the crop cycle, pollen abortion, kernel shrinkage, reduction in seed reserves, anther indehiscence, and reduced growth of the pollen tube are all major effects of high temperatures due to the alteration of different physiological, biological, and biochemical process in wheat (Asseng et al. 2015) which results in reduced global crop yields (Lobell and Gourdji 2012; Rezaei et al. 2015). Exposure of high temperature during the vulnerable growth stages have significant impact in reducing yield. High temperature also effects the other viz. growth, yield, and quality parameters differentially in other crops such as chili, *Brassica,* etc. (Das 2021; Das et al. 2016, 2020). Heat stress during seed germination affects seedling emergence, radicle and plumule growth, and germination percentage, resulting in abnormal seedlings with low seedling vigor (Hasanuzzaman et al. 2013). Heat stress decreases photosynthetic and leaf area expansion in later vegetative stages, resulting in decreased biomass production; stress during the reproductive stages of development, on the other hand, results in fewer as well as smaller sized seeds, resulting in reduced capitulate (Bita and Gerats 2013; Prasad et al. 2017). Starch and protein content of wheat grains are also altered by HS. HT brings more membrane damage by generating excess of reactive oxygen species (ROS), such as the superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), and lipid peroxidation (Djanaguiraman et al. 2018; Narayanan et al. 2016). Current analysis conducted indicates that the average global temperature on Earth has increased by ~0.8 °C since 1880. Two-thirds of the warming has occurred since 1975, at a rate of roughly 0.15–0.20 °C per decade (Lorenz et al. 2019). As a result of climate change, the normal surface temperature of the globe is

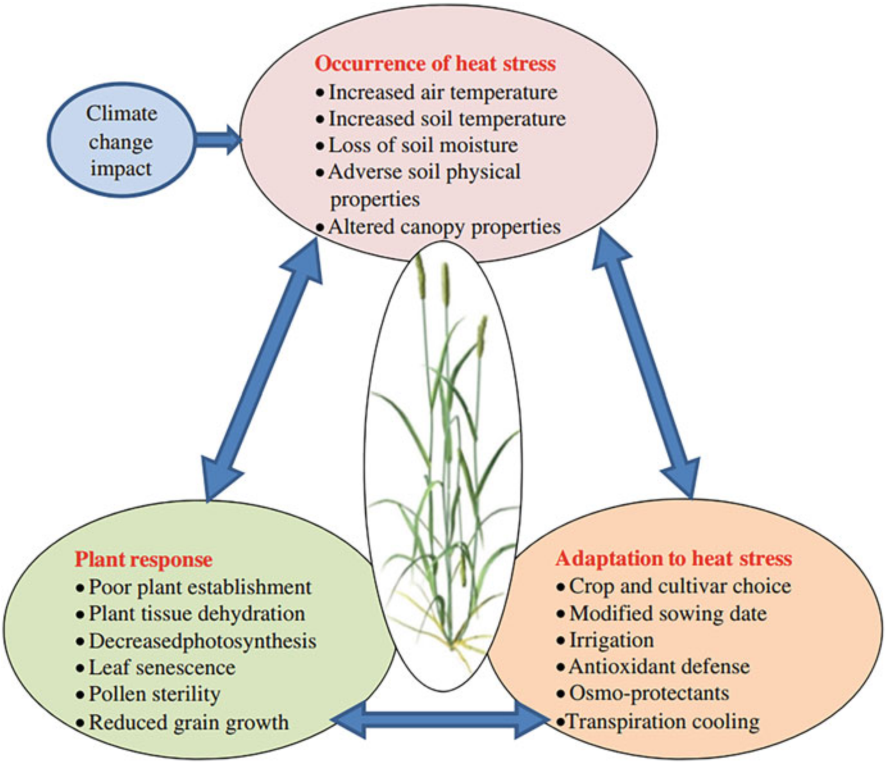

Fig. 5.1 Linking between climate-induced heat stress occurrence, plant responses to heat stress, and their adaptation

enhancing, which can be proven disturbing for all stages of crop growth, mainly in tropical and subtropical countries (Li et al. 2018) (Fig. 5.1). Short episodes of high temperatures during the reproductive period can substantially affect the production of agriculturally important crops, making heat stress one of the biggest threats to global food security (IPCC 2007). Every crop variety has their own cardinal temperatures, below or above which might lead to stress.

Ortiz et al. (2008) reported that global rise in temperature might also highly impact wheat yield, accounting about 21% of grain yield and 200 million ha of agriculture land globally. Temperature accelerates the developmental process in plants, causing senescence to occur earlier and the growth cycle to be shortened (Bita and Gerats 2013). Heat stress is a major abiotic stress that has a significant impact on wheat growth and yield (Joshi et al. 2007a, b; Dwivedi et al. 2015). In dry and semi-dry conditions, lower yields are obtained as a result of continuous temperature rises that coincide with the anthesis and grain filling stages of crops (Moral et al. 2003; Dwivedi et al. 2015). Therefore, as a result of global warming and changing climatic patterns, it is critical to mitigate the impacts of heat stress, understand the reaction to high temperatures, and find viable approaches to improve heat tolerance for wheat production success and productivity in high-temperature environments.

5.2 Effect of High Temperature on Wheat

High-temperature stress is a complex process that affects crop growth and physiologies, resulting in reduced grain yield and quality (Mondal et al. 2013). It can be proven destructive (direct and indirect) on every phases of crop life cycle (Kaushal et al. 2013). The vulnerability of phenological stages to heat stress varies between species and genotypes within the same species. Heat stress can bring certain alteration on wheat anatomy, morphology, physiology, and biochemistry, ultimately affecting the grain yield in wheat (Fig. 5.2).

5.2.1 Effect on Morphology

The primary effect of heat stress in many crops, including wheat, is the impediment of seed germination and poor stand establishment. Heat stress mostly affects the plant meristems and reduces plant growth by inhibition of chlorophyll biosynthesis and promoting leaf senescence and abscission and by dropping photosynthesis (Kosova et al. 2011). Heat stress also causes decrease in root growth which

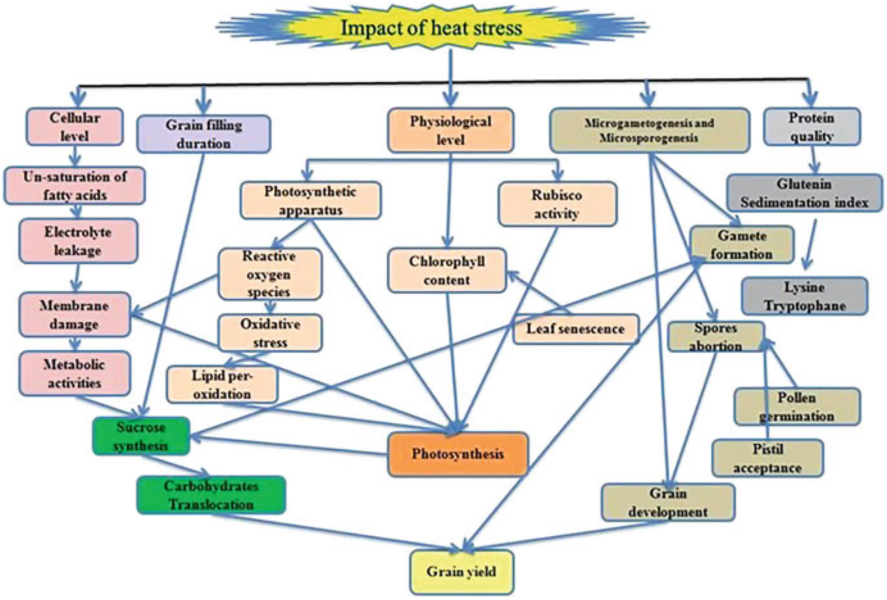

Fig. 5.2 Schematic illustration of the high-temperature impact on wheat associated with the grain yield. (Adapter from Khan et al. (2021))

ultimately affects the crop production (Huang et al. 2012). Elevated temperature (45 °C) also hampers reproductive development in plants causing negative impact on pollen tube formation and pollen mortality (Oshino et al. 2011), impairing development of embryonic cells, followed by improper germination and emergence which leads to poor crop stand (Essemine et al. 2010). High temperature is found to be more hazardous if they prevail in reproductive stage in wheat production (Nawaz et al. 2013). High temperature affects the survivability of the productive tiller, which results in decreased grain yield (53.57%) and tiller number (15.38%) in wheat (Din et al. 2010). The optimum temperature for flowering and grain filling ranges from 12 °C to 22 °C (Sharma et al. 2019a, b). Early stage of gametogenesis is harmed when HS occur during meiosis (Ji et al. 2010). At floral initiation stage, heat stress has a deleterious impact on microspore and pollen cell growth (Kaur and Behl 2010). The grain development phenomenon is influenced by the rate and duration of grain filling, which is particularly sensitive to HS (Gourdji et al. 2013; Lobell and Gourdji 2012). The life cycle of wheat shortens in HS than in normal temperature situation (Alam et al. 2014). 1–2 °C rise in temperature lowers seed weight due to decrease in period of grain filling (Nahar et al. 2010). Short-term heat stress during grain filling may result in loss production to about 23% (Mason et al. 2010). Heat stress during the terminal phase of the plant also inhibits starch biosynthesis which in turn reduces the normal grain size (Kushwaha et al. 2011). Wheat productivity is reduced remarkably due to harmful effect of high temperature in growth process (Janjua et al. 2010). When wheat is subjected to ambient temperature (>35 °C) for a short period of time, it can result in significant loss in grain yield (Sharma et al. 2017).

5.2.2 Effect on Anatomy

High temperature effects plant anatomy by altering leaf area, stomatal aperture, plant height, and pattern of plant developmental stages. The long-term effects of high temperatures on developing seeds resulted in poor germination and vigor, which inhibited emergence and seedling establishment. Heat stress has also been linked to scorching of leaves and twigs, leaf senescence, reduced canopy growth, and low yields (Vollenweider and Gunthardt-Goerg 2005). At the whole plant level, heat stress causes a reduction in cell size, partial closure of stomata to limit water loss, and an increase in the number of xylem vessels in the root and shoot (Anonymous 2007). Significant changes in the chloroplast structure occur at the subcellular level, causing variations in the normal photosynthetic process such as thylakoid membranes and PS-II which are considered the most heat-labile cell components (Ristic et al. 2007). Thylakoid membranes under high temperature show swelling, increased leakiness, physical separation of the chlorophyll light harvesting complex II from the PS-II core complex, and disruption of PS-II-mediated electron transfer (Ristic et al. 2008). The collective effect of the heat stress induced morpho-anatomical alterations may lead to poor canopy formation and decreased productivity.

5.2.3 Effect on Physiology

Physiological responses of wheat to terminal heat stress have been found to be well determined by genotype resistance or susceptibility (Almeselmani et al. 2012). Heat stress has been described as a complex phenomenon affecting plant growth and physiology, ultimately resulting in poor yield and grain quality (Mondal et al. 2013).

5.2.4 Water Relations

Plant water status is one of the important parameters under changing temperatures as it is severely impeded by heat stress (Machado and Paulsen 2001). Heat stress increases evapotranspiration leading to drought stress in crop plants (Lamaoui et al. 2018); thus water relations of the plants are severely affected. Plant water status is generally found to be most erratic under changing ambient temperature. The water relation and content in the plant are affected by high temperatures. Under HS, cell dehydration occurs due to a decrease in osmotic potential (Ahmad et al. 2010). Canopy temperature affects the leaf relative water content, stomatal conductance, and rate of transpiration (Sharma et al. 2019a, b). High temperatures appear to cause dehydration in plant tissue, which inhibits plant growth and development. A temperature of 31 °C is commonly considered the upper limit for maintaining a crop's water status during flowering (Atkinson and Urwin 2012). Farooq et al. (2009) reported that when wheat plants were exposed to heat stress substantially decrease occur in the water potential and the relative water content in leaves, and eventually reduce photosynthetic productivity. After tillering, high temperatures (35/25 °C) considerably reduced wheat water potential, with the drop being greater in genotypes susceptible to heat stress (Almeselmani et al. 2009). Heat stress improves the hydraulic conductivity of cell membranes and plant tissues, mostly due to enhanced aquaporin activity (Martinez-Ballesta et al. 2009) and, to a lesser extent, due to reduced water viscosity (Cochard et al. 2007). When water is limiting, high temperatures have a significant impact on water relations. As a result, improving wheat's thermotolerance could improve its ability to acclimatize to both high temperatures and drought (Machado and Paulsen 2001).

5.2.5 Photosynthesis

The most significant physiological process in plants is photosynthesis, which is greatly influenced by high temperatures leading to poor growth performance in wheat (Feng et al. 2014). Reduced photosynthesis due to decreased leaf area expansion, impaired photosynthetic machinery, premature leaf senescence, and concomitant reduction in wheat yield is a major impact of heat stress (Ashraf and Harris 2013; Mathur et al. 2014). High temperature (~40 °C) results in permanent alternation of RuBisCO, Rubisco Activase (Fig. 5.4), and Photosystem-II (Mathur et al. 2011). Upon exposure of wheat in HS condition, the RuBisCO enzyme was

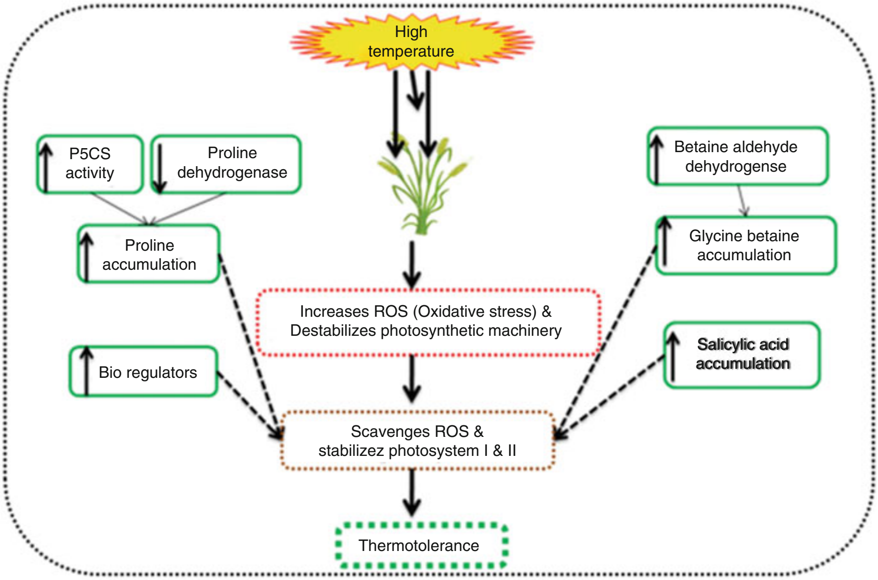

Fig. 5.4 Schematic illustration of osmolytes associated with thermotolerance in wheat

found to be deactivated in less than 7 days (Kumar et al. 2016). Rubisco activase breakdown under heat stress causes decrease in photosynthetic capacity (Raines 2011). Photosystem-II is highly responsive to heat stress in photosynthesizing tissues (Marutani et al. 2012), but photosystem-I is relatively stable (Mathur et al. 2014).Within the PS-II complex, the D1 protein—the main member of PS-II core—is the most sensitive to heat stress (Li et al. 2016). Heat stress also causes change in the fluidity of thylakoid membrane and separation of light harvesting complex II from the photosystem-II. Heat stress damaging and disordering of thylakoid membranes is responsible for the cessation of photophosphorylation (Dias and Lidon 2009). Stroma and thylakoid lamellae are most sensitive to heat stress in wheat where carbon metabolism and photochemical reactions occur, respectively (Mathur et al. 2014). Heat stress causes thylakoid membrane disruption in wheat, which inhibits the activities of membrane-associated electron carriers and enzymes, resulting in a reduced rate of photosynthesis (Ristic et al. 2008). In the absence of a detoxification system, heat stress causes oxidative stress damage by producing reactive oxygen species (ROS). Due to the high temperature, chlorophyll molecules become overexcited, causing ROS generation. During the leaf senescence, ROS production is clearly visible (Chen et al. 2012) and they have deleterious impact on lipid, protein, and DNA, which disrupts cell function. Thermostability of membranes is reduced by 54% as a result of oxidative damage caused by heat stress (Savicka and Škute 2010). ROS accumulated under HS promotes protein denaturation and formation of unsaturated fatty acid, which finally increases cell membrane permeability (Cossani and Reynolds 2012). The production of reactive oxygen species (ROS) by heat stress in the thylakoid membrane has been linked to the degradation of the D1 protein and the inactivation of PS-II (Yamashita et al. 2008).

5.2.6 Leaf Senescence

Protein and nucleic acid degradation, and chloroplast disintegration are accompanied by a highly regulated form of programmed cell death, i.e., leaf senescence (Simeonova et al. 2000). Different biotic or abiotic stresses cause speeding up of senescence. Senescence is attributed by structural changes in the chloroplast due to vacuolar collapse; loss in membrane integrity, disturbance of cellular homeostasis of leaves which are the most distinctive symptoms of heat injury. In the case of wheat premature or mistimed leaf senescence results in the insufficient flow of leaf or stem metabolites to reproductive parts, i.e., spikes and grains, which results into lower grain yield as well as less nutritional value (Gregersen et al. 2013). Thus, senescence-mediated metabolic changes occur in heat stressed wheat plants (Ciuca and Petcu 2009). Reduction in biosynthesis of chlorophyll results in leaf senescence due to elevated temperature (>34 °C) (Pandey et al. 2019). Exposure to heat stress during maturity enhances leaf senescence in wheat plant by turning down of photosystem-II-mediated electron transport and by emphasizing the loss of chloroplast integrity (Haque et al. 2014). However, a large temperature variation diurnally is also responsible for the furtherance of flag leaf senescence in wheat plant (Zhao et al. 2007).

5.2.7 Assimilate Partitioning

Global yield irregularity is mainly contributed by climate-related factors which is about 30–50% (Frieler et al. 2017; Zampieri et al. 2017). Studies reveal that increase in 1 °C global temperature might cause reduction in global wheat yield by 4.1% to 6.4% depending on the measure used for yield forecast (Liu et al. 2016a, b). By hindering the source-sink movement heat stress severely decreases the grain yield in plants. Symplastic and apoplastic pathways which involve assimilate partitioning in the plant system, play significant role in transfer and partitioning of assimilate under heat stress (Taiz and Zeiger 2006; Yang et al. 2002) by reducing the growth and development of crop plants. Source and/or sink limitations can also lead to restriction in seed-set and filling (Lipiec et al. 2013). When heat stress hinders photosynthesis, stem reserves are used as a source of carbon for supporting grain filling during pre-anthesis period (Mohammadi et al. 2009). Carbohydrate translocation increases due to high temperature at the pre-anthesis period from stem to grain which results in the less reduction of starch content in grains of wheat at the post-anthesis heat stress (Wang et al. 2012). Thus heat stress led to reduction in current leaf and ear photosynthesis has serious detrimental effect on grain filling of wheat.

5.3 Effect on Biochemistry

5.3.1 Oxidative Stress

As a consequences of different kind of abiotic stress like heat stress secondary stresses (oxidative stress) may also appear. Catastrophic ROS, including singlet oxygen ($^1O_2$), superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^-) formation takes place in plants exposed to heat stress which in turn leads to oxidative stress (Marutani et al. 2012; Suzuki et al. 2012). Cell injury in many plants including wheat is due to reactive oxygen species that affects the semi-permeability of cellular and subcellular membrane through membrane lipid peroxidation (Xu et al. 2012). Piling up of ROS in cell plasma membrane may occur by depolarizing cell membrane, activating ROS producing enzyme RBOHD and setting off of programmed cell death due to continuous heat stress in plants. For dealing with harmful ROS, plants have antioxidant mechanisms for which they generate various anti-oxidants like catalase (CAT), superoxide dismutase (SOD), and ascorbate peroxidase (APX). H_2O_2 is produced during superoxide dismutase (SOD) mediated detoxification of O_2^-, which is further removed by CAT and APX, thereby ameliorating heat stress in wheat (Suzuki et al. 2011; Caverzan et al. 2016).

5.3.2 Respiration

Effect of high-temperature stress on respiration is yet to be understood clearly (Al-Khatib and Paulsen 1999). Along with the increase in temperature, rate of respiration also increases at such a level that rate of photosynthesis cannot cope up with respiratory losses, which leads to an unpleasant condition called carbon starvation (Almeselmani et al. 2012). Respiration is affected by altered mitochondrial activities under heat stress. In the rhizosphere it changes the solubility of CO_2 and O_2, and the kinetics of Rubisco (Cossani and Reynolds 2012), by enhancing the rate of respiratory carbon loss, reduced production of ATP and also enhances the generation of ROS (Huang et al. 2012). Generally, with increasing temperatures from 0 to 35 or 40 °C (reaches the maximum around 40 to 50 °C) respiration exponentially increases and then again decreases with further increases in temperature above 50 °C (Almeselmani et al. 2009). The rate of respiration in flag leaf of heat susceptible varieties in wheat was notably higher when grown under heat stress (35/25 C day/night)as compared to that of control (23/18 C day/night) (Almeselmani et al. 2012).

5.3.3 Starch Synthesis

Starch is the major constituents of wheat which is about 60–75% of its total dry weight. and is made up of amylose and amylopectin. Starch quality is mainly determined by quantity of amylose content and starch characteristics are affected by variation in the amount of it. High temperature has an adverse effect on dry weight, content of amylose: amylopectin ratio at maturity. Heat stress significantly decreases starch biosynthesis in grains of wheat but at the same time increases the total soluble sugar and protein content (Sumesh et al. 2008; Asthir and Bhatia 2014). Key enzymes that involved in starch biosynthesis are ADP-Glucose Pyrophosphorylase (AGPase) and starch synthase which is available in two forms viz. soluble starch synthase and granule bound starch synthase (Sharma et al. 2019a, b). At high temperature, starch content is reduced in mature wheat grains up to one-third of total endosperm starch and this is because of the decreased efficiency of enzymes involving in starch biosynthesis (Liu et al. 2011). Chauhan et al. (2011) noticed that around 97% of activity of soluble starch synthase is lost at 40 °C, followed by reduced grain growth and starch accumulation in wheat. Asthir and Bhatia (2014) observed increase in total soluble sugar and protein but at the same time lesser amount of starch biosynthesis in wheat grain under heat stress. Wheat grain quality is also dependent on protein content and composition. Iqbal et al. (2017) also reported increased total grain protein content with more essential amino acids fractions, leaf nitrogen content, and sedimentation index.

5.4 Effect on Yield

Temperature range of 12 to 22 °C is suitable for wheat anthesis and grain filling (Shewry 2009). According to Prasad and Djanaguiraman (2014) exposure to the temperature above 24 °C during reproductive stage causes reduction in the grain yield at a significant rate. Again Liu et al. (2016a, b) reported that each degree Celsius increase reduces wheat yield (Fig. 5.3) by 4.1% to 6.4%. Studies of Akter and Islam (2017) revealed that other yield parameters like vegetative weight and number and weight of grain are also affected by high temperature. High temperature accelerates the development of the spike and reduces the number of spikelets and grains per spike (Farooq et al. 2011), thereby affecting the total wheat grain yield. The rate of grain filling in wheat cultivars were more at day and night temperature of 25 and 15 °C respectively which is reduced to a lower level at day and night temperatures of 32 and 22 °C, respectively (Hu et al. 2015). Heat stress influences both size of grains and their numbers as per growth stages. When the heat treatment is forced particularly at an early stage of growth, grain mass is much reduced after anthesis. At temperatures above 20 °C during spike initiation and anthesis stage, an enhanced growth of spike can be observed but the number of spikelet reduces considerably (Semenov and Halford 2009). Heat stress causes shortening of grain filling duration, affecting starch and storage protein deposition (Altenbach 2012). If temperature rises above 30 °C during grain filling, the activity of starch synthesis enzymes reduce (Hurkman et al. 2003), decreasing even more starch accumulation.

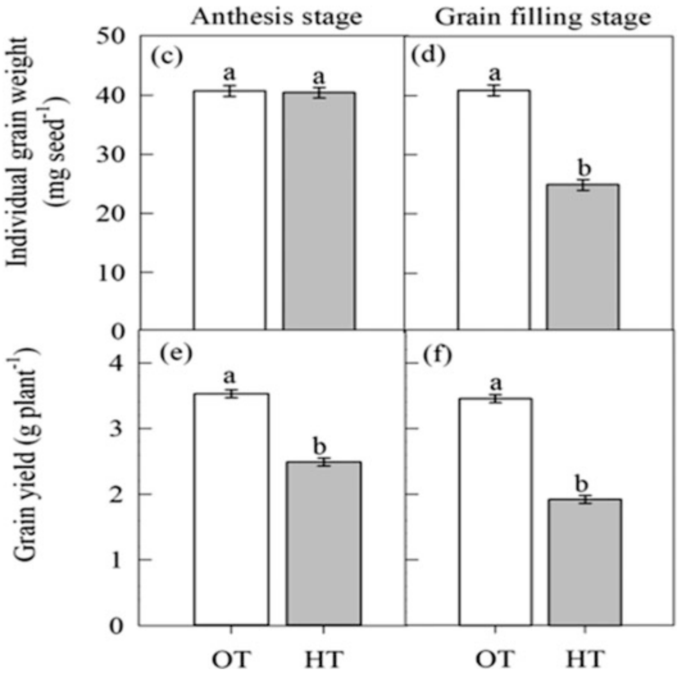

Fig. 5.3 Effect of temperature regimes [optimal temperature (OT: 24/14 °C) and high temperature (HT: 32/22 °C)] on grain yield viz. individual grain weight (mg seed−1), and grain yield (g plant−1) during anthesis and grain filling stage, respectively

5.5 Thermotolerance Mechanism in Wheat

For survival under heat stress plants generally adapt two types of mechanisms: (1) basal thermotolerance (capability of heat tolerance achieved inherently) and (2) acquired thermotolerance (prompted by pre-exposure to higher but non-lethal heat stimulus) (Bokszczanin and Fragkostefanakis 2013). The later has an important role to play in plant survival (Kotak et al. 2007). Hence, the heat stress response is controlled genetically and it can be stimulated by mild or semi-lethal temperatures which further sets off heat stress response in plants (Charng et al. 2006). Moreover, wheat plants endure several physiological mechanisms to deal terminal heat stress including by maturing early (Mondal et al. 2013), remaining green (Kotak et al. 2007; Talukder et al. 2014), reducing canopy temperature (Pinto and Reynolds 2015), accumulating more stem water soluble carbohydrates (Blum et al. 1994) and biomass (Pinto and Reynolds 2015) to convert assimilates into yield. Thus, plants undergo several physiological alterations to cope with high temperature in adverse conditions.

5.5.1 Heat Shock Proteins

The mechanism of synthesis and further folding of polypeptide chain in its native 3D structure determines the functionability of a protein which is disrupted by HS state (Sharma et al. 2019a, b). Protein misfolding has a profound impact on the cell's operating mechanism. Stress causing factors are generated in cell under high temperature which can interfere with the important metabolic activities viz. replication of DNA, transcription, and translation till the cell recover (Biamonti and Caceres 2009). Wheat crop generates heat shock proteins (HSPs) when expose to a temperature range of 32–34 °C which can contribute in protecting against heat stress (Grigorova et al. 2011; Wang et al. 2004). Elevated temperature attacked proteins associated with the biomembrane in crops but stimulates the expression of heat shock genes, encoding the HSPs (Gupta et al. 2013; Lin et al. 2008; Caeiro et al. 2008). These HSPs safeguard the cell from unfavorable effect of high temperature by improving photosynthesis, upregulation of proteins, and metabolic processes (Nadeem et al. 2018). HSPs can be categorized into different families such as HSP100, HSP90, HSP70, HSP60, and small HSPs. Small HSPs of wheat genome assemble with further homo-oligomers and promote binding in ATP independent manners. It associates with HSP90 to avoid unfolding and refolding of proteins under heat stress (Grigorova et al. 2011; Wang et al. 2004). HSP60 expresses constitutively in chloroplast and mitochondria (Hemantaranjan et al. 2014). Large subunit binding protein (chHSP60) of Rubisco is a cofactor of HSP60, which controls the folding of Rubisco enzyme elevated temperature (Xu et al. 2011). HSP70 is a greatly conserved protein, which can bind only with short sequence of the polypeptide chain, temporal and restrain aggregation of non-native protein at elevated temperature (Ye et al. 2020). HSP110 is a subfamily of HSP70; however, it has more capacity to reduce the protein aggregation than the HSP70 (Sumesh et al. 2008). HSP90 regulates transcription, cellular signaling, and managing protein folding through assembling molecular proteins including HSP40 and HSP70 (Sumesh et al. 2008; Lu et al. 2019), whereas HSP100 interacts with different smHSPs and HSP70 to prevent the aggregation of protein (Kumar et al. 2020). Various mechanisms are involved in the expression of genes that encode for HSPs, including temperature sensing, signal transfer to Hsfs, and lastly binding of Hsfs to the heat shock element (HSE) in DNA for gene expression initiation (Al-Whaibi 2011).

5.5.2 Reactive Oxygen Species and Antioxidative Defense Mechanism

HS leads to cell and tissue damage of the plant by producing excessive amount of antagonistic reactive oxygen species (ROS) (Marutani et al. 2012). The cell undergoing oxidative stress produces greater ROS than their scavanging capacity (Mullineaux and Baker 2010) or decrease in free radicals scavenging capacity of cell (Xin et al. 2019). HS associated increase in ROS signals to switch on the antioxidative defense mechanism by activating the free radical scavenging related enzymes (Kumar et al. 2012) which is necessary to save plant from oxidative damage (Xin et al. 2019). Antioxidant enzymes initiate detoxification by converting oxygen and hydroxyl free radicals into H_2O_2, which is then followed by the water molecule. These enzymes scavenge ROS and maintain growth, development, metabolism, and overall productivity, and balance ROS production/elimination from oxidative stress (Caverzan et al. 2016). Antioxidant enzymes viz., POD (peroxidase), SOD (superoxide dismutase), CAT (catalase), and GR (glutathione reductase) usually generate under a high temperature of 35/28 °C day/night in wheat (Caverzan et al. 2016; Wang et al. 2014a, b). The SOD enzyme converts the O^{-2} to H_2O_2, which is a less toxic form than the free radicals (Awasthi et al. 2015). CAT and POD convert H_2O_2 into H_2O, but the CAT activity is higher than other antioxidant enzymes in wheat (Jing et al. 2020). CAT reduces several millions of H_2O_2 molecules into H_2O and oxygen per minute (Sarfraz-Ardakani et al. 2014). GR protects the plant from oxidative stress by reducing oxidized glutathione (Gill et al. 2013). Glutathione peroxidase (GPx) efficiency depends on high γ-glutamyl cysteine synthetase and glutathione synthetase activity for the reduction of H_2O_2 into H_2O (Weydert and Cullen 2010). Although ROS production is linked to oxidative stress, it can also act as a signaling molecule in response to various abiotic stresses, triggering tolerance to such stresses. As a result, ROS should not be fully eliminated and should be maintained at a level that prevents oxidative injury.

5.5.3 Phytohormones

Phytohormones play central roles in the ability of plants to adapt to different environments by mediating growth, development, nutrient allocation, and source/sink transitions (Peleg and Blumwald 2011; Ahammed et al. 2016). Growing evidence shows that the plant hormone abscisic acid (ABA) has an important role in regulation of heat tolerance in wheat. Additionally, ethylene has been linked to a yield penalty under heat stress; lower spike-ethylene contents were strongly associated with higher grain yield (Valluru et al. 2017). Compounds viz., proline, glycine betaine, salicylic acid, abscisic acid, and ethylene maintain the physiology at a high temperature through soluble salts accumulation in the cell and reduce H_2O_2 production in wheat (Fig. 5.4).

Glycine betaine accumulates in the chloroplast of leaves and stabilizes PS-II, reaction centers in the thylakoid membrane (Park et al. 2007), Rubisco enzyme, and inhibits the ROS production (Annunziata et al. 2019). It adjusts the osmotic pressure and ameliorates the antioxidant enzymes activity, and photosynthesis under high temperature in wheat (Wang et al. 2014a, b). Proline accumulation is determined by the proline dehydrogenase activity and Δ1-pyrroline-5-carboxylate synthetase/reductase (P5CS) (Sharma et al. 2019a, b). In tolerant wheat seedlings, high temperatures enhance P5CS and decrease proline dehydrogenase. In mitochondria, proline dehydrogenase catalyzes proline degeneration. However, in the presence of P5CS1, glutamate acts as a precursor for proline synthesis and accumulates in plants under heat stress conditions (Fichman et al. 2015). The proline content of wheat seedlings is directly linked to a high temperature of 35–40 °C, which improves the defensive mechanism (Sattar et al. 2020).

Bioregulators stimulate the antioxidant defense system and keep the PS-II stable at high temperatures. The antioxidant enzyme activity, chlorophyll content, total soluble protein, amino acid, and grain weight in wheat are all increased by foliar application during the grain filling stage and seed priming with a 6.6 mM solution of thiourea (Asthir et al. 2013). Foliar application of 50 ppm dithiothreitol also ameliorates the adverse effect of high temperature in wheat (Agarwal et al. 2017).

5.5.4 Stay Green

The Stay green (SG) linked genotype sustains photosynthesis and grain filling under HS environments through late expression of senescence-associated genes (Vijayalakshmi et al. 2010). It represents the chlorophyll retention and longevity of photosynthetic apparatus for the adaptation of wheat under high temperature (Kamal et al. 2019). SG conserves photosynthetic area and increases nitrogen remobilization to mature grains, making it an essential mechanism for HS tolerance in wheat (Poiroux Gonord et al. 2013). Chlorophyll biosynthesis enzymes determine the senescence in wheat, which influences the assimilation and translocation of photosynthates into grains during grain filling (Shantanu et al. 2018). Increased photosynthetic activity due to SG helps to maintain continuous sugar supply in growing anther and pollen, thus it helps to retain pollen and ovules viability (Ruan 2014). A research was conducted to relate SG traits with canopy temperature depression (CTD) (Dolferus et al. 2011). They found a higher CTD (air temperature-canopy temperature) value in SG genotypes under HS conditions, implying that SG is strongly associated to CTD. As a result, the SG trait in wheat genotypes can be utilized as a selection criterion under heat stress.

5.6 Biotechnological Approach for Improving Heat Tolerance

Heat tolerance can be improved by genetic engineering and transgenic approaches, which can help to alleviate the adverse consequences of heat stress (Chapman et al. 2012). It entails the incorporation of desirable genes into desired plants in order to improve plant tolerance to heat stress (Zheng et al. 2012). However, due to the complexity of the genomic pattern, wheat genetic modification research is challenging. Heat stress for a longer period increases the protein synthesis elongation factor (EF-Tu) in the chloroplast, which is linked to heat tolerance in wheat. In transgenic wheat, constitutive expression of EF-Tu protects leaf proteins from thermal aggregation, reduces thylakoid membrane disruption, improves photosynthetic capacity, and protects against pathogenic microbe infection (Fu et al. 2012). Wheat genotypes with higher EF-Tu were more resistant to heat stress than those with lower EF-Tu (Ristic et al. 2008). Many transcription factors (TFs) involved in various abiotic stresses have recently been discovered and engineered in order to increase crop stress tolerance (Wang et al. 2016). Many plant genome sequences have recently been developed in order to increase stress tolerance. Clavijo et al. (2016) confirmed three previously known and identified one new wheat genomic rearrangements. They used low-cost sequencing technologies, and they expect that researchers will apply the methods shown to sequence multiple wheat varieties.

5.7 Conclusion

Heat stress is a major source of yield loss and the incidence and length of heat events is expected to rise in the future. Further, the frequency of heat stress in wheat is projected to increase worldwide. Wheat is highly sensitive to heat stress, especially during the reproductive and early grain filling stages and as a result, heat stress has become a serious limiting factor in wheat production. Grain setting, duration, rate, quality, and ultimately grain yield are all affected by HS. HS has a genotype-specific effect that is also influenced by the degree, timing, and duration of HS. Despite extensive research into the negative effects of heat stress on wheat, a clear understanding of the mechanism underlying heat tolerance remains elusive. As a result, developing a notable strategy of wheat management under heat stress and foreseeing climate change conditions necessitates the development of a heat stress tolerance mechanism. High-temperature stress alters normal physiological processes resulting in membrane disruption, reduced grain filling period, grain formation, and starch accumulation in grains. It causes oxidative stress by interrupting the photosynthetic machinery and generates reactive oxygen species. The development of a strategy to combat high temperatures necessitates a thorough understanding of the physiological, metabolic, and developmental processes involved in thermotolerance. The tolerance mechanism which includes increased glycine betaine accumulation, antioxidant enzyme activity, heat shock protein and stay green ability could be viable thermotolerance indicators that bring hope to put emphasis on the development of genetically modified more heat tolerant varieties of wheat in future. It is well understood that classical and modern molecular genetics technologies combined with agronomic management practices can overcome the heat syndrome's intricacy.

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Reactive Oxygen Species: Friend or Foe

6

Gyanendra Kumar Rai, Muntazir Mushtaq, Basharat A. Bhat,
Ranjeet Ranjan Kumar, Monika Singh, and Pradeep Kumar Rai

Abstract

Climate change and associated unfavorable abiotic stress conditions, such as drought, salinity, heavy metals, water logging, extreme temperatures, oxygen deprivation, etc., influence plant growth and development to a great extent, eventually disturbing crop yield and quality, finally food security in general. Plant cells produce oxygen radicals and their derivatives, so-called reactive oxygen species (ROS), during different processes related with abiotic stress. Further, the ROS generation is a primary process in higher plants and operates to transmit signaling information at the cellular level in response to the change in environmental conditions. One of the most critical outcomes of abiotic stress is the disruption of the balance between the ROS generation and antioxidant defense systems inducing the excessive ROS accumulation and thus oxidative stress in plants. Remarkably, both enzymatic and nonenzymatic antioxidant defense mechanisms are known to maintain equilibrium between the detoxification and ROS generation under adverse environmental stresses. Even though this area of research has been captivated with massive attention, it mostly remains unfathomed, and our understanding of ROS signaling remains poorly understood.

G. K. Rai (✉) · M. Mushtaq · B. A. Bhat
School of Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Jammu, Jammu and Kashmir, India

R. R. Kumar
Division of Biochemistry, Indian Agriculture Research Institute, New Delhi, India

M. Singh
G.L. Bajaj Institute of Technology and Management, Greater Noida, Nagar, Uttar Pradesh, India

P. K. Rai
Advance center for Horticulture, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, Udheywala, Jammu and Kashmir, India

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In this chapter, we have highlighted the current advancement demonstrating the detrimental effects of ROS, antioxidant defense systems implicated in ROS detoxification during various abiotic stresses, and molecular cross-talk with other key signal molecules such as reactive nitrogen, sulfur, and carbonyl species. Besides, state-of-the-art molecular strategies of ROS-mediated enhancement in antioxidant defense under the acclimation process in response to abiotic stresses in plants have also been covered.

Keywords

Abiotic stress · Antioxidant systems · Oxidative stress · Plant stress tolerance · Reactive nitrogen species · Reactive oxygen species · Stress signaling

6.1 Introduction

Environmental stresses, including salinity, drought, extreme temperature, heavy metals, flooding/waterlogging, etc. are now widespread owing to severe and adverse climate change (Raza et al. 2019; Hasanuzzaman et al. 2020). The aggravation of various abiotic stresses has turned out to be a major menace to global crop production systems. Besides, numerous detrimental effects cause oxidative stress via the overaccumulation of reactive ROS including free radicals (superoxide anion, $O_2^{\bullet -}$; hydroperoxyl radical, $HO_2\bullet$; alkoxy radical, RO•; and hydroxyl radical, •OH) and nonradical molecules (hydrogen peroxide, H_2O_2 and singlet oxygen, $^1O_2$) (Mehla et al. 2017; Hasanuzzaman et al. 2019a, b). The main ROS generation locations in a plant cell are apoplast, chloroplasts, mitochondria, peroxisomes, and plasma membranes (Singh et al. 2019). While ROS are formed in a normal plant cellular metabolism, overaccumulation as a result of stress severely damages indispensable cellular ingredients including carbohydrates, lipids, proteins, DNA, etc. on account of their highly reactive nature (Berwal et al. 2018; Raja et al. 2017). Plants largely respond to oxidative stress by means of an endogenous defense system comprising of different enzymatic (superoxide dismutase, SOD; catalase, CAT; ascorbate peroxidase, APX; glutathione reductase, GR; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione peroxidase, GPX; guaiacol peroxidase, GOPX; glutathione *S*-transferase, GST; Ferritin; nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-like alternative oxidase, AOX; peroxiredoxins, PRXs; thioredoxins, TRXs; glutaredoxin, GRX; etc.) and nonenzymatic (ascorbic acid, AsA; glutathione, GSH; phenolic acids; alkaloids; flavonoids; carotenoids; α-tocopherol; nonprotein amino acids; etc.) antioxidants (Gill and Tuteja 2010; Kumar et al. 2013a, b; Kaur et al. 2019a, b). In plant cells, the antioxidant defensive mechanism and ROS accumulation maintain steady-state equilibrium (Hasanuzzaman et al. 2012). Keeping cellular ROS at an optimum level facilitates accurate redox reactions to take place and the regulation of various processes necessary for growth and development in plants (Mittler 2017). Such optimum level is maintained as a result of equilibrium between ROS production and ROS scavenging (Hasanuzzaman et al. 2019a, b). But, under stress conditions, over-generation of ROS creates imbalance and instigates cell damage, resulting into programmed cell death (PCD), thus reducing crop productivity (Raja et al. 2017). In addition to their damaging activity, ROS are recognized as secondary messengers and are involved in signal transduction to the nucleus via redox reactions using mitogen-activated protein kinase (MAPK) pathway in a number of cellular processes to improve abiotic stress tolerance (Singh et al. 2019). Reactive oxygen species contribute as key molecules during the acclimation process of plants under environmental stimuli by acting as signal transduction molecules, which direct various pathways during the acclimation of the plant under stressed state (Choudhury et al. 2017). A number of investigations have demonstrated that ROS are necessary for the accomplishment of many primary natural processes such as cellular proliferation and differentiation (Mittler 2017). Also, H_2O_2 is an important element in regulation of stress response in plants such as rice (Sohag et al. 2020), wheat (Habib et al. 2020), maize (Terzi et al. 2014), mung bean (Fariduddin et al. 2014), soybean (Guler and Pehlivan 2016), cucumber (Sun et al. 2016), sour orange (Tanou et al. 2012), strawberry (Christou et al. 2014), basil (Gohari et al. 2020), and rapeseed (Hasanuzzaman et al. 2017a, b). Additionally, it is well-known that in addition to ROS, reactive nitrogen species (RNS), reactive sulfur species (RSS), and reactive carbonyl species (RCS) are also involved in signal transduction as well as in a cross-talk in plant tolerance to abiotic stress (Yamasaki et al. 2019). Thus, ROS play a central, dual role in plant biology, exhibiting a fascinating research area for plant biologists. In this chapter, we recapitulate the latest progress of harmful effects of ROS, antioxidant defensive mechanism implicated in ROS detoxification during different abiotic stresses, and as well the cross-talk of RNS, RSS, and RCS with ROS. We also spotlight on development in molecular approaches of ROS-mediated improvement in plant antioxidant defense during the acclimation process against abiotic stress.

6.2 ROS Formation and Types

In plants, ROS are generated in many cellular compartments including chloroplasts, mitochondria, peroxisomes, and plasma membrane (Dmitrieva et al. 2020). In the chloroplast, light quanta are absorbed by chlorophyll (chl) molecules and are excited to their triplet state. If this triplet chl is not quenched well, recombination of charge takes place leading $^3O_2$ to excited $^1O_2$ (Dmitrieva et al. 2020). Though its lifetime is extremely short (3.1–3.9 μs) and diffusion distance is small (190 nm), $^1O_2$ diffuses outside the chloroplast to reach the cell wall, targets plasma membrane, tonoplast, or even cytosolic signaling cascades (Fischer et al. 2013). Furthermore, $^3O_2$ could receive electrons from electron transport chain or nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity generating $O_2^{\bullet -}$ having a half-life of 1–1000 μs (Hasanuzzaman et al. 2019a, b). Additionally, $O_2^{\bullet -}$ reacts with H+ generating $HO_2^{\bullet -}$, which is far more reactive, stable, and permeable through biological membranes. Likewise, H_2O_2 can be generated during the dismutation of

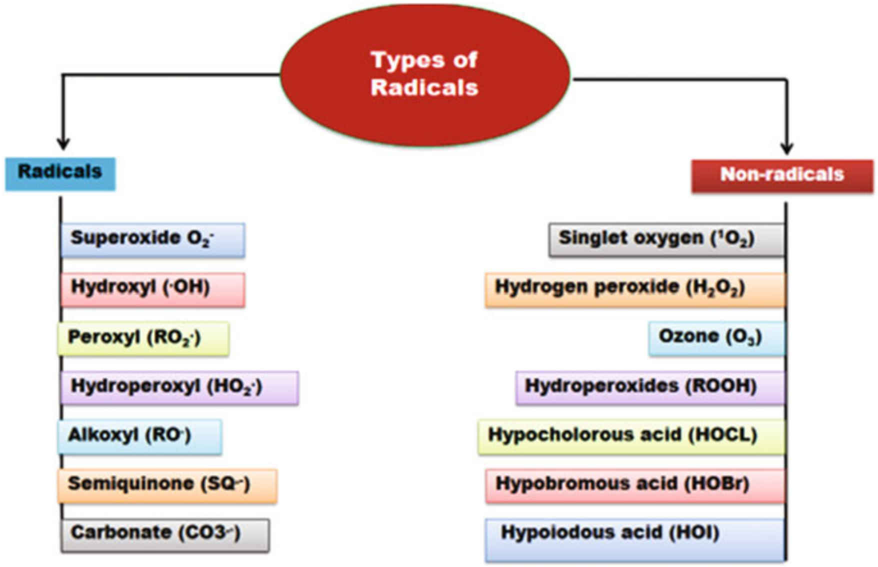

Fig. 6.1 Various types of reactive oxygen species/free radicals generated in plant systems

$O_2^{\bullet -}/HO_2^{\bullet -}$ by SOD isoforms, NADPH oxidases, and heme-containing class III peroxidases (POX) activity (Rejeb et al. 2015; Berwal et al. 2018). Chemically, H_2O_2 is a weak acid with high diffusibility and stability, with a life span of <1 s and can cross the plasma membrane through aquaporins (Mhamdi et al. 2012). Another in place of one more essential ROS •OH can be generated during the Fenton reaction, hydroperoxides activity during sunlight, and inner-sphere electron transfer. Furthermore, proteins, for example heme oxygenases, cytochrome P450s, superoxide reductases, and some photosystem II (PSII) proteins, also generate •OH (Demidchik 2015). The calculated half-life of •HO is about 1 ns and has a short diffusibility of <1 nm.

Cellular ROS constitutes both free radical and non-radicals (Fig. 6.1). Among the free radicals, $O_2^{\bullet -}$, •OH, RO•, and peroxyl radical (ROO•) and non-radicals, H_2O_2, $^1O_2$, and ozone (O_3) are very common (Maurya 2020). Nevertheless, some other non-radicals are also present in plants for example hypochlorous acid (HOCl), hydroperoxides (ROOH), and excited carbonyls (RO*) (Kapoor et al. 2015). In addition, reactive oxygen intermediates (ROI) are also categorized as reactive oxygen molecules generated by incomplete O_2 reduction; thus, ROS comprise all kinds of ROI as well as O_3 and $^1O_2$ (Fig. 6.1). Also, some acids like hypobromous acid (HOBr), hypoiodous acid (HOI), and HOCl and radicals like carbonate radical ($CO_3^{\bullet -}$) and semiquinone ($SQ^{\bullet -}$) are also incorporated into ROS (Waszczak et al. 2018).

Among ROS radicles, $O_2^{\bullet -}$ is a primary reducing agent that forms strong oxidants. Furthermore, RNSs, RSSs, and RCSs are generated on reaction of $O_2^{\bullet -}$

with nitric oxide (NO). These compounds further cause oxidative stress, and play a vital role in "shaping" the intra- and extracellular redox signals (Suzuki et al. 2012).

6.3 Localization and Processes of the Generation of ROS in Plant Cells

In plant cells, ROS is mainly generated in chloroplasts, mitochondria, peroxisomes, plasma membrane, as well as cell wall (Kohli et al. 2019; Kumar et al. 2021). Consequently, compartmental ROS generation in plants sums to its total production (Singh et al. 2019). Chloroplasts are the primary spots for ROS generation depending on the interaction of chl and light, where triplet chl and ETC of PS I and II play main role in main generation of ROS (Dietz 2016; Kim and Dogra 2019; Singh et al. 2019). Mitochondria are considered as the main site of ROS generation in case of nongreen parts of a plant such as roots. In mitochondria, leakage of electrons from both complex I and III of ETC generates $O_2^{\bullet-}$, which later on gets converted into H_2O_2 by Mn-SOD and CuZn-SOD (Singh et al. 2019). The prime source of ROS generation in peroxisomes is glycolate oxidase (GOX) (Kerchev et al. 2016). Also, $O_2^{\bullet-}$ and uric acid are produced in peroxisomal matrix by the activity of xanthine oxidase (XOD), which further dismutates to H_2O_2 by SOD and urate oxidase (UO), respectively (Corpas et al. 2019). In addition to β-oxidation of fatty acids, H_2O_2 is also generated in peroxisomes due to $O_2^{\bullet-}$ disproportionation and flavin oxidase activity (Gilroy et al. 2016). In addition, copper amine oxidase, polyamine oxidase, sulfite oxidase, and sarcosine oxidase enzyme activity also results in generation of H_2O_2 in peroxisome (Corpas et al. 2020). Nevertheless, MDHAR has been established to hydrolyze H_2O_2 through AsA-GSH cycle and regenerate AsA in peroxisomes (Lisenbee et al. 2005), while NADPH oxidase, class III POX, amine and germin-like oxalate oxidases, quinine reductase, and lipoxygenases (LOX) guide the ROS generation in apoplast (Mittler 2017; Choudhary et al. 2020). Fatty acid oxidation as well as also GOX and UO activities produce $O_2^{\bullet-}$ and H_2O_2 in glyoxysomes (Jeevan Kumar et al. 2015). Furthermore, XOD and aldehyde oxidase (AO) potentially contribute to ROS production in cytosol (Jeevan Kumar et al. 2015) (Table 6.1).

6.4 Antioxidant Defense and Plant Abiotic Stress: Recent Approaches

Plants trigger their antioxidant defense system in order to alleviate the unfavorable effects of oxidative stress. However, antioxidant defense role differs between plant species and genotypes, as well as stress types and duration (Table 6.2). Further, various strategies to improve antioxidant defense in plants have also been revealed (Table 6.2).

Table 6.1 Reaction mechanisms of major reactive oxygen species (ROS) scavenging enzymatic antioxidants

Antioxidants	Reactions catalyzed	Catalytic reaction sites
Nonenzymatic		
Ascorbic acid	Scavenges $O_2^{\bullet-}$, H_2O_2, $^{\bullet}OH$, and $^1O_2$	Chloroplast, peroxisomes, cytosol, mitochondria, apoplast
Glutathione	Scavenges H_2O_2, $^{\bullet}OH$, and $^1O_2$	Chloroplast, peroxisomes, cytosol, mitochondria, apoplast
Tocopherol	Scavenges $^{\bullet}OH$, $^1O_2$, $ROO^{\bullet}$, and ROOH	Thylakoid membrane of chloroplast
Carotenoids	Scavenges mainly $^1O_2$	Chloroplast
Flavonoids	Scavenges $O_2^{\bullet-}$, H_2O_2, and $^1O_2$	Chloroplast, vacuole
Phenolic acids	Scavenges $O_2^{\bullet-}$, $^{\bullet}OH$, $ROO^{\bullet}$, and $ONOO^-$	Cell wall
Alkaloids	Scavenges $O_2^{\bullet-}$, $^{\bullet}OH$, H_2O_2, and $^1O_2$	Vacuole
Nonprotein amino acids	Scavenges $O_2^{\bullet-}$, H_2O_2, and $^1O_2$	Cytosol, mitochondria, cell wall
Enzymatic		
Superoxide dismutase (SOD; EC 1.15.1.1)	$^2O_2^{\bullet-} + 2H^+ \rightarrow O_2 + H_2O_2$	Chloroplast, peroxisomes, cytosol, mitochondria, apoplast
Catalase (CAT; EC 1.11.1.6)	$2H_2O_2 \rightarrow 2H_2O + O_2$	Peroxisomes
Peroxidases (POX; EC 1.11.1.7)	$2PhOH + H_2O_2 \rightarrow 2PhO^{\bullet} + 2H_2O$ $2PhO^{\bullet} \rightarrow$ cross-linked substances $PhO^{\bullet} + Asc \rightarrow PhOH + MDHA$ $PhO^{\bullet} + MDHA \rightarrow PhOH + DHA$	Cell wall, apoplast, vacuole
Polyphenol oxidase (PPO; EC 1.14.18.1)	$PhOH + O_2 \rightarrow$ Catechols + $O_2 \rightarrow Q + H_2O$	Thylakoid membrane of chloroplast, cytosol, vacuole
Ascorbate peroxidase (APX; EC 1.11.1.11)	$H_2O_2 + AsA \rightarrow 2H_2O + MDHA$	Chloroplast, peroxisomes, cytosol, mitochondria, apoplast
Monodehydroascorbate reductase (MDHAR; EC 1.6.5.4)	$MDHA + NAD(P)H \rightarrow AsA + NAD(P)^+$	Chloroplast, cytosol, mitochondria
Dehydroascorbate reductase (DHAR; EC 1.8.5.1)	$2GSH + DHA \rightarrow GSSG + AsA$	Chloroplast, cytosol, mitochondria
Glutathione reductase (GR; EC 1.6.4.2)	$GSSG + NADPH + H^+ \rightarrow GSH + NADP^+$	Chloroplast, cytosol, mitochondria
Glutathione peroxidase (GPX; EC 1.11.1.9)	$H_2O_2 + GSH \rightarrow H_2O + GSSG$	Cytosol, mitochondria
Glutathione S-transferase (GST; EC 2.5.1.18)	$R\text{-}X + GSH \rightarrow GS\text{-}R + H\text{-}X$	Chloroplast, cytosol, mitochondria

(continued)

Table 6.1 (continued)

Antioxidants	Reactions catalyzed	Catalytic reaction sites
Peroxiredoxins (PRX; EC 1.11.1.15)	H_2O_2 + PRX-S^- → OH^- + PRX-SOH + GSH → PRX-SSG + H_2O PRX-SSG + GSH → PRX-S^- + GSSG	Cytosol, chloroplasts, mitochondria, nucleus, extracellular spaces
Thioredoxin (TRX; EC 1.8. 1.9)	TRX-RS_2 + NADPH + H^+ → TRX-R$(SH)_2$ + $NADP^+$	Chloroplast, cytosol, mitochondria

6.4.1 Antioxidant Defense in Plants Under Salinity

Regulation of antioxidant mechanism improves the salt stress effects in plants, as delineated in various works (Table 6.2). It has been reported that differential activities of antioxidant enzymes vary in terms of salinity extent, exposure time, and the plant developmental stages (Li et al. 2019). Vighi et al. (2017) recorded differential response in salt-tolerant rice cultivar in contrast to salt-sensitive and revealed that *OsAPX3*, *OsGR2*, *OsGR3*, and *OsSOD3-Cu/Zn* genes were the basic differential markers between tolerant and sensitive rice genotypes. In another study, wheat (salt-tolerant cv. Suntop and salt-sensitive Sunmate) and barley (salt-tolerant cv. CM72) cultivars were compared and revealed that higher antioxidant activity (SOD, peroxidase; POD, APX, GR, and CAT) is strongly associated with the higher tolerance to salinity demonstrating an apparent antioxidant role in enhancement of oxidative stress induced by salinity (Zeeshan et al. 2020). In the same way, Alzahrani et al. (2019) reported higher levels of SOD, CAT, GR, and AsA in *Vicia faba* genotypes, when H_2O_2 concentration increased over 90% during salt stress, thus validating the antioxidant response regulation under salinity stress and its mitigation. Antioxidant activity can be regulated by employing either chemical or natural protectants against salinity has been demonstrated to play vital role in antioxidant response for ameliorating stresses in plants for example salinity (Zulfiqar et al. 2019, 2020). Alsahli et al. (2019) reported that a twofold increase in SOD, CAT, and APX activity resulted into threefold decrease in H_2O_2 in wheat under salinity stress on application of salicylic acid (SA) in contrast to control plants. Also, the application of jasmonic acid (JA) and humic acid together enhanced APX activity, improving salt tolerance in sorghum (Ali et al. 2020), whereas application of polyamines exogenously controlled antioxidant responses in sour orange when grown under high salinity conditions (Tanou et al. 2014).

6.4.2 Role of Antioxidants in Plants Under Water Scarcity and Drought Stress

Various studies have demonstrated the activity of antioxidant defense system under drought stress in various plant species (Table 6.2). In a study carried out by Nahar et al. (2017), decrease in AsA/DHA and GSH/GSSG ratio due to enhanced activities of APX, GR, GPX, and GST in mung bean seedlings compared to control in

Table 6.2 Antioxidant defense in plants under different abiotic stress factors

Plant species	Stress conditions	Antioxidant defense	References
Salinity			
Triticum aestivum	100 mM NaCl; 20 days	Nitrogen supplementations increased the activity of SOD, CAT, GR, MDHAR, and DHAR by twofold and APX threefold, respectively, compared to untreated	Ahanger et al. (2020)
Nicotiana benthamiana	150 mM NaCl; 15 days	Acetylcholine application increased SOD by onefold and POD by twofold	Qin et al. (2020)
Solanum lycopersicum	150 mM NaCl; 5 days	Vanillic acid increased AsA/DHA, GSH/GSSG, MDHAR, GR, GST, SOD, and CAT by 161%, 90%, 18%, 53%, 87%, 43%, and 105%, respectively	Parvin et al. (2020)
Medicago sativa	250 mM NaCl; 2 weeks	Melatonin increased the activities of CAT, POX, and Cu/Zn-SOD	Cen et al. (2020)
Cucumis sativus	150 mM NaCl; 3 days	Melatonin increased CAT, SOD, POD, and APX by 23%, 29%, 15%, and 16%, respectively	Zhang et al. (2020a, b)
T. aestivum	100 mM NaCl; 20 days	Sodium nitroprusside (SNP) and glucose solely increased Cys and GSH content by 86% and 79%, and 19% and 18%, respectively, whereas SOD, CAT, APX, and GR increased by 75% and 65%, 49% and 37%, 97% and 57%, and 60% and 57%, respectively. Combined SNP and glucose application increased the activity of these antioxidant enzymes (SOD, CAT, APX, and GR) by 138%, 61%, 271%, 127% and 44%, 17%, 119%, 23%, respectively, compared to the control and glucose-treated plants	Sehar et al. (2019)
C. sativus	200 mM NaCl; 7 days	H2S increased ASA content by 42.6% and GR activity by 9.1%. Reversed decreased SOD and POD activity	Jiang et al. (2019)
Brassica juncea	100 mM NaCl; 15 days	Nitric oxide increased SOD, CAT, APX, and GR activity by 91%, 33%, 114%, and 49%, respectively	Jahan et al. (2020)

Water deficit and simulated drought			
Zea mays cv. Run Nong 35, Wan Dan 13 and Dong Dan 80	Mild drought (80% FC), moderate drought (60% FC), and severe drought (40% FC)	Increased activities of APX, MDHAR, and DHAR by 24%, 13%, and 29% in Dong Dan. 80% and 16%, 11%, and 10% in Wan Dan 13, respectively, under severe drought. Higher SOD activity as well as AsA and DHA contents under moderate and severe drought in both maize hybrids	Anjum et al. (2017)
Glycine max and *G. tomentella*	Water deficit, flowering stage, 12 days	A substantially increased SOD and GR enzymes activities with the highest value during eighth day of stress treatment in *G. max*. A gradual increase in GR activity till the end of drought treatment was observed in *G. tomentella*	Hamim et al. (2017)
Brassica napus cv. Binasarisha-3	Osmotic stress (10% and 20% PEG), 48 h	MDHAR activity was higher under 10% PEG. DHAR activity increased under both stress level. GR and GST activity was higher by 26% and 23% and 25% and 31% at both stress level, respectively	Hasanuzzaman et al. (2017a, b)
Phaseolus vulgaris cv. Bn-150 (drought-tolerant) and Bn-16 (drought-sensitive)	Moderate drought (50% FC) and severe drought (0% FC), 14 days	Significantly increased total phenolic contents of Bn-150 by 223% and 265%, respectively, under moderate and severe drought. SOD, CAT, APX, and GR activities were increased in tolerant genotypes (Bn-150) than the sensitive one (Bn-16)	Kusvuran and Dasgan (2017)
Vigna radiata cv. BARI Mung-2	Osmotic stress (5% PEG), 48 h	Decreased AsA/DHA ratio by 54%. Increased APX and GR by 20% and 42%, respectively. Reduced CAT and MDHAR activity by 13% and 26%, respectively	Nahar et al. (2017)
B. napus cv. Binasarisha-3	Osmotic stress (10% and 20% PEG), 48 h	Moderate stress increased AsA content, GPX, and GST activity but reduced CAT activity, whereas severe stress enhanced APX activity but reduced MDHAR, DHAR, and GR activities. Both levels of stress increased GSH and GSSG contents by 31% and 26%; and 83% and 225%, respectively, compared to control	Hasanuzzaman et al. (2018a, b)
B. rapa cv. BARI Sharisha-15	Osmotic stress (20% PEG), 2 days	AsA and GSH contents increased by 10% and 72%, respectively. APX, GR, CAT, and GPX activity increased by 23%, 81%, 29%, and 26%, respectively	Bhuiyan et al. (2019)

(continued)

Table 6.2 (continued)

Plant species	Stress conditions	Antioxidant defense	References
Oryza sativa var. *japonica* cv. Nipponbare	Osmotic stress (20% PEG-6000), 5 days	Decreased SOD, APX, and CAT activities, but POD activity increased by 59% compared to control	Liu et al. (2019)
S. lycopersicum cv. Login 935	Drought stress (60% FC), 20 days	Enhanced SOD, CAT, and APX activities by 110%, 66%, and 77%, respectively. Increased AsA, GSH, and α-tocopherol contents by 81%, 93%, and 103%, respectively	Rady et al. (2020)
G. max	Osmotic stress (5%, 10%, and 15% PEG), 3 weeks	Highest activities of CAT, APX, and PPO were observed at mild osmotic stress (5% PEG), whereas increased SOD and POX activities were found at severe osmotic stress (15% PEG). Total phenol and tocopherol contents increased by 51%, 32%, and 44%, and 26%, 26%, and 21% at three levels of osmotic stress intensities, respectively, compared to control	Rezayian et al. (2020)
Toxic metals/metalloids			
Brassica napus cv. BINA Sharisha-3	0.5 and 1.0 mM $CdCl_2$, 2 days	Reduction of AsA content, whereas higher GSSG content and GST activity. APX and GR activity increased, but CAT, MDHAR, and DHAR activity reduced	Hasanuzzaman et al. (2017a, b)
V. radiata cv. BARI mung-2	0.5 mM $AlCl_3$, 2 and 3 days	AsA content reduced, but GSH and GSSG increased. The activity of SOD, GST, GPX, APX, and GR increased, but MDHAR, DHAR, and CAT decreased	Nahar et al. (2017)
Oryza sativa cv. BRRI dhan54	0.25 and 0.5 mM $NiSO_4 \cdot 7H_2O$, 3 days	64% lower AsA and 146% higher GSH content at 0.5 mM Ni stress. APX, MDHAR, DHAR, and GR activities increased by 114%, 116%, 31%, and 104% at 0.5 mM Ni stress, respectively	Hasanuzzaman et al. (2019a, b)
Pisum sativum	100 μM $NiCl_2$, 3 days	GSH accumulation increased by fivefold. SOD activity increased by 14-fold, CAT and APX activities both by sixfold, and GR activity by almost threefold	El-Amier et al. (2019)
P. sativum	100 μM $CdCl_2$, 3 days	GSH accumulation increased by threefold and GSSG by twofold. SOD activity increased by tenfold, CAT and APX activities both by eightfold, and GR activity by almost fourfold	El-Amier et al. (2019)

B. juncea cv. BARI Sharisha-11	0.5 and 1.0 mM CdCl2, 3 days	42% lower AsA and 200% higher DHA at severe stress, whereas 44% and 72% higher GSSG content under mild and severe stress, respectively. 44% higher SOD and 31% higher GPX activity at severe stress	Al Mahmud et al. (2018)
T. aestivum cv. Pradip	0.5 and 1.0 mM $Pb(NO_3)_2$, 2 days	APX activity increased, but MDHAR and DHAR decreased; GR increased initially and then declined. 35% higher SOD, 44% higher GST along with 31% lower CAT, and 28% lower GPX activities were reported	Hasanuzzaman et al. (2018a, b)
Extreme temperature			
Cicer arietinum (sensitive genotype: ICC14183, ICC5912; tolerant genotypes: ICCV07110, ICCV92944)	30/20, 35/25, 40/30, and 45/35 °C; 2 days for flower and 8 days for three leaves stage	Reduced APX (by 38–49% and 43–50% at 40/30 °C) and GR (by 30–46% and 44–49% at 45/35 °C) activity as well as AsA (by 13–18% and 28–32% at 40/30 °C), and GSH (by 24–33% and 37–44% at 45/35 °C) content in sensitive genotypes	Kumar et al. (2013a, b)
Cucumis sativus	35 ± 1 °C; 7 days	Improved SOD (by 16.6%), CAT (by 13%), APX (by 25.2%), GR (by 14.4%), and POD (by 35.4%) activity	Ding et al. (2016)
Sorghum bicolor	36/26 and 39/29 °C; until 7 days after full anthesis	In pollen, decreased the SOD (58–87%), CAT (44–56%), and POX (36–60%) activity. In pistil, decreased the SOD (59–77%), CAT (35–60%), and POX (42–78%) activity	Djanaguiraman et al. (2018)
Gossypium hirsutum	45/30 ± 2 °C; 120 days	Enhanced SOD and CAT activity	Sarwar et al. (2018)
O. sativa	38 °C; 5 days	Decreased the activity of SOD and CAT. Enhanced POD (by 32.1%) activity	Liu et al. (2019)
O. sativa cv. DM You 6188	12 °C; 6 days	Enhanced SOD (by 1.4%), CAT (by 1.58%), and GSH/GSSG (by 2.42-fold)	Han et al. (2017)
Calendula officinalis	4 °C; 24, 48, 72, 96, and 120 h	Elevated GR (161%), SOD (46%), and APX (82%) activity at 120 h	Jan et al. (2018)
Capsella bursa-pastoris	10 °C; 24, 48, 72, 96, and 120 h	Elevated GR (70%), POD (79%), and CAT (70%) activity at 120 h	Wani et al. (2018)
Citrus reticulata	1, −1, and −3 °C; 3 h	Enhanced CAT (1.35-fold) and APX (twofold) activities	Mohammadrezakhani et al. (2019)

(continued)

Table 6.2 (continued)

Plant species	Stress conditions	Antioxidant defense	References
Vitis vinifera	5 °C; 6, 12, 24, 48, and 72 h	Elevated GR (20.26%), DHAR (7.64%), and MDHAR (16.60%) activities with increased AsA (12.13%), DHA (7.89%), and GSH (56.09%) contents	Chen et al. (2019)
Waterlogging			
S. bicolor cv. JN01 and JZ31	Waterlogged soil, 12 days	Increased SOD (by 1.38- and 1.5-fold) and CAT (by 1.43- and 1.36-fold) in JN01 and JZ31, respectively	Zhang et al. (2019)
S. lycopersicum cv. Roma	Waterlogged soil, 15 days	AsA content reduced by 31%. SOD, CAT, and POD activities increased by 7%, 33%, and 57%, respectively, compared with control samples	Rasheed et al. (2018)
Sesamum indicum cv. BARI Til-4	Waterlogged soil, 2, 4, 6, and 8 days	GSH and GSSG increased by 45% and 150%, respectively, whereas AsA content decreased by 38% after 8 days WL. APX and MDHAR activity increased by 61% and 55%, but DHAR and GR activity reduced by 59% and 23%, respectively, after 8 days WL	Anee et al. (2019)
Deschampsia antarctica	Waterlogged soil, 7 days	Increment of CAT activity by 91%	Park and Lee (2019)
Hordeum vulgare cvs. TF57 and TF58	Waterlogged soil, 21 days	SOD, POD, and CAT activities increased in both WL-sensitive TF57 and WL-tolerant TF58 genotype	Luan et al. (2018)

response to drought stress, which resulted into drought-induced tolerance to oxidative stress. Akram et al. (2018) reported the increase in total phenolics and POD and CAT activities in the two *B. napus* cultivars under drought stress (60% FC, 21 days). A group of researchers studied the two *Sorghum bicolor* L. cultivars, M-81E (tolerant) and Roma (sensitive) and observed the increased H_2O_2 concentration in both M-81E and Roma, respectively, in contrast to control, when activities of SOD and APX increased respectively, thus improved tolerance to drought stress (Guo et al. 2018). Another study conducted by Hassan et al. (2020) reported decreased CAT activity but increased GPX activity under drought stress in *Triticum aestivum* cv. Sakha-94 (Hassan et al. 2020).

6.4.3 Antioxidant Defense in Plants Under Toxic Metals/Metalloids

Various investigations have demonstrated the positive correlation between tolerance to metals/metalloids toxicity with improved antioxidant activities for ROS detoxification and metal chelation (Table 6.2) (Gratao et al. 2019). Among major antioxidants, GST assists GSH to reduce toxicity to metals/metalloids on conjugation with them (Kumar and Trivedi 2018). In addition, GSH functions as a cytosolic precursor of phytochelatins (PC), binds to metals and allows the transport of compound into cell vacuole by catalyzing the transport of metal ions and other xenobiotics (Chakravarthi et al. 2006). Hasanuzzaman et al. (2019a, b) reported an increase in both the GSH and GSSG in rice seedlings under Ni stress, but under the application of exogenous Si, GSH content was further enhanced while GSSG level decreased, indicating the function of Si in upregulating GSH. Ahanger et al. (2020) reported an enhancement in both GSH and tocopherol content together with SOD, GST, and DHAR activities with elevated H_2O_2 and $O_2^{\bullet-}$ concentrations in *V. angularis* seedlings under Cd stress, while AsA levels and CAT activity were found to be reduced. On the contrary, activities of SOD, CAT, POX, and GR were increased with elevated levels of H_2O_2 under Cd stress in two *Mentha arvensis* genotypes indicating the induction of an antioxidant defense mechanism in response to Cd toxicity (Zaid et al. 2020). The authors also observed a further upregulation of antioxidant defense activity after application of gibberellic acid, triacontanol, or SA.

6.4.4 Antioxidant Defense in Plants Under High Temperature

Like other abiotic stress factors, the antioxidant defense mechanism is also activated to cope with high temperature (HT) stress in plants (Table 6.2) (Ding et al. 2016), but in general antioxidant activity varies between species as well as tolerant and sensitive genotypes (Hasanuzzaman et al. 2012). According to Kumar et al. (2013a, b), APX and GR activities were considerably reduced in sensitive chickpea cultivars with approximately twofold H_2O_2 increase under high temperature conditions compared to tolerant genotypes. Liu et al. (2019) reported reduced activities of SOD and CAT with subsequent decreased OsSOD, OsCAT, and OsAPX2 expression, causing elevated levels of H_2O_2 in germinating rice seeds in response to high temperature stress. Sarkar et al. (2016) reported increased activity of CAT and POX in wheat in response to high temperature stress. In another study, Zandalinas et al. (2017) reported enhanced GSH and AsA levels in Carrizo citrange under HT stress (40 °C) with enhanced SOD and CAT activities in Cleopatra mandarin.

6.5 Plant Antioxidant Defense System

Antioxidants have been shown to either directly or indirectly scavenge reactive oxygen species (ROS) and/or inhibit ROS generation (Carocho and Ferreira 2013). Nonenzymatic antioxidants such as tocopherols, phenolic compounds (PhOH), flavonoids, alkaloids, AsA, GSH as well as several nonprotein amino acids make up the plant antioxidant defense system (Hasanuzzaman et al. 2019a, b). In order to limit the ROS production, the nonenzymatic antioxidants operate in a coordinated approach with antioxidant enzymes such as SOD, POX, CAT, APX, MDHAR, DHAR, GR, GPX. TRX, GST, PRX, and polyphenol oxidase (PPO) (Fig. 6.2) (Laxa et al. 2019). In plant defense system, the catalytic reactions occur in the cellular organs and between enzymatic and nonenzymatic antioxidants as represented in Table 6.1. In plants, the SOD enzyme plays a crucial role linked directly to the stress tolerance and has been considered as first line of defense by converting O_2 into H_2O_2 (Table 6.1) (Del Río et al. 2018). This generated H_2O_2 further converts into H_2O with the help of enzymes such as CAT, GPX, and APX or it can be catalyzed in the AsA-GSH cycle. The AsA-GSH cycle, also known as the Asada-Halliwell cycle, considered as a major antioxidant defense system in plants and plays a crucial role to catalyze H_2O_2. The cycle consists of four antioxidant key enzymes such as APX, MDHAR, DHAR, and GR as well as low molecular weight nonenzymatic antioxidants like AsA and GSH. In plants, the AsA-GSH cycle plays critical function in the antioxidant defense system by minimizing H_2O_2 concentration and maintaining the redox homeostasis (Fotopoulos et al. 2010). Furthermore, detoxification of H_2O_2 and xenobiotics requires two vital enzymes such as GPX and GST (Fig. 6.2) (Hasanuzzaman et al. 2018a, b). Among the nonenzymatic antioxidants, AsA and GSH are the most abundant soluble antioxidants in the higher plants (Foyer and Noctor 2011). These play an important role as electron donors and actively scavenge ROS via the AsA-GSH cycle (Hasanuzzaman et al. 2019a, b). In addition, the concentration of cellular ROS lowers by interaction of beta-carotene with OH, O_2, and ROOH (Kapoor et al. 2019).

6.5.1 Nonenzymatic Antioxidants

In plants, there are a number of nonenzymatic antioxidants playing an important role in the ROS scavenging. Among the nonenzymatic antioxidants, ascorbate contributes momentous role to scavenge ROS molecules through AsA-GSH cycle by donating electrons and endures stability due to delocalization of electrons caused

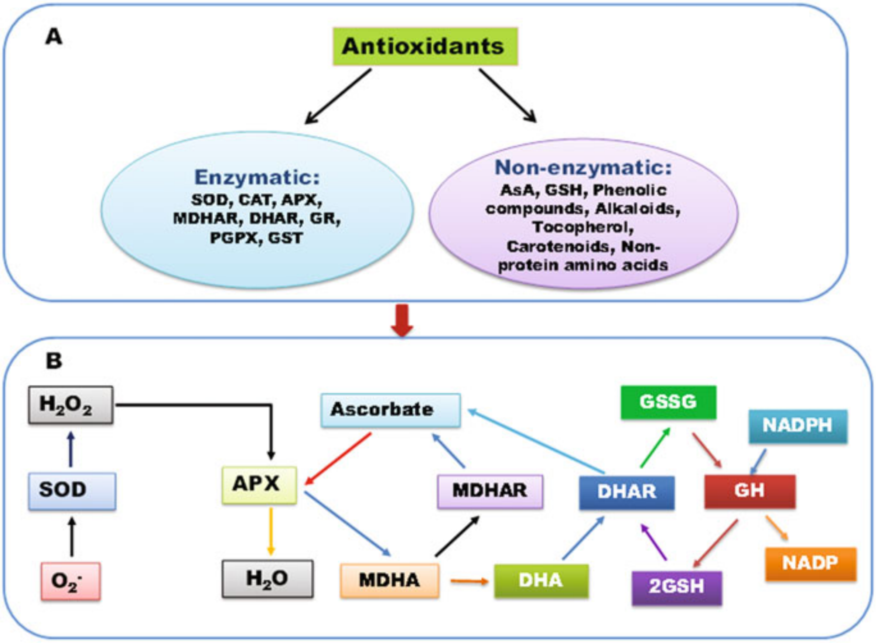

Fig. 6.2 Overview of plant antioxidant defense system: (**a**) types of antioxidants and (**b**) combined mechanisms of enzymatic and nonenzymatic antioxidants. See the text for a more detailed description. APX, ascorbate peroxidase; AsA, ascorbate; CAT, catalase; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; GPX, glutathione peroxidase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione S-transferase; H_2O_2, hydrogen peroxide; MDHA, monodehydroascorbate; MDHAR, monodehydroascorbate reductase; NADPH, nicotinamide adenine dinucleotide phosphate; $O_2^{\bullet -}$, superoxide anion; POX, peroxidases; PRX, peroxiredoxins; R, aliphatic, aromatic, or heterocyclic group; ROOH, hydroperoxides; –SH, thiolate; SOD, superoxide dismutase; –SOH, sulfenic acid; TRX, thioredoxin; X, sulfate, nitrite, or halide group

by the resonance phenomenon between the two forms (Hasanuzzaman et al. 2019a, b). AsA regenerates α-tocopherol (Vitamin-E) from tocopheroxyl radical by scavenging •OH and $O_2^{\bullet -}$ radicals and also regulates a number of phytochrome biosynthesis production pathways (Naz et al. 2016). Another important nonenzymatic antioxidant such as GSH also scavenges ROS molecules and maintains homeostasis (Hasanuzzaman et al. 2019a, b). Tocopherol, on the other hand, is an important component of the antioxidant defense system that protects the chloroplast and keeps photosynthesis by scavenging ROS, mainly $O_2^{\bullet -}$ and •OH (Kumar et al. 2013a, b). Another group of nonantioxidant molecules called carotenoids also play an important role to protect light harvesting complex proteins and thylakoid membrane integrity by scavenging free radicals (Terzi et al. 2014). Some other low molecular compounds such as flavonoids, particularly dihydroxy B-ring substituted flavones and flavanols, also play a promising role for scavenging ROS free molecules and reducing lipid peroxidation and induced cell damage (Tiong et al. 2013). Furthermore, abiotic stressors increase the expression of genes which are involved in activated antioxidant defense system and production of flavonoids (Mehla et al. 2017). One more important group of nonenzymatic antioxidants called phenolic acids are made up of hydroxybenzoic and hydroxycinnamic acids, which function as chelators and scavengers of free radicals, particularly $O_2^{\bullet}$, $^{\bullet}OH$, ROOH, and $ONOO^-$ (Carocho and Ferreira 2013). As free radical scavengers, alkaloids also decrease H_2O_2 in the cells and induce oxidation in the plants (Tiong et al. 2013). Nonprotein amino acids (gamma-aminobutyric acid, ornithine, and citrulline) are also thought to be nonenzymatic antioxidants (Vranova et al. 2011).

6.5.2 Antioxidant Enzymes

Antioxidant enzymes are a group of enzymes which scavenge ROS molecules in plants. Among these the most critical antioxidant enzyme called SOD (EC 1.15.1.1) is characterized into three types, namely Cu/Zn-SOD, Fe-SOD, and Mn-SOD, which lead the frontline defense enzyme in the antioxidant defense system in plants (Berwal and Ram 2018; Rai et al. 2017). This enzyme dismutates the $O_2^{\bullet}$ free radical into H_2O_2 and prevents the production of •OH (Gill et al. 2015; Rai et al. 2018). Another important antioxidant enzyme known as catalase (EC 1.11.1.6) is a tetrameric heme-containing enzyme for ROS detoxification in the antioxidant defense system. A research study concluded that this enzyme can catalyze 26 million H_2O_2 molecules into H_2O in one minute (Mehla et al. 2017). Peroxidase (EC 1.11.1.7) primarily oxidizes PhOH to produce phenoxyl radical (PhO•), also known as QA, in which H_2O_2 takes an electron and is transformed to H_2O. Phenoxyl radical (PhO•) cross-interacts producing suberin, lignin, and quinines in the absence of AsA but in the presence of AsA, PhO• reacts with AsA, resulting in monodehydroascorbate (MDHA) and, eventually, DHA (Fig. 6.2 and Table 6.2) (Jovanovic et al. 2018).

Polyphenol oxidase (EC 1.14.18.1) is one more antioxidant enzyme to scavenge ROS molecules. The enzyme is predominantly located in the chloroplast's thylakoid membrane and can directly affect the photosynthesis process. In the ROS scavenging reaction, the enzyme polyphenol oxidase may interact with the peroxidase or water-water cycle. Another important function of PPO is that it oxidizes PhOH to QA and H_2O using available O_2 (Boeckx et al. 2015). Plant cells contain one more antioxidant enzyme known as AsA-dependent APX (EC 1.11.1.1) which is present in various isoforms (mitochondrial APX (mtAPX), chloroplastic APX (chlAPX), and cytosolic APX (cAPX)).The APX is the only enzyme capable of scavenging H_2O_2 in the chloroplasts of plants because CAT enzyme is absent and peroxisomal/glyoxysomal APX (including mAPX) and other H_2O_2 help to produce monodehydroascorbate (MDHA) through AsA-GSH cycle in plants (Pandey et al. 2017). In plants, the produced MDHAR (EC 1.6.5.4), a NADPH-dependent flavin adenine dinucleotide enzyme found in two isoforms found in diverse cellular sites (Hasanuzzaman et al. 2019a, b). The enzyme plays an important role in plant life by converting MDHA to AsA. By phenoxyl radical reduction, monodehydroascorbate reductase enzyme contains a thiol group which regenarates AsA (García-Caparrós et al. 2019). Monodehydroascorbate reductase is further reduced to DHA nonenzymatically, which is then recycled to AsA by the activity of GSH-dependent DHAR (EC 1.8.5.1) (García-Caparrós et al. 2019). Furthermore, GSH is oxidized to GSSH, which is then reduced to GSH by the NADPH-dependent GR (EC1.6.4.2) enzyme, which is also an important enzyme for redox homeostasis regulation (Couto et al. 2016).

In plants, GPX (EC 1.11.1.9) is a nonheme-containing POX family antioxidant enzyme with a highly reactive thiol group that scavenges H_2O_2, reducing lipids, and organic acids via GSH and TRXs (Bela et al. 2015). GST (EC 2.5.1.18) metabolizes xenobiotics (particularly herbicides and other pharmaceutically active compounds) and transports them into plant vacuoles by conjugating GSH and electrophilic substrates at its active sites (Xu et al. 2015; Christou et al. 2016). GST enzyme also plays an important role in peroxide breakdown, hormone production and stress signaling as well as GPX activity acceleration (Nianiou-Obeidat et al. 2017). Another critical antioxidant enzyme in plants which plays an important role in ROS scavenging is TRX (EC 1.8.1.9). The enzyme has different isoforms (f, m, h, o, y, and z) and contains an enzyme active redox site known as (WCG/PPC). This enzyme reduces disulfide bonds into dithiol by H_2O_2 and regulated target proteins quicker than GSH enzyme or dithiothreitol (Calderón et al. 2018). In chloroplast organelle of plants, the two isoforms of TRX enzyme (TRXx and TRXy) regulates the redox homeostasis by reducing 2-Cysteine (Cys) PRX, whereas TRXo1 activates antioxidant defense in mitochondria by interacting with PRX and sulfiredoxin (Sevilla et al. 2015).

Another thiol-based PRX enzyme (EC 1.11.1.15), a POX-like antioxidant enzyme in plant cells, neutralizes peroxides (H_2O_2 and ROOH) in the cytosol, chloroplasts, mitochondria, and nucleus (Liebthal et al. 2018). PRXs enzymes are thiol-dependent (GSH or any other thiol group) and have ability to reduce diverse organic and inorganic peroxides and also play an important role in regulation of ROS molecules (Fig. 6.2 and Table 6.1) (Hasanuzzaman et al. 2017a, b).

6.6 Reactive Oxygen Species Signaling in Plant Defense

Excess ROS are generated in response to various abiotic stresses as a result of the disturbance of various metabolic activities and physiological disorders (Choudhury et al. 2017). The antioxidant defense pathways for example, AsA-GSH pathway uses energy in the form of NADPH, and once this energy is used up, these pathways would be unable of evading ROS toxicity (Choudhury et al. 2017). Though, the functions of ROS (especially H_2O_2) in plant stress biology came into the attention at the end of the twentieth and the beginning of the twenty-first century. Few scientific groups identified H_2O_2 as a signaling molecule, which induces acclimation processes and increases tolerance to various environmental stresses (Neill et al. 2002). Reactive oxygen species evolved in the chloroplast under stress may divert electrons from the photosynthetic apparatus inhibiting overload of the antenna and consequent damage. Reactive oxygen species also guard mitochondria in a same way (Asada 2006). Cell wall peroxidase may contribute to generation of ROS in relation to signaling where H_2O_2 uses Ca^{2+} and MAPK pathway as a downstream signaling cascade. In addition, phytohormones, particularly ethylene (ET) and abscisic acid (ABA), are implicated in various responses to different environmental stresses via cross-talk with ROS and thus augment stress tolerance, which indicates the dual role of ROS under various stresses (Kar 2011). Apart from signal transduction and communication with hormones, ROS can also involve in metabolic fluxes under abiotic stresses, which mutually direct plant acclimation processes where redox reactions check transcription and translation of proteins and enzymes related to stress adaptation, eventually defending plant cells from injury (Choudhury et al. 2017). Moreover, H_2O_2 controls NO and Ca^{2+} signaling pathways, which manage plant growth and development, and other cellular and physiological responses under varied abiotic stresses (Janicka et al. 2019). Since endogenous H_2O_2 plays pivotal role in enhancing abiotic stress tolerance, exogenous application of H_2O_2 is gaining interest and has proved its efficiency at a large scale (Savvides et al. 2016; Hasanuzzaman et al. 2017a, b). In Table 6.3, we have mentioned some key findings highlighting the effect of H_2O_2 treatment in response to various abiotic stress conditions. Furthermore, ROS interact with RNS, RSS, and RCS under stress and collaborate in signal transduction pathways (Kaur et al. 2019a, b). Antioxidant levels in the cell may vary in order to alter generation of ROS and play a specific role to signaling (Hancock and Whiteman 2016). In contrast, RSS affect the generation, perception, and further signaling of ROS and RNS (Kaur et al. 2019a, b), whereas RCS act downstream of ROS as signal mediators in response to a variety of stresses (Biswas et al. 2019).

6.7 Cross-talk of Reactive Nitrogen, Sulfur, and Carbonyl Species with ROS

Apart from ROS, other reactive species are produced in plant cells during adverse environmental conditions, including RNS, RSS, and RCS (Fig. 6.3) (Nawaz et al. 2019). All these reactive species are involved in a molecular cross-talk and have a particular role in cellular signaling cascades [23]. Therefore, the following subsections discuss the intimate relationship among ROS, RNS, RSS, and RCS. Cross-Talk of Reactive Nitrogen, Sulfur, and Carbonyl Species with ROS. Apart from ROS, other reactive species are produced in plant cells during adverse environmental conditions, including RNS, RSS, and RCS (Fig. 6.3) (Nawaz et al. 2019). All these reactive species are involved in a molecular cross-talk and have a particular role in cellular signaling cascades. Therefore, the following subsections discuss the intimate relationship among ROS and RNS.

Table 6.3 Effect of exogenous H_2O_2 in plants under different abiotic stress factors

Plant species	Stress condition	H_2O_2 treatments	Positive effects	References
Triticum aestivum cv. Zhengmai no. 004	150 mM NaCl; 2 days	Cotreatment; 0.05 µM, 2 days	Decreased MDA content and $O_2^{\bullet-}$ generation. Increased GSH and carotene content by 21% and 33%, respectively. Increased SOD, POD, CAT, and APX activity. Increased growth and biomass	Li et al. (2011)
Cucumis sativus cv. Jinchun no. 4 and Lvfeng no. 6	Osmotic stress; (10% PEG 6000); 2 days	Pretreatment as spraying; 1.5 mM	Decreased MDA and H_2O_2 content. Increased AsA and GSH content. Increased activity of GPX, CAT, APX, GR, MDHAR, and DHAR	Liu et al. (2010)
C. sativus cv. Jinchun no. 4	Low light; 100 mol m^{-2} s^{-1}; 144 h	Pretreatment as spraying; 1.5 mM	Decreased $O_2^{\bullet-}$, H_2O_2, and MDA content. Increased CAT, SOD, APX, GR, MDHAR, and DHAR activity	Zhang et al. (2011)
Vigna radiata L. Wilczek) cv. SML-668	Cu ($CuSO_4 \cdot 5H_2O$); 50 and 100 mg kg^{-1} of soil	Spraying; 2.5 mM	Increased relative water content (RWC) and SPAD value. Increased pro content. Enhanced activity of SOD and CAT. Increased growth	Fariduddin et al. (2014)
Zea mays	Osmotic stress (3% PEG 6000), 12 h	Pretreatment; 10 mM, 6 h	Decreased water loss, MDA, and H_2O_2 content. Increased levels of soluble sugars and proline. Increased Put, Spd, and Spm content by 72%, 106%, and 68%, respectively, over control	Terzi et al. (2014)
Glycine max cv. Merrill 537	Drought; withholding irrigation, 4 and 7 days	Foliar spray; 1 mM, 3 days	Improved water status, pigment content, and alleviated lipid peroxidation. Decreased MDA and H_2O_2 content. Increased activity of SOD (by 93% and 190%), CAT (by 49% and 120%), APX (by 106% and 194%), and GR (by 31% and 229%) on day 4 and 7 of the drought period, respectively, over control	Guler and Pehlivan (2016)
C. sativus L.	Drought; 60 ± 5% FC	Spraying; 1.5 mM (100 mL pot^{-1})	Decreased MDA content and ROS ($O_2^{\bullet-}$, H_2O_2) generation. Increased activity of SOD and POD. Increased soluble sugar and proline content. Increased chl and RWC	Sun et al. (2016)

(continued)

Table 6.3 (continued)

Plant species	Stress condition	H_2O_2 treatments	Positive effects	References
Brassica napus cv. Binasarisha-3	Cd; 0.5 mM (mild) and 1.0 mM (severe) $CdCl_2$; 2 days	Pretreatment; 50 µM, 24 h	Decreased MDA content (by 23% and 25%) under both Cd toxicity levels, respectively. Decreased H_2O_2 content. Increased AsA (by 32% and 30%); GSH content (by 38% and 25%) under both Cd stresses, respectively. Enhanced GSH/GSSG ratio. Increased activity of APX (by 40% and 39%), DHAR (by 77% and 67%), GR (by 36% and 79%), GST (by 44% and 43%), CAT (by 79% and 47%), and glyoxalase II (by 47% and 55%) under both Cd stresses, respectively. Enhanced GPX activity (by 40%) under severe stress and glyoxalase I activity (by 35%) under mild stress	Hasanuzzaman et al. (2017a, b)
T. aestivum cv. Fsd-2008 and S-24	Drought; withholding irrigation, 6 weeks	Seed priming; 1.5 mM, 16 h	Decreased MDA and H_2O_2 content. Enhanced the activity of SOD, CAT, and POD. Increased photosynthetic pigments. Increased GB and Pro content	Habib et al. (2020)
Oryza sativa cv. BRRI dhan29	Osmotic stress (15% PEG-6000)	Foliar spray; 5 and 10 mM	Decreased MDA and H_2O_2 content. Increased activity of CAT and GPX. Protected photosynthetic pigments	Sohag et al. (2020)

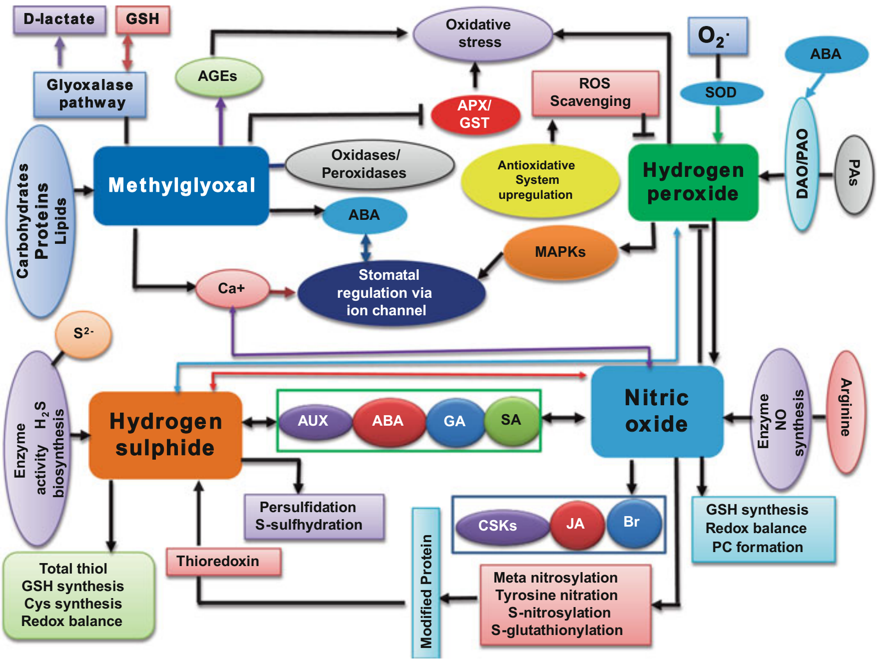

Fig. 6.3 Cross-talk among vital ROS (H_2O_2), RNS (NO), RSS (H_2S), and RCS (MG) in plant cells for oxidative stress and defense response in plants. APX, ascorbate peroxidase; AUX, auxin; ET, ethylene; ABA, abscisic acid; ROS, reactive oxygen species; GSH, reduced glutathione; JA, jasmonates; MAPKs, mitogen-activated protein kinases; SA, salicylic acid; AEGs, advanced glycation end products; PAs, polyamines; MG, methylglyoxal; NO, nitric oxide; H2S, hydrogen sulfide. Dotted lines represent activation/enhancement

6.8 Transgenic Approach in Enhancing Antioxidant Defense in Plants

From the last 20 years, transgenics have been extensively used to improve plants under oxidative stress. Therefore, transgenic plants can be engineered to improve abiotic stress tolerance and the antioxidant enzyme defense mechanism activity. Here, we have highlighted transgenic plants with enhanced responses of antioxidant defense systems under several stresses which are presented in Table 6.4. Kiranmai et al. (2018) observed lower concentrations of MDA, H_2O_2, and $O_2^{\bullet-}$ and increased activities of SOD and APX in groundnut due to overexpression of *MuWRKY3* gene under drought stress. Another study conducted by Sun et al. (2018) demonstrated the enhanced drought stress tolerance and activities of CAT and POD in transgenic apple cultivars due to overexpression of *MdATG18a*. Results also denoted that tolerance to stress was improved because of a high frequency of autophagy and inhibition of oxidative damage. Kumar et al. (2020) demonstrated that chickpea

Table 6.4 Antioxidant defense systems and their expression pattern in crop plants

Stress condition and duration	Plant system	Source plant	Gene	Impact on antioxidant defense systems	References
Salinity					
0, 50,100, and 150 mm Nacl; 7 and 15 days	*Solanum tuberosum*	*Potentilla atrosanguinea* and *Rheum australe*	PaSOD and RaAPX	Enzyme activities are enhanced in transgenic plants as of SOD by two- to sixfold in PaSOD and one- to threefold in double transgenic plants (DTP); APX by 5-fold to 11-fold in APX and four- to eightfold in DTP	Shafi et al. (2017)
100, 200, 400 mm; 1, 5, 10, 15 days	*Chrysanthemum*	*Dendranthema grandiflorum*	*DgNAC1*	Enzyme activities are enhanced in transgenic plants as of SOD by two-, CAT by two-, and POD by threefold	Wang et al. (2017)
150 mM NaCl; 3, 6, 9, 12, and 24 h	*Arabidopsis thaliana*	*Vitis vinifera*	*VvWRKY30*	Enzyme activities are significantly enhanced in transgenic plants, i.e., POD, CAT, and SOD	Zhu et al. (2019)
150 or 200 mM NaCl, till germination	*Glycine max*	*Glycine max*	*GmMYB84*	Enzyme activities are significantly enhanced in transgenic plants, i.e., SOD, POD, and CAT	Zhang et al. (2020a, b)
Water deficit and simulated drought					
Osmotic stress (20% PEG); 1, 3, 6 12, 24, and 48 h	*Nicotiana tabacum*	*Spinacia oleracea*	*SoCYP85A1*	Overexpressed lines improve the activity of POD by 1.3–1.5 and SOD by 1.36–1.39-fold	Duan et al. (2017)
Withholding water for 14 and 21 days	*Malus domestica*	*Malus domestica*	*MdATG18a*	Enzyme activities are enhanced in transgenic plants as of CAT and POD by 1.57–2.05-fold in overexpressed lines	Sun et al. (2018)
Withholding water till the wilting stage	*Arachis hypogaea*	*Macrotyloma uniflorum* Lam. Verdc.	*MuWRKY3*	Enzyme activities are enhanced in transgenic plants as of SOD by three- to five- and APX by three- to sevenfold	Kiranmai et al. (2018)
Osmotic stress (15% PEG); 60 days	*N. tabacum*	*Zea mays*	*ZmSO*	Overexpressed lines increase the activity of GSH 64% and 88%	Xia et al. (2018)

Osmotic stress (15% and 25% PEG); 7 days	*A. thaliana*	*Cicer arietinum*	*CaMT*	Enzyme activities are enhanced in transgenic plants as of APX 488%, POD 135%, GPX 134%, and GRX 186%	Dubey et al. (2019)
Withholding water for 12 days	*A. thaliana*	*Malus prunifolia*	*MpDGK2*	Enzyme activities are enhanced in transgenic plants, i.e., CAT, APX, and POD	Tan et al. (2020)
Drought stress	*Solanum Lycopersicon*	*Tomato*	*Antioxidant gene*	Enhanced the SOD, APX gene expression	Rai et al. (2018)
Toxic metals/metalloids					
As(III) [5 and 10 μM ($NaAsO_2$)], As(V) [50 and 100 μM (Na_2HAsO_4)], Cd [30 and 50 μM ($CdCl_2$)] and Cr($K_2Cr_2O_7$)	*A. thaliana*	*Oryza sativa*	*OsSultr1;1*	Enzyme activity is enhanced in transgenic plants, i.e., GSH with As(III) toxicity	Kumar et al. (2019)
300 μM $CdCl_2$ and 300 μM $NiCl_2 \cdot 6H_2O$; 1, 12, 24, and 48 h	*N. tabacum*	*Salicornia brachiate*	*SbMYB15*	Enzyme activities are enhanced in transgenic plants as of CAT and SOD and also increase the expression of Mn-SOD at 100 μM (1.69-fold) and 300 μM (3.2-fold) of CdCl2 and CAT1 by 62.19- and 9.8-fold at 100 and 300 μM	Sapara et al. (2019)
Extreme temperature					
45 °C; 0.5, 1, 2, 3, 6, 9, 12, and 24 h	*N. tabacum*	*Triticum aestivum*	*TaFBA1*	Enzyme activities are enhanced in transgenic plants, i.e., SOD, POD, and APX, while CAT activity was decreased under heat stress	Li et al. (2018)
48 °C; 6 h	*M. domestica*	*M. domestica*	*MdATG18a*	Enzyme activities are enhanced in transgenic plants, i.e., SOD, POD, CAT, AsA, and GSH, whereas a decreased ratio of GSH/GSSG was reported	Huo et al. (2020)

(continued)

Table 6.4 (continued)

Stress condition and duration	Plant system	Source plant	Gene	Impact on antioxidant defense systems	References
4 °C; 5 days	*Solanum lycopersicum*	*A. thaliana*	*AtDREB1A*	Enzyme activities are enhanced in transgenic plants as of SOD by 29.49% and CAT by 21.34%	Karkute et al. (2019)
4 °C; 6, 12, 24, 36, and 48 h	*S. tuberosum*	*S. tuberosum*	*StSOD1*	Enzyme activities are enhanced in transgenic plants as of SOD by 1.38-, POD by 1.24-, and CAT by 1.37-fold	Che et al. (2020)
Waterlogging					
2 cm waterlogging; 3, 6, 12, 24, and 72 h	*A. thaliana*	*Brassica napus*	*BnERF2.4*	Enzyme activities are enhanced in transgenic plants, i.e., SOD, POD, and CAT	Lv et al. (2016)
Soil–atmosphere interface for 1 weeks	*A. thaliana*	*Mentha arvensis*	*MaRAP2–4*	Enzyme activities are enhanced in transgenic plants, i.e., CAT, GPX, and SOD	Phukan et al. (2018)
5 cm waterlogging; 24 and 48 h	*A. thaliana*	*Dioscorea alata*	*DaAPX*	Enzyme activity is enhanced in transgenic plants, i.e., APX but no significant effect on CAT	Chen et al. (2019)
3 cm standing water; 14 days	*Chrysanthemum morifolium*	*Chrysanthemum morifolium*	*CmSOS1*	Overexpressed plants enhance the activities of SOD and CAT by 171%	Wang et al. (2019)
Overexpressed plants enhance the activities of SOD and CAT by 171%	*A. thaliana*	*Hordeum vulgare*	*HvERF2.11*	Enzyme activities are enhanced in transgenic plants as of SOD by 55%, 48%, and 45%, POD by 64%, 65%, and 70%, CAT by 2.2%, 2.1%, and 2.1%, alcohol dehydrogenases by 2.1-, 2.3-, and 1.9-fold in three transgenic lines, respectively	Luan et al. (2018)

CaGrx gene was overexpressed in *A. thaliana* with maximal activities of GRX, GR, GPX, GST, and APX under heavy metal stress in comparison to controls, while activities of CAT, SOD, and MDHAR were also considerably enhanced. Authors recommended that CaGrx can be an appropriate candidate gene to surmount metal stresses in other crops as well (Kumar et al. 2020). Karkute et al. (2019) reported the increased activities of SOD, CAT, and POD and in turn tolerance to chilling stress due to overexpression of *A. thaliana AtDREB1A* gene in tomato. They observed 29% and 21% increase in activities of SOD and CAT respectively in transgenic plants, demonstrating better chilling stress tolerance. Che et al. (2020) showed that the activities of SOD, POD, and CAT were enhanced on overexpression of the potato *StSOD1* gene during cold stress and enhanced cold tolerance in transgenic potato plants. Similarly, Wang et al. (2019) revealed the overexpression of *CmSOS1* gene increases SOD and CAT by 171% in transgenic Chrysanthemum plants under waterlogging conditions.

6.9 Conclusions and Future Perspectives

Abiotic stresses are major limiting factors that affect growth and development of plants all over the globe. Consequently, there is a need to decipher the physiological, biochemical, molecular, and cellular abiotic stress response mechanisms and tolerance and to establish potential mitigation approaches that would lead to global food and agricultural sustainability. Abiotic stresses cause ROS accumulation, which leads to oxidative injury in plants. In the beginning, ROS were believed to cause toxicity and considered as outcome of aerobic metabolism, present in some subcellular compartments. The ROS metabolism is essential in growth, development, and adaptation of crop plants under various environmental stresses. The generation and scavenging of ROS are of utmost importance to plant defense processes. In order to enhance resistance to various abiotic stresses, modulation and overexpression of candidate genes governing production of various ROS-detoxifying enzymes are extensively used. Nonenzymatic antioxidant systems are known to play dynamic role in maintaining equilibrium between detoxification and ROS generation in plants under stressful conditions. Remarkably, ROS are well-known to play a dual part in plant biology owing to molecular cross-talk with other signaling molecules for example RNS, RSS, and RCS. On the basis of previous works, ROS is incredibly essential player for different biological mechanisms and are well-known for its signaling role at low concentrations. On the other hand, ROS toxicity explicitly destroys cells via oxidative stress as a result of ROS-activated machinery accountable for cellular degradation. Besides, there exists a correlation between ROS, RCS, RSS, and RNS and metabolic activities in normal and stressed conditions; nevertheless, a few reports have addressed these interactions. Both ROS and RNS can generate oxidative and nitrosative stress exclusively or in concert cause nitro-oxidative stress although both are also involved in signaling cascade of higher plant species principally under harsh environment. Alternatively, both ROS and RSS signaling pathways are indistinguishable and signal via interaction with Cys, but the RSS signaling seems to be more widespread in comparison to ROS signaling. On the contrary, RCS can maintain metabolism of ROS as these molecules are direct outcome of oxidative stress and have the capability to operate as its sensors. Thus, these four reactive molecules possibly will be the novel gateway of attention for the plant scientists. Even though amassing of information regarding signaling pathways of such reactive molecules has been accelerated over the period of time, more comprehensive research is desirable to illuminate their roles in plant stress biology. With the latest advances in molecular and genetic techniques, considerable advancement has been made in enhancing plant stress tolerance through transgenics with improved activities of antioxidant enzymes. Based on the available literature, there is a need to identify and report candidate genes that can considerably enhance the tolerance and yield of transgenic plants under stressful environments. Additionally, chemical priming is a smart way to genetic engineering so as to accomplish similar targets, often through the regulation of the antioxidant defense apparatus. At some point, systems biology approaches such as genomics, transcriptomics, proteomics, and metabolomics may possibly help introducing novel alternatives for the improving plant stress tolerance. Integrating abovementioned approaches can be employed to identify key and stress-related regulators, genes, proteins, and metabolites. Moreover, identification and exploitation of pathways related to ROS-detoxifying regulators could be improved to produce genotypes tolerant to abiotic stresses. As we know, plants undergo a wide range of stresses simultaneously; therefore, identification of genes that can confer multiple abiotic stress tolerance is of utmost importance. Also, state-of-the-art genome-editing technologies such as CRISPR/Cas system could modify the plant genome through the development of mutants with single or multiple genes, e.g., ROS-detoxifying regulators for sustainable growth and development in plants and to improve the antioxidant defense mechanisms. Recently, speed breeding has also come to light as a powerful means to enhance the plant growth and development under desired circumstances. Thus, in order to save time plant genome editing could be integrated with speed breeding to generate transgenic plants with induced antioxidant potential that are tolerant to different stresses and will thus contribute to feed ever-growing population and to guarantee global food security.

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CDPKs Based Signalling Network: Protecting the Wheat from Heat

7

Ranjeet Ranjan Kumar, Sindhu Sareen, Jasdeep C. Padaria, Bhupinder Singh, and Shelly Praveen

Abstract

Wheat, being a staple food grain crop, is highly sensitive to terminal heat stress. The mechanism underlying heat stress tolerance in wheat has yet not been elucidated. Diverse signalling networks has been adopted by the plants in order to regulate their biological functions and protect the cells from the vageries of nature. Out of all the signalling pathways known and characterized, Ca^{2+} – secondary messenger-linked pathways are very predominant in different biological functions. Calcium Dependent Protein Kinases, showed the presence of N-terminal domain (which is variable), a protein kinase domain (shows phosphorylation activity), an auto-inhibitory region, and a calmodulin-like domain with EF-hand Ca^{2+}-binding sites. CDPKs has been reported to acts as temperature sensing device or thermometer for the plants. It plays very important role in the regulation of guard cells and in ABA-regulated stomatal signalling in Arabidopsis. *OsCDPK7* and *OsCDPK13* present in rice have been reported to modulate the tolerance level against cold, salt, and drought stresses. CDPKs have also been reported to involved in ROS homeostasis and protection of cells against abiotic stresses. CDPKs has also been observed to modualte the carbon fixation process under adverse conditions. The actual sensory and signalling molecules and/or the primary targets

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R. R. Kumar (✉) · S. Praveen
Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India

S. Sareen
Indian Institute of Wheat and Barley Research, Karnal, Haryana, India

J. C. Padaria
National Institute for Plant Biotechnology (NIPB), New Delhi, India

B. Singh
Centre for Environment Science and Climate Resilient Agriculture (CESCRA), Indian Agricultural Research Institute, New Delhi, India

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of CPKs mediated regulation of photosynthesis and carbon assimilation metabolism under heat stress are still unclear. It is worthwhile to examine the role of CDPKs in altering the photosynthesis and source-to-sink carbon transfer in wheat under heat stress.

Keywords

CDPKs · Heat stress · Thermotolerance · Wheat · Signalling network · SAGs · HSFs · HSPs

7.1 Introduction

Heat stress has adversely affected the production and productivity of agriculturally important crops like wheat, rice, maize, etc. The environmental temperature is predicted to increase by 1.5 °C in the next few decades. This increasing average surface temperature has a severe effect on plant growth and development (Raza et al. 2019). Wheat, being a staple food grain crop, has inherent ability to adapt to diverse ecological conditions, though it is highly prone to fluctuation in environmental temperature. A recent study has reported that there is a decrease of 4% in the yield of wheat for every 1 °C increase in temperature (Kumar et al. 2019). Heat stress alters different physicochemical, and biological processes in wheat (Asseng 2015) at all the growth stages of wheat by accelerating plant growth and shortening the various stages (Akter and Rafiqul Islam 2017). However, pollination and fertilization stages are reported to be more sensitive to heat stress than vegetative phase (Kumar and Rai 2014). Episodes of heat stress around anthesis lead to both structural and functional abnormalities in the anthers, reduced pollen fertility, poor fertilization, abnormal ovary development, and abortion of ovules and thus poor seed-setting (van Es 2020; Kumar et al. 2020). During grain-filling, the size and weight of individual grains are reduced due to reduction in the endosperm cell division and short duration of grain-filling (He et al. 2019). The duration of grain-filling is reduced by ~10 days under heat stress (Shirdelmoghanloo et al. 2016). The yield losses due to terminal heat stress vary from region to region and are also based on the intensity of heat stress at the time of grain-filling. On average, it causes 30–40% yield losses every year worldwide, especially in the Indian subcontinent (Guerrini et al. 2020).

7.2 Calcium-Dependent Protein Kinases: The Thermometer of Plants

Plants have developed very diverse signal networks in order to regulate their biological functions and to mitigate the adverse effects of environmental climatic factors. Different signalling molecules work in tandem in order to operate the signal transduction pathways in efficient manner, and out of all the signalling pathways known and characterized, Ca^{2+} − secondary messenger-linked pathways are very predominant in different biological functions (Wen et al. 2020; Bennett et al. 2012).

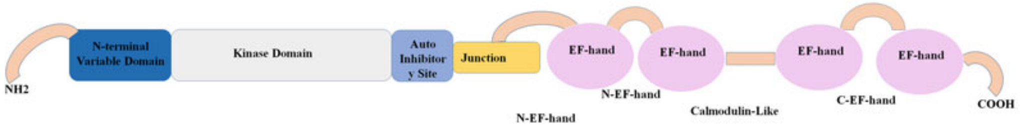
Any sort of fluctuation in the environmental factors leads to changes in the concentration of Ca^{2+}, which is sensed by different sensors or Ca^{2+}-binding proteins (CBPs). The CBPs have been classified into three different types in plants—calcium-dependent protein kinases (CDPKs), calmodulins (CaMs), and CaM-like proteins 6 and calcineurin B-like proteins.

The CDPKs have been identified and characterized in most of the agriculturally important crops, though few crops like wheat, having a complex genome, have yet not been completely characterized. The structure analysis of CDPK showed the presence of N-terminal domain (which is variable), a protein kinase domain (shows phosphorylation activity), an auto-inhibitory region, and a calmodulin-like domain with EF-hand Ca^{2+}-binding sites (Fig. 7.1; Yip Delormel and Boudsocq 2019). The secondary messenger Ca^{2+} causes activation of CDPKs, and further, the activated CDPKs regulate the expression of downstream transcription factors/genes.

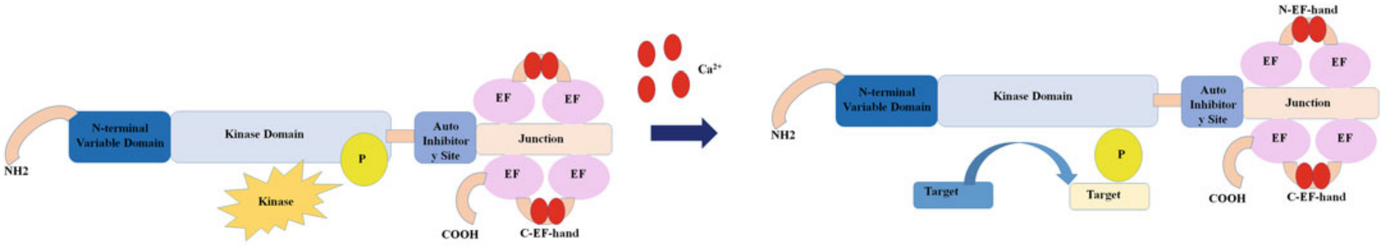
CDPKs constitute a large multigene family in various plant species, though most of the identification and characterization has been mostly done in modal crop plants like *Arabidopsis thaliana* (34 CDPK genes) and *Oryza sativa* (29 CDPKs) (Shi et al. 2018). The CDPKs have been reported to be upregulated by various factors like hormones, abiotic stresses, and biotic stresses. The localization of CDPKs has been reported in different subcellular locations, such as nucleus, cytosol, plasma membrane, endoplasmic reticulum, and mitochondrial outer membrane.

7.3 Genome-Wide Identification of CDPKs

CDPKs are vastly distributed in various plant species. Genome-wide identification of the CDPKs has become very possible largely due to the whole-genome sequencing of plant species. Till date, 34 genes encoding CDPKs were identified in *Arabidopsis thaliana* (Cheng et al. 2015), 20 in wheat (Li et al. 2013), 31 in rice (Ray et al. 2007), and 29 in tomato (Hu et al. 2016). Further, studies revealed that CDPKs are mostly expressed in the leaves, roots, meristems, and flowers of the plants, with some expressing only in specific tissues (Simeunovic et al. 2016). In our lab, we also performed de novo transcriptome sequencing of diverse genotypes of wheat grown under control (22 ± 2 °C) and HS-treated (38 ± 2 °C) conditions. We have identified 47 diverse transcripts of CDPKs in wheat through transcriptome analysis, which were further characterized and classified into four distinct families (Kumar et al. 2016; 2020). Phylogenetic analysis of CDPKs reported from different plant sources showed the classification of CDPKs into four different groups—group I consist of majority of CDPKs found in model plants (Fig. 7.2; Hamel et al. 2014). Very few CDPKs from the model systems were observed in group IV (Hu et al. 2016). Most of the CDPKs identified in plant systems have been reported to be highly conserved across the species (Kumar et al. 2016). Gene duplication has been observed to be the reason behind the existence of tandem duplication of CDPKs (Hamel et al. 2014; Hu et al. 2016).



Structure of Calcium Dependent Protein Kinase



Mechanism of phosphorylation of enzyme and target proteins

Fig. 7.1 Structure of calcium-dependent protein kinase (CDPK) protein in plants; the presence of N-terminal domain, a protein kinase domain, an auto-inhibitory region, and a calmodulin-like domain with EF-hand Ca^{2+}-binding sites were observed

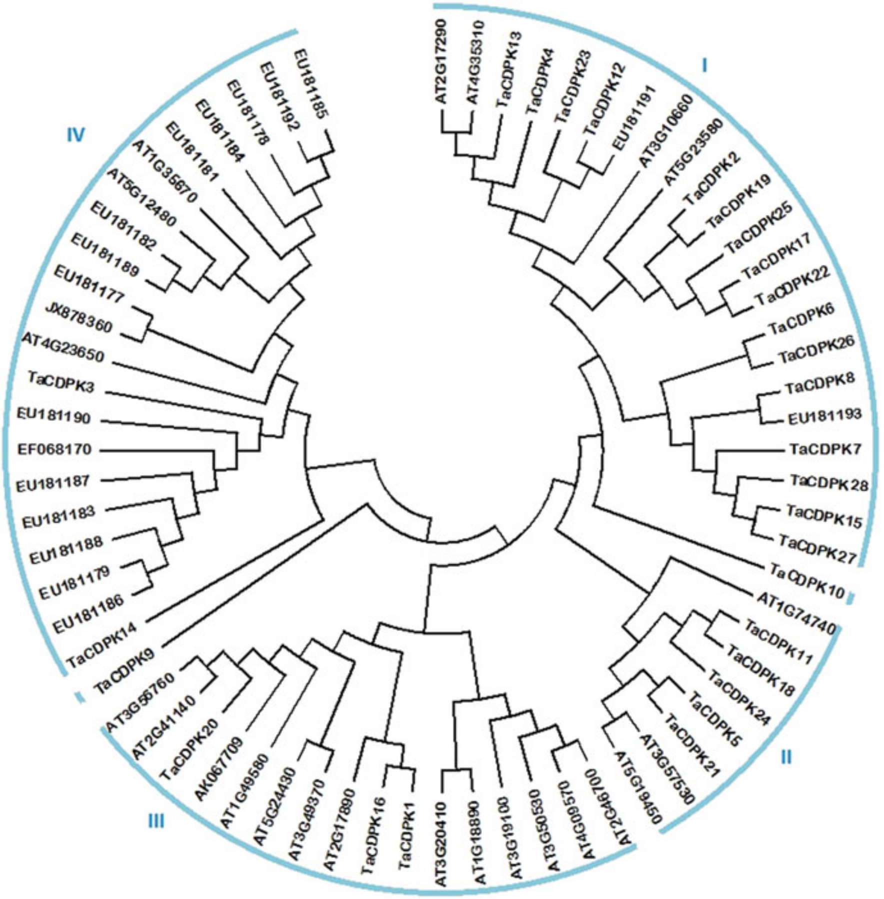


Fig. 7.2 Phylogeny tree analysis of calcium-dependent protein kinases (CDPKs) in wheat; CDPK transcripts identified from wheat through de novo transcriptomic approach were used for the evolutionary pattern analysis

7.4 Role of CDPKs in Phytohormone signallinig and Thermotolerance

7.4.1 CDPKs and ABA Signalling

The phytohormones, especially ABA has been reported to play very important roles in tolerance to different abiotic stresses in plants. CDPK is involved in ABA signalling pathways, other than the tolerance to abiotic stresses. CDPKs have been characterized to play a very important role in the regulation of guard cells and in ABA-regulated stomatal signalling in Arabidopsis (Zou et al. 2015; Geiger et al.

2009). These CDPKs were observed to be positive regulators of the abiotic stress response. The CPK3-dependent regulation under abiotic stress is independent of the MAPK-mediated signalling pathway. Few literatures have reported enhanced tolerance to salt and drought stresses in plants overexpressing CPK6. Similarly, CPK10 reported from Arabidopsis was characterized to modulate the tolerance against drought stress and in opening and closure of stomata in response to ABA and Ca^{2+} (Zou et al. 2010; Munemasa et al. 2015). CPKs were observed to play important role in regulating the stomatal movements through ABA- and Ca^{2+}-mediated signalling (Fig. 7.3).

In different plant species, the CDPKs have been reported to phosphorylate ABA-responsive element binding factors (ABFs) and in turn influence the ABA signalling pathway. It phosphorylates ABA-responsive transcription factors, such as ABF1 and ABF4 and positively regulates the CDPK-mediated ABA signalling pathway. Even some of the CPKs like CPK4, CPK11, CPK21, and CPK23 have been observed to involve in seed germination, seedling growth, stomatal movement, and tolerance to different abiotic stresses (Fig. 7.3; Ali et al. 2020). Similarly, findings based on *Xenopus laevis* oocytes showed that CPK21 and CPK23 control the activation of SLAC1 (anion channel present in guard cells) in response to Ca^{2+} concentrations. Similarly, Open stomata 1 protein kinase (OST1) (also known as CPK23) and ABA-INSENSITIVE1 (ABI1) were associated with the calcium-independent steps of ABA signalling, whereas CPK21/ABI1 regulates SLAC1 in response to cytosolic calcium signalling (Brandt et al. 2015). This makes us to conclude that CDPK signalling network is quite a complex one and is mainly involved in ABA and abiotic stress responses in plants.

Similarly, *OsCDPK7* and *OsCDPK13* present in rice have been reported to modulate the tolerance level against cold, salt, and drought stresses (Abbasi et al. 2004). *OsCPK21* has been observed to enhance the tolerance of the rice plant in response to salt stress. It has positive regulatory effect on ABA and salt signalling pathways (Asano et al. 2011).

Enzymes like NADPH oxidase have been reported to involve in ABA-dependent ROS production and stomatal closure. The ROS produced by NADPH oxidase acts as signalling molecule in triggering the defense network of the plants. CDPKs have been reported to help in phosphorylating the NADPH oxidase. CDPKs like CDPK4, CDPK5 (potato), CPK5/CPK6 and CPK4/CPK11 (Arabidopsis), OsCPK12 (rice) have been reported to regulate ROS production (Asano et al. 2012). It acts as positive regulator of ROS production against biotic and abiotic stresses. The rice CDPK OsCPK12 is reported to be an essential positive regulator of tolerance to salt stress. The overexpression of *OsCPK12* has been reported to increase the tolerance against salinity stress and reduces the accumulation of H_2O_2 in plants. *OsCPK12* has been observed to regulate the expression of antioxidant enzymes like *OsAPx2*, *OsAPx8*, and *OsrbohI*. OsCPK12 has been reported to have functions in multiple signalling pathways and inversely modulates the biotic and abiotic stresses. Several studies suggested that CDPKs play significant roles in abiotic stress tolerance by expressing differently under various levels of ABA, salinity, drought, heat, and cold (Crizel et al. 2020).

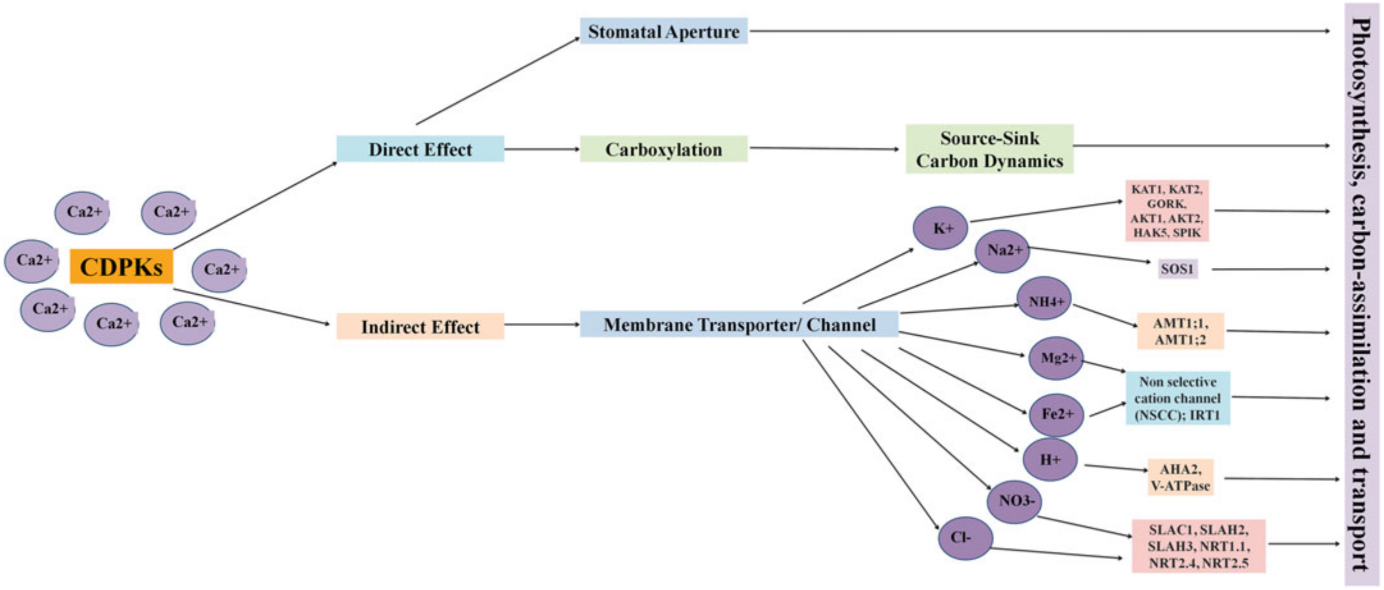


Fig. 7.3 Characterizing the direct and indirect effects of calcium-dependent protein kinases (CDPKs) in regulating the stomatal opening, photosynthesis, carbon assimilation, and transport of ions through membrane

7.4.2 Role of CDPKs in Thermotolerance

Fluctuations in temperature negatively affect plants' growth and survival. A number of CDPKs are reported to be responsive to heat stress. A number of studies identified various CDPKs responsive to heat stress in various plants. Our lab has also worked on spatiotemporal expression analysis of CDPKs in wheat under heat stress and established the modulatory role of CDPKs in thermotolerance (Kumar et al. 2014). Zhao et al. (2021) characterized calcium-dependent protein kinase ZmCDPK7 and reported their role in heat stress tolerance in maize. Similarly, Wang et al. (2016; 2018) worked on Tea plant and established the involvement of CsCDPK20 and CsCDPK26 in heat stress tolerance. Hosseini et al. (2021) worked on Lentil under heat and drought stresses and reported the upregulation of CDPKs under multiple stresses. Many literatures available in public domain reported the role of CDPKs in modulating the tolerance against multiple stresses in agriculturally important crops.

7.4.3 Correlation of CDPKs with Other TFs and SAGs

CPKs, while playing roles in specific abiotic and biotic stress responses, also correlate with other multiple stress-related proteins. AtCPK12 is involved in phosphorylation of a number of salt stress responsive proteins (Yin et al. 2017). Similarly, VaCPK21 (grapevine gene) expressed in Arabidopsis through transgenic approach was found to interact with a lot of salt stress-associated genes, such as AtRD29, AtKIN1, AtNHX1, AtCSD1, and AtCOR47 (Dubrovina and Kiselev 2019). In the same way, VaCPK20 which is responsive to cold and drought stresses regulates the expression of KIN1, COR47, NHX1, or ABF3 in transgenic Arabidopsis (Atif et al. 2019). CPK-encoding genes in plants also function in regulating other abiotic stress-associated genes, such as AtRBOHD, AtABI1, AtHSP70, AtHSFA2, AtP5CS2, and AtAPX1. CsCDPK20 and CsCDPK26 function interactively to confer thermotolerance in tea plants (Wang et al. 2018). In oilseed rape, it was reported that BnaCPK2 correlates with NADPH oxidase-like RbohD to regulate the accumulation of ROS and the death of a cell. Studies show that overexpressing CPK9 and CPK33 interactively function in controlling guard cells' regulation and movement of the stomata (Chen et al. 2013). When VaCPK29 was overexpressed, it led to the upregulation of other stress-associated genes such as dehydration elements (DREs), which function in conferring tolerance to heat and osmotic stresses (Dubrovina and Kiselev 2019). The cross talk between the CDPKs and other proteins leads to the complex nature of the signalling pathways.

7.5 Role of CDPK in Carbon fixation in Wheat under Heat Stress

Photosynthesis, a process critical to plant growth and development, is highly sensitive to heat. Temperature sensitivity of the photosynthetic process may be visualized in terms of variation in the energy metabolism, photosystem electron transport (PSII and PSI), and the carboxylation efficiency, which is guided by the RuBisCo and the RuBisCo activase activities, but takes a hit under the heat stress (Kumar et al. 2019; Zhang et al. 2018). In addition to the direct effect of HS on carbon assimilatory process, the indirect effect of HS on the photosynthetic capacity is also vital and cannot be ignored. An elevated temperature, depending on severity of stress will variably inhibit the photosynthetic carbon assimilation by altering the availability of mineral nutrients and water to the leaves through its indirect effect on water availability in the soil and in the root zone to restrict the rate of transpiration and thus the uptake of nutrient-rich water from the rhizospheric regions (Slattery and Ort 2019). Further, an inhibited carbon fixation under heat stress conditions may further restrict the availability and partitioning of photosynthates toward maintenance of root growth which may decline to consequential levels under continued severe heat stress, so as to further restrict the photosynthesis via a feedback inhibition mechanism. Additionally, an induction in oxidative stress at elevated temperatures, may also increase the degradation of chlorophyll besides causing damage to the photosystem II, proteins, and membrane lipids and a reversal of these ROS-related aberrations call for the presence of a robust antioxidant consortia, i.e., the enzymatic and the nonenzymatic scavengers, that should be present in quantities sufficient enough to outbalance the oxidative stress, to mitigate the toxic free radicals, and savage the related cellular anomalies (Kasajima 2017).

Calcium (Ca^{2+}), a major essential mineral nutrient, also functions as a second messenger and when sensed by cationic sensors or Ca^{2+}-binding proteins such as calcium-dependent protein kinases (CDPKs or CPKs), calmodulins (CaMs), and CaM-like proteins (CMLs) and calcineurin B-like proteins (CBLs), influences various signalling transduction pathways (Demidchik and Shabala 2018). However, the actual sensory and signalling molecules and/or the primary targets of CPKs-mediated regulation of photosynthesis and carbon assimilation metabolism under heat stress are still unclear (Fig. 7.3).

Reports available in the literature point toward the role of molecular temperature sensors that are distributed in different cell compartments and produce receptive signals such as Ca^{2+} and H_2O_2 and activate different TFs through activation of CDPKs and MAPKs (Mittler et al. 2004). CDPKs or CPKs are known to influence different physiological and biochemical processes, stress response, hormone trafficking—signalling to elicit effect on plant growth and development (Regmi et al. 2020). A positive correlation of CDPK with thermotolerance, carbon metabolism, and amylolytic-linked traits, but a negative correlation between CDPK and photosynthetic rate (P_n) has been reported under heat stress in wheat (Dahuja et al. 2020). The above interactive interplay in totality ensures maintenance of normal physiological functions including photosynthesis by inducing the high temperature-related metabolic protection mechanisms, viz., induction of heat shock proteins (HSP's), which are molecular chaperones that impart protection against protein degradation (Song et al. 2021). A critical analysis of literature reflects on the role of CDPKs (*AtCPK4, AtCPK6, AtCPK10, AtCPK11, AtCPK12, OsCPK7, OsCPK13, OsCPK21, NtCDPK2, and NtCDPK3*) in ABA and Ca^{2+}-mediated stomatal regulation under abiotic stress particularly drought, high and low temperature and salinity across Arabidopsis, rice, and tobacco crops (Das and Pandey 2009; Xiao et al. 2017). New insight is now available pertaining to the role of $Ca^{2\ +}$ in regulation of photosynthesis relates to the regulation of Ca^{2+} delivery into the stroma and the thylakoid of the chloroplast where Ca^{2+} is likely to the photosynthetic electron transfer and the light-mediated oxidation–reduction reactions (Zhang et al. 2018). It is also pertinent to mention here that an increase in Ca^{2+} concentration in the cytosol in response to the abiotic stress including high-temperature stress induces the activity of the CDPKs and the downstream signalling pathways, to regulate indirectly the photosynthesis and the carbon assimilation processes by balancing the stress response on the nutrient uptake. CDPKs are considered the regulator of the membrane ion transporters/channels that determine the uptake and in plant homeostasis of sodium, potassium, ammonium, nitrate, chloride, magnesium, and iron to cause an indirect effect on the quantum and specific activity of the enzyme proteins that mediate the carbon assimilation efficiency that depends on the gas exchange attributes, RuBisCo activity, chlorophyll, etc. (Shi et al. 2018). Further, Tang and Luan (2017) showed that the homeostasis of Ca^{2+} and Mg^{2+} in the plant system is highly interdependent and is strictly regulated. Mg^{2+} toxicity is said to induce a short-term increase in cytosolic Ca^{2+} which is then sensed by the CBL2 and CBL3—CIPKs (calcineurin B-like-CBL interacting protein kinase), the tonoplast Ca^{2+} sensors, to positively regulate Mg^{2+} transport to aid the vacuolar Mg^{2+} sequestration. Similar interactive influence has also been documented for iron mediated by its chief IRON REGULATED TRANSPORTER (IRT1). Ca^{2+}-driven CDPKs have been shown to promote EHB1-IRT1 interaction (Khan et al. 2019). Another indirect role of CDPKs originates from its regulation of the K^+ channel (AKT2) activity, which is known to determine the phloem loading of sugars preferably sucrose for its in-plant distribution (Shah et al. 2021). AKT2, via phosphorylation cause K^+ efflux and retrieval of sucrose via phloem membrane repolarization (Sandmann et al. 2011). An increase in AKT2 activity was reported in a Ca^{2+}-dependent manner by the CBL4–CIPK6 complex. In addition to the control of CDPKs over the photosynthesis and the carbon transfer, they (CBL1 and CIPK14) are also known to regulate the plant response to external sugar viz., glucose supplementation, when supplied as the energy source or as a hormone, with its functional role similar to that of the ABA responsive genes (Li et al. 2013; Saito and Uozumi 2020). Additionally, some of the other anion species, i.e., nitrate (NO_3^-) and chloride (Cl^-) complement K+ synergistically in maintaining the turgidity of the guard cell, thus, controlling of stomatal aperture. Two of the CBL-CIPK family members, i.e., CIPK7, CIPK8, and CBL7 are reported to regulate the expression of several nitrate responsive genes viz., NRT2.1/NPF6.3/NRT2.4/NRT2.5 (Fig. 7.3; Kiba et al. 2018). Direct mediation of Ca^{2+}-CDPKs in regulating K+ uptake and transport and in stomatal movement has also been documented and the activity of the two K^+ transport systems, i.e., the K^+ channel AKT1 and the KT/KUP/HAK-type transporter HAK5 depends on CBL1 (or CBL9) and CIPK23. Schulze et al. (2021) generated the *cpk* quintuple mutants by crossing *cpk3/4/6/11* and *cpk5/6/11/23* quadruple mutants to show an impaired CO_2-controlled stomatal opening and closing in *cpk3/5/6/11/23* quintuple mutants. The slowing of stomatal opening at low [CO_2] in *cpk3/5/6/11/23* quintuple mutants clearly suggests an interactive three-way regulatory control of the stomatal movement by CO_2, K^+, and Ca^{2+}. Knowing fully well that a degree increase in temperature may cause about 10% loss of yield in the staple cereal like wheat and that indirect and not alone the direct consequence of the high temperature will regulate the gas exchange and carbon assimilation and transport attributes, it is worthwhile to examine the role of CDPKs in altering the photosynthesis and source to sink carbon transfer in wheat; focus on which as of now is limited, however, there are numerous inklings in respect of the regulatory linkages as have been amply evidenced from the model plant *Arabidopsis*. Wen et al. (2020) identified about 20 CDPK genes from *Triticum aestivum* which are more or less similar in their genomic structure to rice and were observed to respond to abiotic stress including temperature with overlapping regulatory control and convergence of the multiple transduction pathways. However, there is still a long way to go before the functionality of the wheat CDPK genes is completely understood.

7.6 Manipulation of CDPKs for the Development of Climate Smart Crop

CDPKs represent a large and diverse family, as observed in different plant species. It plays a very crucial role in different physicochemical and biochemical processes inside the plant system. It modulates the defense mechanism of the plant against abiotic and biotic stresses by triggering the expression of stress-associated TFs and genes. Some CDPKs have been revealed to be important players in tolerance to various abiotic stresses through modulation of ABA signalling and decreasing the ROS accumulation in plants (Asano et al. 2012). We have many success stories, wherein different types of CDPKs have been manipulated to mitigate the effect of abiotic stresses in plants (Table 7.1).

7.7 Future Prospects

Plants are more affected due to changes in various climatic factors like temperature and carbon dioxide due to nonmobile nature. Heat stress have been reported to compromised the quantity and quality of the grains. The yield of most of the agriculturally important crops has been reported to decrease with increase in environmental temperature. Most of the time, the inherent defense mechanism of the plants are not strong enough to cope up with the climatic factors, as evident from the decrease in the yield. Different signalling pathways are operated inside the plant

Table 7.1 Manipulation of Calcium-Dependent Protein Kinases (CDPKs) and its effect on physiological and biochemical traits linked with abiotic stress tolerance in plants

Plant system	CDPK targeted	Response to abiotic stress	Physiological and biochemical changes	References
Arabidopsis	*CPK23*	Mutant Arabidopsis lines demonstrated noticeably enhanced drought and salt tolerance while reducing stomatal apertures, although overexpression of *CPK23* in Arabidopsis resulted in lines showing more sensitivity to drought and salt stresses	Control the activation state of the slow guard cell anion channel SLAC1 in response to different Ca^{2+} concentrations as well as in a Ca^{2+}-independent manner	Ma et al. (2007)
Arabidopsis	*CPK4* and *CPK11*	Salt and drought tolerance	*CPK4* and *CPK11* are involved in abiotic stress through ABA-regulated signalling pathway	Zhu et al. (2007)
Arabidopsis	*AtCPK6*	Acts as a positive regulator in the salt/ drought stress tolerance	Elevated tolerance of the *AtCPK6* overexpressing transgenic Arabidopsis plants was validated by the change of proline and malondialdehyde (MDA)	Xu et al. (2010)
Nicotiana tabacum	*ZoCDPK1*	ZoCDPK1 acts as a positive regulator of the signalling pathways involved in drought and salinity stress responses in ginger	Overexpression of ginger *CDPK1* gene improved tolerance to salinity and drought stress in *Nicotiana tabacum* as indicated by the high percentage of seed germination, higher relative water content, expression of stress responsive genes, higher leaf chlorophyll content, increased photosynthetic efficiency, and other photosynthetic parameters	Vivek et al. (2013)
Rice	*OsCPK9*	Improved drought stress tolerance	Physiological studies demonstrated that *OsCPK9* improved tolerance to drought	Wei et al. (2014; 2016)

(continued)

Table 7.1 (continued)

Plant system	CDPK targeted	Response to abiotic stress	Physiological and biochemical changes	References
			stress via enhancing stomatal closure and by improving the osmotic adjustment ability of the plant	
G. hirsutum	*GhCDPKs*	Involved in the responses to multiple abiotic stresses such as ABA, cold, drought, salinity, and alkalinity	This study revealed that CDPK family is involved in regulating processes during the cotton fiber developmental stages, including initiation, elongation, and secondary wall thickening	Li et al. (2015)
Strawberry	*FaCDPK*	Differential expression of *FaCDPK* in response to salt and drought stress	Optimal fruit quality, as well as long shelf life, has been revealed to be ABA dependent	Crizel et al. (2020)
Citrus	CsCDPKs	Improved response of citrus CDPK to AMF and drought stress		Shu et al. (2020)
Apple	*MdCPK1a*	Increased tobacco cold tolerance	Increase in cold tolerance in *MdCPK1a* overexpressed plants was a result of ROS scavenging and modulating the expression of stress-related genes	Dong et al. (2020)
B. distachyon	*BdCDPKs*	They seem to be involved in WRKY or MAPK-mediated abiotic stress response	Expression profiles specify that most *BdCDPK* genes were concerned with phytohormones signal transduction pathways and regulated physiological processes in response to multiple environmental stresses. Furthermore, the co-expression network demonstrated that *BdCDPKs* may act both as the activator and the repressor in WRKY transcription	Wen et al. (2020)

(continued)

Table 7.1 (continued)

Plant system	CDPK targeted	Response to abiotic stress	Physiological and biochemical changes	References
			factors or MAPK cascade genes mediated abiotic stress	

system for triggering the defense mechanism against the biotic and abiotic stresses. Out of the characterized signal defense network, the CDPK signalling pathway has been reported to be most decisive one, as evident from its role as a sensor in different biological processes. Though, many variants of CDPKs have been identified and characterized in different crop plants, still the information is very meager in agriculturally important crops like wheat and rice. Even the kinetics of CDPK protein has been studied in only a few plants, and the information about the enzymes, its optimum pH and temperature are available only in a few crops. The interactome of CDPK protein with other candidate proteins and their effect on the overall tolerance level to the plant has not been elucidated. Till-date, very few variants of CDPKs has been identified, cloned and characterized from wheat. There is a need to enrich the CDPK-associated genetic resources available on public domain by identifying novel CDPK genes and their respective proteins. The advanced tools of Omics, especially transcriptomics can be used for the identification and characterization of CDPKs. Focus on enzyme kinetics will help us to manipulate the catalytic efficiency, plasticity, and activity of the CDPK enzyme. We can manipulate the CDPK enzyme to use it as natural thermometer for sensing the heat stress level of the plants. Being the potential members of SAGs, molecular markers, especially SSRs and SNPs, overlying on CDPKs, can be used for evaluating diverse germplasm of crop plants for various traits related to heat stress tolerance. Protein engineering tools can also be used to enhance the structural stability as well as catalytic phosphorylation efficiency of the CDPK enzymes in order to make the signalling network more sensitive against abiotic stresses.

To conclude, CDPK need to be targeted for manipulating the tolerance level of the plant. The information about the CDPKs, enzyme kinetics, and their role in different biological processes need to be further elucidated in order to develop a crop plant with robust signalling mechanisms against different biotic and abiotic stresses. The role of CDPK as sensor can be further exploited to analyze the stress level of the plant well ahead of time in order to protect it using different approaches. CDPKs can be manipulated in plant system to develop climate-smart crops with better nutrition quality of the grains.

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Heat Shock Proteins: Catalytic Chaperones Involved in Modulating Thermotolerance in Plants

8

Anil Dahuja, Suneha Goswami, Ranjeet Ranjan Kumar, Vinutha T, and Shelly Praveen

Abstract

Plants as sessile organisms are exposed to persistently changing stress factors. The primary stresses such as drought, salinity, cold and heat are interconnected in their effects on plants. These factors cause damage to the plant cell and lead to secondary stresses such as osmotic and oxidative stresses. Plants cannot avoid exposure to these factors but adapt morphologically and physiologically by some other mechanisms. Almost all stresses induce the production of a group of proteins called heat shock proteins (Hsps) or stress-induced proteins. The induction of transcription factors of these proteins is a common phenomenon in all living things. These proteins are grouped in plants into five classes according to their approximate molecular weight: (1) Hsp100, (2) Hsp90, (3) Hsp70, (4) Hsp60, and (5) small heat shock proteins (sHsps). Higher plants have at least 20 sHsps and there might be 40 kinds of these sHsps in one plant species. The diversification of these proteins reflects an adaptation to tolerate the heat stress. Transcription of heat shock protein genes is controlled by regulatory proteins called heat stress transcription factors (Hsfs). Plants show at least 21 Hsfs with each one having its role in regulation, but they also cooperate in all phases of periodical heat stress responses (triggering, maintenance, and recovery). There are more than 52 plant species (including crop ones) that have been genetically engineered for different traits such as yield, herbicide and insecticide resistance, and some metabolic changes. In conclusion, major heat shock proteins have some kind of related roles in solving the problem of misfolding and aggregation, as well as their role as chaperones.

A. Dahuja · S. Goswami · R. R. Kumar (✉) · V. T · S. Praveen
Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India

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Keywords

Chaperones · Heat shock proteins · Transcription factors · Stress · Heat stress · Heat tolerance

8.1 Introduction

The constant flow of energy through all biological organisms provides the dynamic driving force for the maintenance of biological processes such as cellular biosynthesis and transport. The maintenance of steady-state results in a metastable condition called homeostasis. Any undesired modulation disrupting homeostasis is known as biological stress. Plants as sessile organisms are exposed to persistently changing environmental stress factors. Biological stress in plants is divided into two categories: abiotic and biotic stress. Abiotic stress is a physical stress (e.g., temperature, drought, chemical, light, and salt) that the environment may impose on the plant. Biotic stress is a biological insult (e.g., insects, pests, and pathogens) to which a plant may be exposed during its lifetime.

Abiotic stresses, especially heat, drought, and salinity stresses are the major problems in agriculture. They significantly affect the growth of plants and productivity of crops. It is considered the major cause of >50% reduction in average yield of major crops. Heat stress is turning out to be a major problem in the cultivation of various crops like wheat. Of late, a drastic decrease in the total seed setting and yield has been observed in many wheat growing regions of India mainly due to the terminal heat stress. The problem of heat stress is likely to exacerbate with global climate change adding to the exasperation of the stakeholders. Heat stress has been shown to influence photosynthesis, cellular and subcellular membrane components, seed setting, protein content, and antioxidant enzyme activity; thereby significantly limiting crop productivity (Georgieva 1999). Besides mitigating heat stress, crop productivity under the stress may be enhanced by adaptation strategies.

Numerous heat-responsive proteins have been identified from different crop species. However, the expression patterns of these genes and proteins under heat stress are still not clear. Different stress-associated proteins have been identified from crops like rice, maize, and *Arabidopsis* and their characterization has also been carried out in response to different stresses.

8.2 Mechanism of Heat Stress

The primary stresses such as high temperature, drought, salinity, cold, and chemicals are interconnected in their effects on plants. These factors cause damage to the plant cell and lead to secondary stresses such as osmotic and oxidative stresses. Plants cannot avoid the exposure to these factors but adapt morphologically and physiologically by some other mechanisms. Heat stress as well as other stresses can trigger some mechanisms of defense such as the expression of stress-associated chaperones, the heat shock proteins (HSPs), which was not expressed under "normal" conditions (Kumar et al. 2016). Almost all stresses induce the production of a group of proteins called heat shock proteins (HSPs) or Stress-induced proteins. Heat stress/shock response is a universal phenomenon and heat shock proteins (HSPs) form the most crucial defense system in all living systems at the cellular level (Katschinski 2004). The cytoprotective effects of HSPs were attributed primarily to their chaperone activities, which minimize the proteotoxicity induced by the accumulation of unfolded or denatured proteins upon stress (Katschinski 2004). HSP synthesis is tightly regulated by different members of heat shock transcription factors (Hsfs) at transcriptional level (Morimoto 1998). Hsfs alone can function in the maintenance of cellular homeostasis that include regulation of cell cycle, cell proliferation, redox homeostasis, and cell death mechanisms (Katschinski 2004; Sreedhar et al. 2006).

8.3 Heat Shock Proteins

During evolution, plants have developed sophisticated mechanisms to sense the subtle changes in growth conditions, and trigger signal transduction cascades, which in turn activate stress-responsive genes and ultimately lead to changes at the physiological and biochemical levels. Abiotic stress especially thermal stress adversely affects the functioning of cellular and metabolic pathways in plants. One of the main effects is on functioning of normal cellular proteins. Under thermal stress there is aggregation and misfolding of important cellular proteins occurred. Plants have developed different defense mechanisms to adapt to these adverse conditions. Under the course of defense mechanisms at molecular level, transcription and translation of a special set of proteins like Heat Shock Proteins (HSPs) occur (Kotak et al. 2007; Kumar et al. 2016). Diversification in HSPs may reflect an adaptation to tolerate heat stress. These molecular chaperones assist in protein refolding under stress conditions, protects plants against stress by re-establishing normal protein conformation, and thus cellular homeostasis.

Under stressful conditions, cell response triggered the production of heat shock proteins (HSP). They were named heat shock protein as first described in relation to heat shock, but are now also known to be expressed during other stresses like exposure to cold, UV light, during wound healing, or tissue remodeling. Many HSPs also functions as chaperone by stabilizing new proteins or by helping the refolding of damaged proteins of the cell due to stress (Fig. 8.1). This increase in the expression of HSPs are transcriptionally regulated and the dramatic upregulation of the heat shock proteins is a key heat shock response and is induced primarily by heat shock factors (Hsfs) that are located in the cytoplasm in an inactive state. These factors are considered as transcriptional activators for heat shock (Baniwal et al. 2004; Hu et al. 2009). HSPs are found in virtually all living organisms, from bacteria to plants and humans.

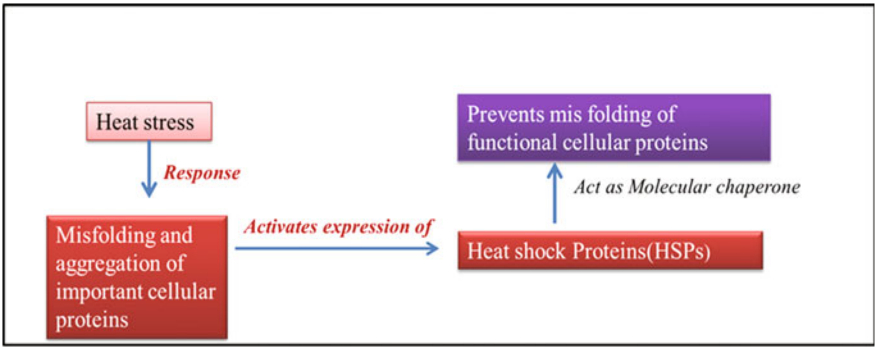

Fig. 8.1 Schematic representation of functional overview of HSP

8.4 Thermal Stability of HSPs

Incorrect protein folding into cells can cause several conformational disorders and in order to prevent such structural misfolding and to maintain homeostasis, cells have evolved an efficient protein quality control system (PQC) as an endogenous process. This PQC system needed molecular chaperones (including all HSP families) and their main function is to prevent inappropriate interactions, avoiding protein aggregation by assisting their correct folding and if protein correction is not possible, guiding them to cell degradation system. To maintain the thermal stability of proteins, the chaperone system changes from a folding to a storing function at heat shock temperatures. The temperature at which this change occurs depends on the presence of a thermosensor in at least one of the components of the chaperone systems. One of the most important chaperones is the Heat shock protein 90 kDa (HSP90), which is responsible for the correct folding of a wide range of proteins. In the folding process, it is essential that HSP90 form complexes with co-chaperones, and providing a cooperative action during the maturation cycle of client proteins.

8.5 Classification of Heat Shock Proteins

The expression of heat shock protein gene was first observed by Italian Scientist F. Ritossa in the chromosome puffing of *Drosophila melanogaster* in response to heat shock. An increase in protein synthesis was observed that occurred also by the use of other stress factors such as azide, salicylate, and 2,4-dinitrophenol (Ritossa 1962). After that report, these proteins were identified and named as heat shock protein (HSP) (Tissieres et al. 1974). Thereafter, various studies were started to find out the relationship between the synthesis of these proteins and the tolerance of stresses. On the other hand, Lin et al. (1984) reported that the exposure of *Glycine*

Table 8.1 Families of HSPs in human beings, their site, and suggested functions

HSP families	Cellular location	Proposed functions
HSP27 (sHSP)	Cytosol, nucleus	Microfilament stabilization, antiapoptotic
HSP60	Mitochondria	Refolds proteins and prevent aggregation of denatured proteins, proapoptotic
HSP70		Antiapoptotic
HSP72 (HSP70)	Cytosol, nucleus	Protein folding, cytoprotection
HSP73 (HSP70)	Cytosol, nucleus	Molecular chaperones
HSP75 (mHSP70)	Mitochondria	Molecular chaperones
HSP78 (GRP78)	Endoplasmic reticulum	Cytoprotection, molecular chaperones
HSP90	Cytosol, endoplasmic reticulum, nucleus	Regulation of steroid hormone receptors, protein translocation
HSP110/104	Cytosol	Protein folding

max seedlings to heat shock (from 28 to 45 °C) for 10 min (longer periods killed the seedlings) induce the synthesis of HSPs at the cost of other proteins synthesis.

Several types of heat shock proteins have been identified in almost all organisms (Bharti and Nover 2002). HSPs are mainly characterized on the basis of the presence of a carboxylic terminal called heat shock domain (Helm et al. 1993). HSPs having molecular weights ranging from 10 to 200 kDa are characterized as chaperones where they participate in the induction of the signal during heat stress (Schoffl et al. 1998). Heat shock proteins of archaea have been classified on the basis of their approximate molecular weight as (1) Heat shock protein of molecular weight 100 kDa: HSP100, (2) HSP90, (3) HSP70, (4) HSP60, and small heat shock proteins (sHSPs) where the molecular weight ranges from 15 to 42 kDa (Trent 1996). Schlesinger (1990) reported that in eukaryotic organisms, the principle heat shock proteins of human beings do not differ from those of bacteria except for the presence of HSP33. Later, the HSPs of human beings were grouped into five families (Kregel 2002) as in Table 8.1.

In plants, according to molecular weight, amino acid sequence homologies and functions, five classes of HSPs are characterized: (1) HSP 100, (2) HSP 90, (3) HSP 70, (4) HSP 60, and (5) small heat shock proteins (sHSPs) (Kotak et al. 2007; Gupta et al. 2010).

The high molecular weight HSPs are characterized as molecular chaperones. Higher plants have at least 20 sHSPs and there might be 40 kinds of these sHSPs in one plant species. The name of HSPs in bacteria differs from those in eukaryotic cells as given below but the nomenclature for sHSPs are same in both organisms (Kotak et al. 2007).

Escherichia coli	Eukaryotic cells
ClpB	HSP100
HtpG	HSP90
Dnak	HSP70
GroEL	HSP60

8.6 Role of Different HSPs

Under thermal stress, the general role of HSPs is to act as molecular chaperones and regulate the protein folding, accumulation, localization, and degradation of proteins in all plants and animal species (Hu et al. 2009; Gupta et al. 2010), indicated that HSPs protect the cells from injury and facilitate recovery and survival after a return to normal growth conditions. On the other hand, under nonthermal stress, their function could be different: as it may protect the protein from damage and maintain the correct protein structure (Timperio et al. 2008). As chaperones, these proteins prevent the irreversible aggregation of other proteins and under heat stress, they participate in refolding of proteins (Tripp et al. 2009). Each group of these HSPs has a unique mechanism and the role of each is as follows.

8.6.1 Class: HSP 100

This class of proteins is responsible for the reactivation of aggregated proteins (Parsell and Lindquist 1993). They basically re-solubilize the nonfunctional protein aggregates and help to degrade irreversibly damaged polypeptides (Kim et al. 2007). This class HSPs function is not restricted only to acclimation to high temperatures, but they also provide housekeeping functions, essential for chloroplast development (Lee et al. 2006), and facilitating the normal situation of the organism after severe stress (Gurley 2000).

8.6.2 Class: HSP 90

HSP90 can bind with HSP70 to form chaperone complexes and act as molecular chaperones, playing important role in signaling protein function and trafficking (Pratt and Toft 2003; Kumar et al. 2012), regulating the cellular signals such as the regulation of glucocorticoid receptor (GR) activity (Pratt et al. 2004). Cytoplasmic HSP 90 reacts with resistance protein (R), the signal receptor from the pathogen, and participates in providing resistance from pathogens. Thus, HSP90 is considered the essential component of innate immune response and pathogenic resistance in rice (Thao et al. 2007). Yamada et al. (2007) reported that in *A. thaliana*, in the absence of heat stress, cytoplasmic HSP90 negatively inhibits the Hsf, but under heat stress this role is temporarily supressed, so that Hsf is active.

8.6.3 Class: HSP 70

The HSP 70 plays role as a chaperone for newly translated proteins and prevents their accumulations as aggregates, helps in their proper folding, protein import and translocation, and proteolytic degradation of unstable proteins by targeting the proteins to lysosomes or proteasomes (Su and Li 2008). HSP 70 along with sHSPs play a crucial role in protecting plant cells from the detrimental effects of heat stress (Rouch et al. 2004; Kumar et al. 2016). HSP 70B is present in the stroma of chloroplasts, also involved in photo-protection and repairing of photosystem II during and after photoinhibition (Schroda et al. 1999). A study on *A. thaliana* reported that HSP70 was found in the stroma of chloroplast involved in the differentiation of germinating seeds (Su and Li 2008). Structurally, HSP70 consists of a highly conserved N-terminal ATPase domain of 44 kDa and a C-terminal peptide-binding domain of 25 kDa. HSP70 family chaperones are considered to be the most highly conserved HSPs, with, 50% identical residues between the *Escherichia coli* homolog DnaK and the eukaryotic HSP70.

8.6.4 Class: HSP 60

A well-known chaperonin, responsible for assisting plastid proteins is Rubisco (Wang et al. 2004). This class of HSPs participates in folding, aggregation, and transport of many mitochondrial and chloroplast proteins (Lubben et al. 1989). HSP60 prevents the aggregation of newly transcribed protein before their folding (Parsell and Lindquist 1993). Functionally, plant chaperonins are limited and stromal chaperones (HSP 70 and HSP 60) are involved in attaining functional conformation of newly imported proteins to the chloroplast (Jackson-Constan et al. 2001).

8.6.5 Class: HSP 40

HSP40 proteins regulate complex formation between polypeptides and HSP70 by different mechanisms. First, HSP40 interacts with HSP70-polypeptide to stimulate its ATPase activity (Cyr et al. 1992). Second, HSP40 proteins have polypeptide-binding domains (PPDs) that bind and deliver specific proteins to HSP70 (Cheetham and Caplan 1998). Third, within the same cellular compartment, specialized members of the HSP40 family are localized to different sites, which facilitate the interaction of different HSP70–HSP40 complexes to bind unique proteins at that site (Shen et al. 2002). This class of protein is also known as J-domain-containing protein (J-protein). It acts as a co-chaperone component of the HSP70 system, increasing HSP70 affinity for proteins (Kampinga and Craig 2010). It has a conserved 70-amino acid J-domain that interacts with the nucleotide-binding domain (NBD) of HSP70 and participates in various virus–plant interactions. Similar to HSP70, the function of HSP40 in viral pathogenesis has been well established. For example, the coat protein of *Potato virus Y* interacts with DnaJ-like protein (HSP40), which is important for cell-to-cell movement (Hofius et al. 2007). The functions of HSP70 and HSP40 in plant immunity have been generally identified as chaperones in microbial pathogenesis, particularly, in viral movement. Several HSP70 and HSP40 were demonstrated as positive regulators in plant immunity. Overexpression or knockdown of these HSPs enhance resistance and susceptibility to pathogen infections respectively, although the mechanisms remain unclear.

8.6.6 Class: sHSPs (Small HSPs)

The genes encode for small HSPs, their expression is limited in the absence of environmental stress and occurs in some stages of growth and development of plants such as embryogenesis, germination, development of pollen grains, and fruit ripening (Sun et al. 2002). Structurally these proteins have a common alpha-crystalline domain of 80–100 amino acid residues in the C-terminal region (Seo et al. 2006; Kumar et al. 2013). Functionally, these proteins are responsible for the degradation of the proteins having unsuitable folding. The representative protein of this class of HSPs is the enzyme-bound ubiquitin (molecular weight is 8.5 kDa) (Ferguson et al. 1990). Unlike chaperones, these proteins have ATP-independent activity (Miernyk 1999). sHSPs can bind to partially folded or denatured proteins, preventing irreversible unfolding or wrong protein aggregation but they cannot refold the non-native proteins (Sun et al. 2002). Nakamoto and Vigh (2007) concluded that under stress conditions, small heat shock proteins play an important role in controlling the membrane quality and maintaining membrane integrity.

8.7 HSPs/Chaperones Network

In the protective mechanism of HSPs/chaperones, many chaperones act in concert with the chaperone machinery network. During stress, several enzymes and structural proteins undergo detrimental structural and functional changes. Therefore, maintaining proteins in their functional conformations, preventing from aggregation of non-native proteins, refolding of denatured proteins to regain their functional conformation, and removal of nonfunctional but potentially harmful polypeptides (arising from aggregation, misfolding, or denaturation) are particularly important for cell survival under stress. Therefore, the different classes of HSPs/chaperones cooperate in cellular protection and play complementary and sometimes overlapping roles in the protection of proteins from stress. Small HSPs (sHSPs) bind to non-native proteins and prevent their aggregation, thus providing a reservoir of substrates for subsequent refolding by members of the HSP70/HSP100 chaperone

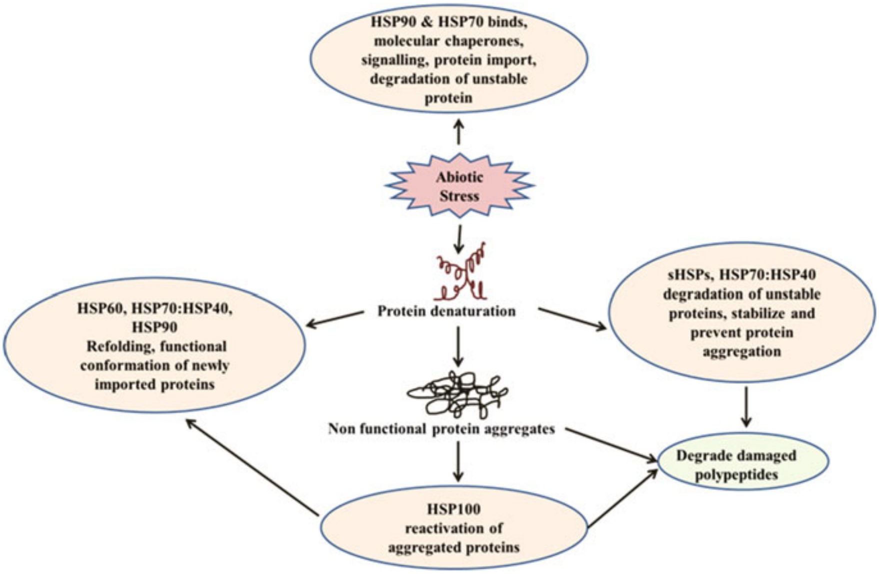

Fig. 8.2 The heat shock proteins (HSPs) and chaperone network under abiotic stress

families. The Chaperone/HSPs network under stress, how they regulate different proteins' stability/degradation is presented in Fig. 8.2.

The response of plants to heat shock resulted in changes in the level of enzymes, cellular membrane structure, photosynthesis activity, and protein metabolism (Singla et al. 1997). It has been reported that high temperature changed the properties of membranes of nucleus, endoplasmic reticulum, mitochondria, and chloroplasts of rice plant, *O. sativa* (Pareek et al. 1998). Lipids in the thylakoid membranes of the chloroplast are very important to improve photosynthesis and hence stress tolerance.

The transcription of these genes is controlled by regulatory proteins called heat shock transcription factors (Hsfs) located in the cytoplasm in an inactive state. So these factors are considered transcriptional activators for heat shock (Baniwal et al. 2004; Hu et al. 2009). Plants are characterized by a large number of transcriptional factors (Baniwal et al. 2007). These factors have been classified (Tripp et al. 2009) into three classes according to the structural differences in their aggregation in triples, i.e., oligomerization domains as follows:

- Plant HsfA such as HsfA1 and HsfA2 in *L. esculentum*
- Plant HsfB such as HsfB1 in *L. esculentum*
- Plant HsfC

The synthesis of HSPs depends upon activity of special class of transcription factors called Heat Shock Factors (Hsfs). Hsfs are modular transcription factors encoded by a large gene family in plants. Hsfs have three highly conserved features: the amino terminal DNA binding domain of approximately 100 amino acids

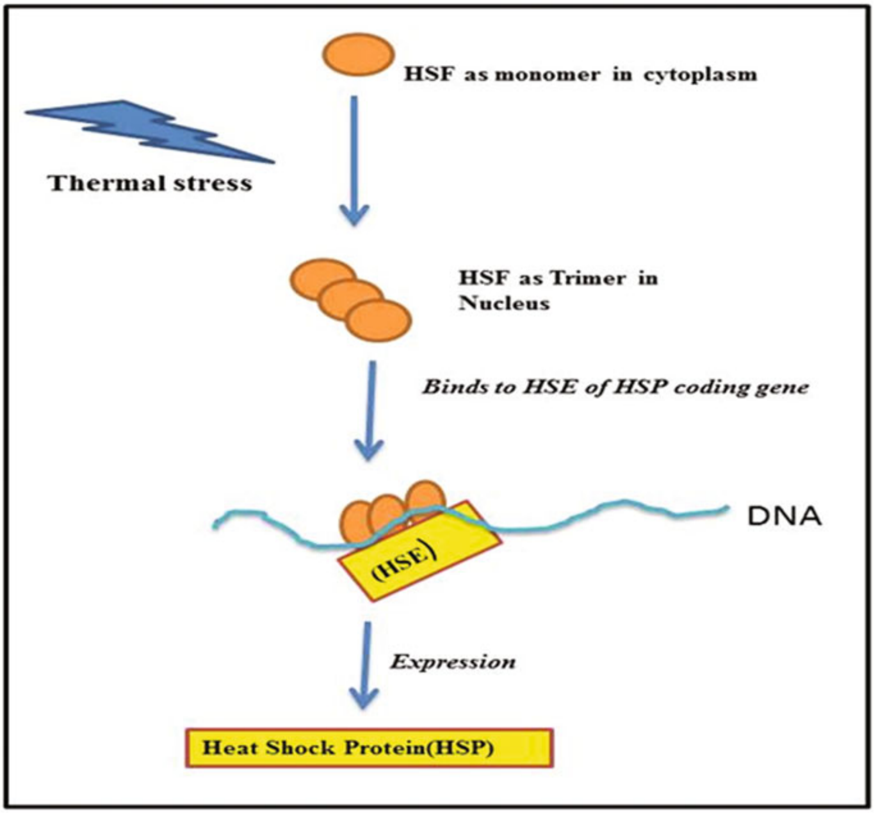

Fig. 8.3 Mechanism of action of Heat Shock Proteins

(Harrison et al. 1994) and a domain having three leucine zippers mediating multimerization (Wu et al. 1994; Swamynathan 1995) and an additional leucine zipper motif at the carboxy terminus. Hsfs trimerizes via the formation of a triple-stranded α-helical coiled coil, involving the three conserved leucine zippers next to the DNA binding domain (Peteranderl and Nelson 1993). Hsfs bind to heat shock elements (HSE) in a sequence-specific and reversible manner, leading to the activation of transcription of heat shock proteins (Morimoto et al. 1994; Goswami et al. 2016; Fig. 8.3).

8.8 Genetically Modified Plants for Heat Stress Tolerance

Several plant species (more than 52) have been genetically modified for different traits including crop plants like tomato, potato, soybean, maize, rice, and cotton. Other non-crop transgenic plants were also developed for different abiotic stress tolerance in laboratory. High-temperature stress is one of the major abiotic stresses

Table 8.2 Transgenic attempts to enhance thermotolerance of plants

Phenotypes	Gene	Function	Plant
Heat stress tolerant	HsfA1	Transcription factor	Tomato
Heat stress tolerant	Hsf3	Transcription factor	Arabidopsis
Heat stress sensitive	HSP70	HSP	Arabidopsis
Heat stress tolerant	HSP17.7	HSP	Carrot
Heat stress tolerant	Hvapx1	Active oxygen species (AOS) metabolism	Barley
Heat stress tolerant	Fad7	Fatty acid desaturation	Tobacco

Source: Sample of a larger table of Sung et al. (2003)

for crop plants. The plant reaction to high-temperature stress resulted in changes in cell membrane stability, photosynthesis activity, enzyme denatured, and protein synthesis (Goswami et al. 2015). High temperature also changes the properties of membranes of mitochondria, chloroplast, endoplasmic reticulum, and nucleus. Lipids in the thylakoid membrane of chloroplast are important for membrane stability and also for photosynthetic efficiency which may be disturbed due to very high or very low temperatures. By increasing the expression of glycerol 3-phosphate acyltransferase enzyme in tobacco plant, the degree of lipid unsaturation was increased which makes the plants cold tolerant. An increase in the degree of saturation of membrane lipids may lead to an increase in the heat tolerance of the plants. Other ways to develop thermotolerance of plants, by changing the level of HSPs expression, Hsfs expression, increase in the synthesis of osmolytes in the cells, modifying the endogenous genes of crop plants such as rubisco activase, oxygen-evolving enhancer proteins, signaling molecules like calcium-dependent protein kinase (CDPK), mitogen-activated protein kinases (MAPK), and genes involved in starch biosynthesis pathways through site-directed mutagenesis and make them thermotolerant. Some examples of the attempts taken for developing thermotolerant crop plants are given in Table 8.2.

8.9 Conclusion

Although, many attempts have been made in the past to develop genetically modified plants for stress tolerance, but with limited sucess. Most attempts were for one trait, while in nature the prevailing conditions is quite complex and require cohorted effort to protect the plant from the vageries of stress. Heat shock proteins basically works as catalytic chaperones preventing the heat stress from inducing protein aggregation/ denaturation and helps in maintaining different metabolic reactions under extreme condition. Out of all the HSPs, sHSPs showed very high-fold increase in the expression in response to heat stress and has been observed to store unfolded proteins. Few HSPs also acts as proteases protecting the cell from damage under abnormal condition. The HSPs has been reported to facilitate the restoration of normal cell function by assisting the refolding of denatured/ aggregated proteins, along with protection of nascent proteins. It also helps in removing the irreparable proteins from the cells. The HSP90 family has been observed to play very important role in signalling as well as defense against biotic and abiotic stresses. The HSP70 family represents one of the most highly conserved classes of heat shock proteins. HSPs ensure proper protein folding and their transfer to final location. There is a need to explore the gene-protein networks of HSPs operating inside the cells and their correlation with the signalling pathways in order to understand the mechanism underlying heat stress tolerance in plants. The information generated will pave the way for the development of climate-smart crop.

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Starch Metabolism under Heat Stress

9

Suneha Goswami, Ranjeet Ranjan Kumar, Suman Bakshi,
and Shelly Praveen

Abstract

Heat stress (HS) is an important abiotic stress which created challenges for the agricultural sector. It is a key barrier; pose one of the most important constraints to growth and productivity of major cereal crops and also severely impact on world food security. Plant growth, physiological processes, and the final yield of plant produce are all influenced by HS to varying degrees, reflecting the physical damages, physiological disturbances, and biochemical modifications that occur at various phases of plant growth. HS has detrimental impact on starch metabolism of the plant. It affects various physiochemical and molecular parameters such as photosynthetic rate, activity and expression analysis of enzymes involved in starch metabolism, total starch, amylose and amylopectin content, and total amylolytic activity. In counteract to HS, plants remobilize their reserve starch in order to release energy, sugar, and other metabolites to alleviate stress and help plants to survive. To generate HS-tolerant crop varieties, conventional breeding, biotechnological, and molecular techniques are the preferred approach. Recent advances in omics approaches have resulted in a better understanding of the molecular pathways involved in HS and application of CRISPR/Cas-mediated genome editing approach could develop non-genetically modified (Non-GMO) crop variety having tolerance to multiple abiotic stresses and also have potential for public acceptance in the future.

S. Goswami (✉) · R. R. Kumar · S. Praveen
Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India

S. Bakshi
Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, India

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Keywords

AGPase · Heat stress · Starch biosynthesis · Starch metabolism · Amylose · Amylopectin · Starch synthase · Starch branching enzyme · Starch debranching enzyme

9.1 Introduction

Plants store majority of their carbohydrates in the form of starch, which accumulates in the plastids. To assess the significance of starch in overall plant carbon allocation, one must first comprehend the molecular mechanism that control starch structure and metabolism, as well as how cellular and the environmental factors influence them. Starch is an insoluble, nonstructural carbohydrate composed of two glucan polymers, amylose and amylopectin, both of which consist of α-(1,4)-linked glucose (Glc) chains that are occasionally branched via α-(1,6)-glycosidic linkage of glucose moiety (Badenhuizen 1963). The major component of starch (70–80%) is amylopectin, which makes up the structural framework and provide highly organized and crystalline structure to the starch granule. Amylopectin has a high degree of polymerization ($\underset{\sim}{}10^5$) and 4–5% of its linkages are $\alpha-1,6$-branch points while amylose is considerably smaller with very few branching points. Amylose fills the semicrystalline structure formed by amylopectin and makes starch granules as a dense molecule, and causes water insolubility and osmotically inactive. Starch is mostly associated with storage organs such as rhizomes, roots, stems, seeds and tubers, and in leaves; it acts as a buffer for nighttime carbon starvation (Zeeman et al. 2010). In many industrial processes, starch is used as a binding agent. There are two forms of starch found in higher plants: Storage starch, which is made in the amyloplast and is used to store energy for a long time; and transient starch, which is synthesized and degraded in chloroplasts according to the diurnal cycle (Lloyd and Kossmann 2015). Various reports revealed that non-photosynthetic cells such as wheat (*Triticum aestivum* L.) peduncle parenchyma (Scofield et al. 2009) and Arabidopsis (*Arabidopsis thaliana* L.) floral organs (Hedhly et al. 2016), also produce transient-like starch in storage plastids for temporary carbon storage and rapid remobilization during floral development.

Among the ever-changing elements of the natural environment, one of the most detrimental stresses is the steadily increasing ambient temperature. Heat stress (HS) is defined as an increase in temperature above a threshold level for an extended period of time that causes irreparable damage to flora growth and development processes. Heat shock, or HS, is a transitory spike in temperature of 10–15 degrees Celsius over ambient temperature (Wahid and Close 2007). Agriculture production is threatened by future changes in global climatic conditions, which are expected to increase by 1.5–5.8 degrees Celsius, leading to global warming (Hemantaranjan et al. 2014; Kumar et al. 2019). Extreme climatic conditions, such as extremely hot temperatures, are expected to have a severe impact on plant growth and development, resulting in a catastrophic loss of food quality all over the world (Kumar et al.

2017; Yuan et al. 2017). Drying of the stigmatic surface, defunct pollen, inappropriate fertilization, breakdown of starch granules, creation of tiny and pleated granules, unfilled voids, and shrivelled grains are all symptoms of heat stress (Kumar et al. 2016). Heat stress disturbs essential processes by denaturing/aggregating crucial enzymes such as RuBisCo, RuBisCo activase, transporters, and enzymes involved in the starch manufacturing pathway (Kumar et al. 2018). It also affects the expression of transcription factors that control the expression of genes linked to stress. Under HS, the accumulation of metabolites is also affected, resulting in a shift in the redox potential of cells.

This chapter considers starch biosynthesis, multi-dimensions of starch metabolism, effect of heat stress on starch metabolism and the mechanisms involved in the tolerance development of plants.

9.2 Types of Starch Granules

Starch granule shapes and sizes can vary with different species (Table 9.1). The size distribution of starch granules in amyloplasts, as well as the content and characteristics of starch granules, alter during granule formation. Starch granules exist in A-type and B-type. A-type starch granules are large and lenticular and found in wheat, barley, and rye. B-type starch granules are small, spherical, or polyhedral. Rice, oats, and maize starch granules are irregular and polyhedral in shape. Although rice starch granules are comparable in size to wheat and barley B-type starch granules while those of maize are larger. Starch is made up of amylose and amylopectin, with amylose levels ranging from 20% to 30% in most grain starches (Katz et al. 1993). In the case of certain varieties of maize, barley, and rice, the starch is entirely composed of amylopectin; called as "waxy" starch while amylomaize has high amylose (52%) found. A fraction of the amylose in the starch granule is complexed with lysophospholipids depending on the stage of endosperm development at which the amylose is produced (Morrison 1993). B-type granules emerge later in the grain development process than A-type granules. During wheat and barley grain growth, the quantities of amylose and lysophospholipids increased in

Table 9.1 Variations in the shape and size of starch granules in diverse species of plants

Source	Granule shape	Granule size (nm)	Amylose content (%)
Wheat	Lenticular or round	20–25	22
Maize	Round or polyhedral	15	28
Waxy maize	Round	5–15	1
High amylose	Round or irregular sausage shaped	25	52
Barley	Round or elliptical	20–25	22
Rice	Polygonal	3–8	17–23
Oat	Polyhedral	3–10	23–24

Adapted from Lineback (1984)

both A-type and B-type starch granules (Morrison and Gadan 1987; McDonald et al. 1991).

9.3 Starch Biosynthesis in Plant

Starch synthesis takes place in plastids-chloroplasts in leaves and specialized amyloplasts in starch-storing tissues of staple crop. In the process of starch synthesis and accumulation, more than 30 major enzymes participate in the metabolism of carbohydrates during endosperm development in cereal grains. Among them, five enzymes play key roles in this process, which are ADP-glucose pyrophosphorylase (AGPase), granule-bound starch synthetase (GBSS), soluble starch synthase (SSS), starch branching enzyme (SBE), and starch debranching enzyme (DBE) (Yang et al. 2001; Hannah and James 2008; Chen and Bao 2017; Wenting et al. 2021).

Starch polymer formation involves the synthesis of adenosine diphosphate glucose (ADP)-glucose by ADP-glucose pyrophosphorylase (AGPase) (Fig. 9.1). This is the first and limiting step of the starch biosynthesis reaction (Tuncel et al. 2014).

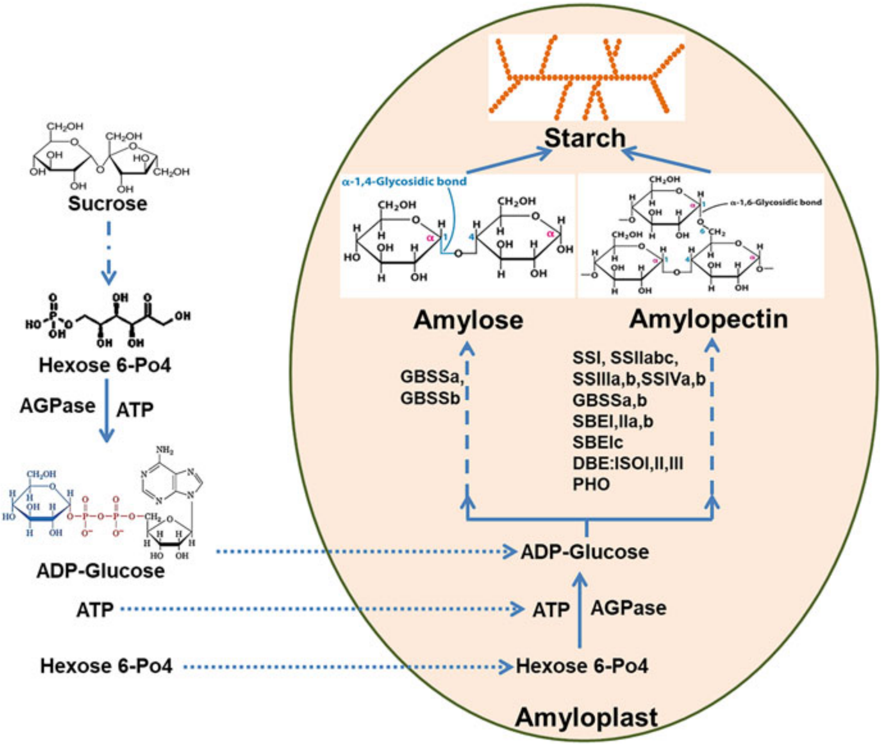

Fig. 9.1 Starch biosynthesis pathway in plant. *AGPase* ADP-glucose pyrophosphorylase, *GBSS* Granule-bound starch synthetase, *SSS* Soluble starch synthase (SSS), *SBE* Starch branching enzyme (SBE), *DBE* Starch debranching enzyme, *ISP* Isomerase

Glucose-1-phosphate generated in the Calvin–Benson cycle along with ATP is catalyzed by AGPase into ADP-glucose and pyrophosphate (PPi). Increasing AGPase activity can regulate starch synthesis and hence increases seed weight (Smidansky et al. 2003; Tuncel and Okita 2013). Starch synthase (SS) uses (ADP)-glucose to form linear α−1,4 glucan chains. The glycosyltransferase (GT) moiety of SSS catalyze the transfer of the glucosyl moiety of ADP-glucose to the nonreducing end of an existing glucosyl chain, and creating an α-1,4 bond and elongating the chain. In starch biosynthesis, five SS classes are involved: four are soluble in the stroma (or partially bound to the granule), and one is entirely attached to the granule. Amylopectin synthesis is carried out by the soluble SSs (SSI, SSII, SSIII, and SSIV), while amylose synthesis is carried out by the granule-bound SS (GBSS) (Pfister and Zeeman 2016; Nazarian-Firouzabadi and Visser 2017). The branch points within amylopectin are introduced by starch branching enzymes (SBEs). SBEs catalyzes the formation of α-1,6- glycosidic linkage and add branches in the existing chain via glucanotransferase reactions. Starch branching enzymes belong to the α-amylase superfamily of enzymes and it cleave an α-1,4-glucan chain and transfer the cleaved portion to the C6 position of a glucose unit from the same or another chain, and create an α-1,6-linked branch (Pfister and Zeeman 2016; MacNeill et al. 2017). In doing so, SBEs also generate additional substrates for the SSs (i.e., nonreducing ends of chains). Starch debranching enzymes (DBEs) hydrolyze α-1,6-linkages and release linear chains (Satoh et al. 2003; Fujita et al. 2009; Jeon et al. 2010). DBEs belong to the glycoside hydrolase family 13 (Lombard et al. 2014) and share the central α-amylase domain and a starch-binding domain with BEs. They can be further divided into two types: isoamylases (ISAs) and limit-dextrinase (LDA). The two forms of DBEs can be distinguished based on protein sequences and substrate specificity, as only LDAs can efficiently digest pullulan (Zeeman et al. 2010; Hizukuri et al. 2006). Plant genomes encode three classes of isoamylase—ISA1, ISA2, and ISA3—and one LDA. ISA1 and ISA2 are involved in debranching during the synthesis of amylopectin while ISA3 and LDA primarily debranch starch during its degradation (Dinges et al. 2003; Wattebled et al. 2005; Delatte et al. 2006; Yun et al. 2011). All these enzymes have been implicated in total starch synthesis, amylose and amylopectin accumulation from nucleotide-sugar precursors in the cereals endosperm (Collins et al. 2021). The starch biosynthetic enzymes are highly conserved across plant species, implying a common ancestor (Deschamps et al. 2008).

9.4 Effect of Elevated Temperature on Starch Granule and Grain Structure

Elevated temperature occurring during the grain filling stage is an important factor affecting starch synthesis and accumulation in cereal grain and causes negative effects on grain yield and quality. The elevated temperature shifted the timing of the starch biosynthetic process. The ideal temperature for strong activity of ADP-glucose pyrophosphorylase and soluble starch synthase is about 22 °C. At

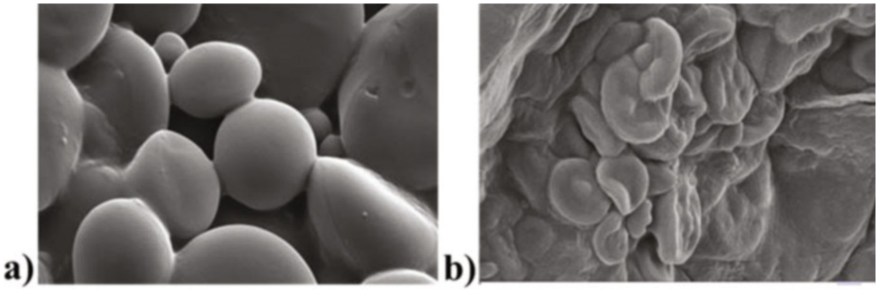

Fig. 9.2 Scanning Electron Microscope picture of starch granule: (**a**) Starch granule under control condition and (**b**) Starch granule under elevated temperature

higher temperature during grain filling stage, increases the activities of starch branching enzyme and suppressed the activities of granule-bound starch synthase and starch debranching enzyme, which results hindering of starch synthesis and accumulation. The high temperature had a noticeable influence on the augmentation of grain α-amylase activity during kernel filling, and the starch granules becomes vulnerable to enzymatic hydrolysis, which disintegrates and distorts starch granule. High temperature results chalkiness of the grain. Scanning electron microscope study showed cereals variety susceptible to high temperature has defragmented, pleated, loosely arranged starch granule with less numbers of granules formation occur (Fig. 9.2). High temperature stress reduced the accumulation of amylose and starch.

9.5 Multi-dimensions of Starch Metabolism

Starch metabolism has a variety of activities. The mechanism of starch metabolism is well studied in leaves, where starch is produced during the day and mobilized at night to ensure a constant supply of carbon and energy when photosynthesis is not available. Under fluctuating photoperiods or in reaction to a sudden early or late nightfall, the rate of starch degradation is adjusted to guarantee adequate carbon supply for continuing growth during the night (Stitt and Zeeman 2012). Starch is destroyed by an extensive network of events including the synergistic activity of numerous enzymes. β-Amylase is the major hydrolytic enzyme involved in night-time starch degradation in leaf chloroplasts, and break the nonreducing end of starch to liberate maltose (Fulton et al. 2008). Debranching enzymes along with β-amylase, hydrolyze the $\alpha -1,6$-branches of starch into short soluble malto-oligosaccharides. The hydration condition of the granule–stroma interface is increased by starch phosphorylation, which disrupts its crystallinity and hence facilitates the actions of the glucan-hydrolyzing enzymes. Starch performs number of important roles in plant physiology along with its well-known role in promoting nighttime metabolism. For example, Starch is rapidly destroyed in the guard cells that surround the stomatal pores and govern water and CO_2 exchange with the environment when exposed to light, assisting in the production of organic acids and sugars that raise guard cell turgor and encourage stomatal opening (Horrer et al. 2016). In such cells, the predominant starch degrading enzyme is β-amylase, which works in tandem with α-amylase to efficiently digest starch (Valerio et al. 2011; Horrer et al. 2016). Under normal conditions, loss of β-amylase and α-amylase alone or in combination had no effect on starch metabolism in mesophyll cells, indicating that they are tissue-specific enzymes (Horrer et al. 2016; Thalmann et al. 2016). Starch turnover in reproductive tissues is functionally connected to cell division and differentiation rather than storage functions during floral, early embryo and silique development stages (Andriotis et al. 2010; Hedhly et al. 2016). The potential translocator of Glc6P for starch production in reproductive organs has been identified as the glucose-6-phosphate (Glc6P)/phosphate antiporter GPT1. Since mesophyll cells are unable to import Glc6P efficiently, demonstrates that starch turnover in different cell types requires the activity of specialized enzymes, and also explains that why there are so many starch-related enzymes, some of which are involved in nighttime metabolism in leaves while others are required for starch metabolism in different plant cell types. Small amount of starch is also present in the amyloplasts of the root-cap columella cells, which are required for full gravity perception and sensitivity (Weise and Kiss 1999). However, the glucan enzymes that ply in this cell type, as well as the molecular mechanisms that support them, are mostly unknown.

9.6 Starch Metabolism During Heat Stress

Starch is a major molecule that modulating plant responses under heat stress. Heat stress alters the plant photosynthetic rate, starch accumulation, and expression of enzymes involved in starch biosynthesis (Kumari et al. 2020; Kumar et al. 2017, 2019b). Various reports confirmed that in cereal crops such as wheat (Kumar et al. 2017) and maize (Yang et al. 2018) heat stress during grain filling stage reduces the starch content, and increases the protein content. This change in grain components and contents was due to changes of enzymes involved in starch and protein biosynthesis (Fig. 9.3). The activities of adenosine diphosphate glucose pyrophosphorylase (AGPase), aldolase, acid invertase, and acid phosphatase decreased by heat stress during grain filling (Duke and Doehlert 1996). Grain starch synthesis is regulated by adenosine diphosphate glucose pyrophosphorylase (AGPase), SSS and SBE. Heat stress during grain filling decreases the activities of sucrose synthase (SuSy), AGPase, glucokinase, soluble starch synthase (SSS), and starch branching enzyme (SBE), which restricted the accumulation of starch (Singletary et al. 1994; Wilhelm et al. 1999). Sucrose is the main transport substrate for starch formation in cereals. Heat stress also decreases the activities of sucrose phosphate synthase (SPS) and sucrose synthase (SuSy) during grain filling, which inevitably led to decreased sucrose content of grain (Zhao et al. 2013). Heat stress significantly lowered the expression of genes related to sucrose and starch synthesis-related enzymes, producing chalky grains as a result of reduced starch deposition (Hakata et al. 2012; Phan et al. 2013).

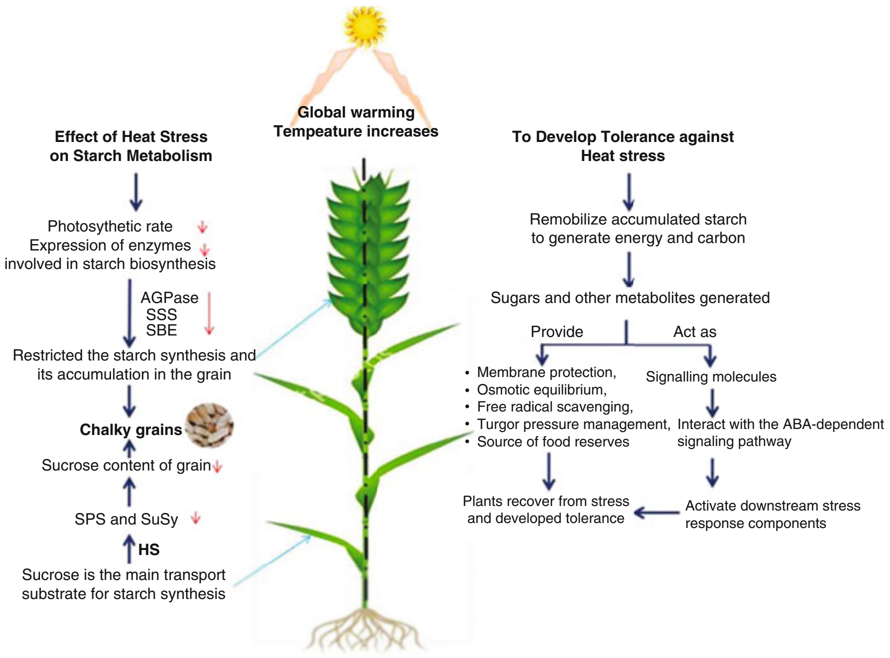

Fig. 9.3 Starch metabolism during Heat stress. *AGPase* adenosine diphosphate glucose pyrophosphorylase; *SSS* Soluble starch synthase; *SBE* Starch branching enzyme; SPS: Sucrose phosphate synthase; *SuSy* Sucrose synthase; *HS* Heat stress

The starch metabolism is a critical factor of plant fitness during abiotic stress in most plant species. To combat the detrimental effect of heat stress and to survive under stress, plants remobilize accumulated starch to generate energy and carbon when photosynthesis rate is also potentially reduced. Thalmann and Santelia (2017) reported upregulation of starch degrading enzymes (β-amylase and α-amylase) during heat stress and suggested their role in inducing sugar (maltose) accumulation. Sugars and other metabolites generated during heat stress contributes to membrane protection, osmotic equilibrium, free radical scavenging, turgor pressure management, and a source of food reserves to help plants recover from adverse condition or to counteract the stress damaging effects (Krasensky and Jonak 2012; Kumar et al. 2017; Chandrakar and Keshavkant 2018; Seth et al. 2021). Sugars released from starch metabolism can also function as signaling molecules, and interact with the ABA-dependent signaling pathway to activate downstream stress response components (Krasensky and Jonak 2012; Thalmann et al. 2016; Kumar et al. 2012). For example, the desiccated shoots of the moss *Polytrichum formosum* were devoid of starch (Presselet al., 2006); and degraded upon heat stress in woody plants such as the lychee tree (*Litchi chinensis*) and herbaceous species such as wheat (*Triticum aestivum*) (Kumari et al. 2020; Kumar et al. 2017), barley (*Hordeum vulgare*) (Villadsen et al. 2005; Damour et al. 2008). Seth et al. (2021)

reported the interaction of various heat shock proteins (HSPs) and heat shock factors (HSFs) with unigenes actively involved starch metabolism and develop tolerance against heat stress in tea plant. Although starch degradation in response to heat stress has been linked to increased tolerance, the reason for this discrepancy is unclear.

9.7 Strategies to Mitigate the Effect of Heat Stress

9.7.1 Conventional Breeding Strategies

Conventional breeding strategies focused on genotype selection and inter-mating result in a limitation of genetic variation for domesticated and selected traits. Thus, to improve crop performance under stress conditions, extensive research is required to sort out genetic heterogeneity in both wild and domesticated plant species. Genetic features related to stress tolerance are complicated because they are controlled by multiple genes and are very sensitive to environmental factors. To improve HS tolerance, wild type land races of crop plants must be investigated for HS-tolerant genes and quantitative trait loci (QTLs) (Fernie et al. 2006; Hassan et al. 2020). Physiological trait-based breeding strategies have a competitive advantage over traditional breeding methods. For heat stress tolerance adaptation, breeding approaches must target a variety of physiological features associated to plant canopy structure such as membrane stability, plant stay green character, leaf with waxy cuticle, improved stomata conductance and photosynthetic rate, better filling duration and fruit setting, and grain yield (Cossani and Reynolds 2012; Gupta et al. 2012; Fahad et al. 2017).

9.7.2 Molecular and Biotechnological Approaches

To find HS-related genes/QTLs, molecular biology and molecular markers are required fields and approaches, respectively. Biotechnological approaches played key role in finding heat stress tolerant genes in crops such as tomato, wheat, and maize, where many genes have been discovered which are important for heat shock proteins (HSPs) synthesis (Momcilovic and Ristic 2007). Variations in transcription and translation of stress-associated genes/proteins can influence HS tolerance in plants. To improve HS tolerance in plants, two biotechnological techniques are commonly used: (i) Genetic transformation and (ii) Marker Assisted Selection (MAS).

The application of MAS implies the identification of appropriate genetic markers associated with QTLs/genes responsible for HS tolerance at the whole plant level. Several genetic markers linked to various abiotic stresses have recently been discovered in a number of crops. Several QTLs with excellent impacts on the HS have been found and characterized using this method in many plant species (Hassan et al. 2020). Gene expression and genetic transformation study developed a better understanding of the biochemical and genetic bases of plant HS tolerance, which will serve as the foundation for breeding genotypes with improved tolerance to HS. Transgenic approaches have resulted in increase in HS tolerance in a variety of plant species by changing gene expression and producing targeted genes products, i.e., proteins and enzymes. In conclusion, biotechnology efforts to producing HS-tolerant cultivars are promising. However, more research is needed to understand the physiological, molecular, and genetic bases for developing cultivars with enhanced tolerance to HS.

9.7.3 Omics Approaches in Developing Heat Stress Tolerance

Omics are approaches that allow for the identification of translational, transcriptional, and posttranslational mechanisms, as well as signaling networks that significantly affect plant responses to HS. Omics also aid in the systematic examination and determination of links between alterations in the genomes, micromes, and proteomes of various plants in response to heat tolerance. Using gene expression and genetic screenings, a plethora of genes with possible roles against HS have been found. Plants can regulate post-transcription gene expression by using microRNAs (Chinnusamy et al. 2007). Several microRNAs have been identified and characterized that play a crucial role in plant osmolyte accumulation in response to HS. MicroRNAs play many roles in plants; for example, stress-downregulated microRNA leads to the accumulation of targeted RNAs and helps plants to develop tolerance against heat stress, on the other hand stress-upregulated microRNA fails to target the specific RNAs and does not contribute to heat tolerance. Furthermore, a thorough understanding of microRNA functions in cell tolerance, transcriptome homeostasis, and developmental plasticity will aid in the development of HS-tolerant cultivars by genetic engineering.

Microarray technology is being developed as an important tool for analyzing the expression profiles of various genes triggered by HS (Liu et al. 2011; Yeh et al. 2012). Microarray studies also broaden our understanding of the various functions related with HS tolerance. Rizhsky et al. (2004) used an Arabidopsis genome array to evaluate transcript alterations in response to HS, drought stress, and the two stresses together and reported 262% increase in transcript response to HS. With the help of microarray analysis, Penueli et al. (2003) claimed that HSPs are also expressed under other stresses, despite the fact that HSP expression dominate under heat stress. These studies suggest that omics approach can be a promising tool for the identification of suitable candidate genes to develop a HS-tolerant crop variety.

9.7.4 CRISPR/Cas-Mediated Genome Editing

Advances in genome editing technology open up new avenues for crop development. The identification of candidate genes is a crucial step for the success of achieving the desired features. These genes could fall into two major categories, structural and regulatory genes. Structural genes encode proteins that directly give stress tolerance, whereas regulatory genes work indirectly by regulating the expression of other genes involved in various cellular processes. Furthermore, cis-regulatory sequences are also critical for attaining stress tolerance. The targeting of these regulatory and/or structural genes, along with cis-regulatory sequences, by using the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system as a robust, efficient, and practical approach for developing crop varieties resistant to adverse climate condition. CRISPR/Cas-mediated genome editing approach is an efficient and effective technique for developing non-genetically modified (Non-GMO) novel plant types having tolerance to multiple abiotic stresses and also have potential for public acceptance in years to come.

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Heat Stress and Grain Quality

10

Sneh Narwal, Sonia Sheoran, Dinesh Kumar, Antim Kundu, and Ajeet Singh

Abstract

Cereals are the main source of energy for the majority of world population. The composition and quality of the cereal grains are affected by different types of stresses. High-temperature stress, especially during grain filling, has a severe impact on grain quality mainly due to changes in accumulation and distribution of different macro and micronutrients. The impacts of heat stress during grain filling may vary among different crop species depending on the intensity, duration, and timing of the stress. Exposure to high temperatures during grain filling alters the expression of many genes related to starch biosynthetic pathways, starch transporters, transcription factors, and storage proteins. The activities of the main enzymes involved in starch synthesis are reduced leading to the reduced content of starch, changes in the shape and size of starch granules, alterations in the amylose: amylopectin ratio, and branching patterns. Heat stress, even for a few days during the grain filling period can have a major impact on protein composition. The changes in glutenin: gliadin ratio in wheat flour significantly

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S. Narwal (✉) · A. Singh
Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India
e-mail: Sneh.Narwal@icar.gov.in

S. Sheoran
Department of Crop Improvement, ICAR-Indian Institute of Wheat and Barley Research, Karnal, India
e-mail: sheoran_sonia@yahoo.co.in

D. Kumar
Barley Network Programme, ICAR-Indian Institute of Wheat and Barley Research, Karnal, India
e-mail: Dinesh.Kumar3@icar.gov.in

A. Kundu
Chaudhary Charan Singh Haryana Agricultural University, Hisar, India

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alter the dough strength. The changes in starch and protein composition lead to the changes in rheological and physicochemical properties of flours which may finally affect the quality of the end products. In this chapter, the effects of heat stress on four major cereals, i.e., wheat, rice, maize, and barley, are discussed separately because of the differences in quality requirements for their utilization in the production of different types of end products.

Keywords

Heat stress · Grainfilling period · Grain quality · Starch · Protein

10.1 Introduction

Grain composition (quality) of the cereals is mainly controlled by the genotype, but many other factors like growing conditions, growth locations, and sustained or episodic environmental stresses like heat and drought. Under stress conditions, the relative abundance of macromolecules like starch and proteins and micronutrients mainly minerals are affected. High temperature-associated changes in grain quality vary depending on the duration of the stress and the stage of grain development. Heat stress during the grain filling period is mainly responsible for the changes in the grain composition which in turn is associated with the altered processing, technological and nutritional quality. Environment x genotype interactions also have a significant effect on grain quality. During heat stress, the source-sink relationship is highly affected mainly due to the limitation of assimilates and reduced nutrient mobilization. As a result, the seed-setting and grain filling is restricted leading to reduced grain numbers and grain weights. Post-anthesis heat stress episodes affect synthesis of storage proteins, carbohydrate metabolism primarily due to changes in the activity of critical enzymes. The heat stress alters the activities of sensitive starch biosynthetic enzymes, like those involved in substrate production, the elongation of the α-1, 4-glucan chains and their branching, and the maintenance of granule crystallinity. Changes in these enzymes substantially alter the structure and composition of starch granules. The starch biosynthetic enzymes in different genotypes are affected differently by changes in temperature conditions (Beckles and Thitisaksakul 2014).

Starch structure and composition is an important indicator of the processing quality, palatability, and health beneficial effects of any food, the nutritional value of cereal grains as an animal feed and its suitability as a feedstock for biofuels. Both, high soil and air temperatures reduce the starch production and its functionality (Tester 1997). Diurnal variations in the heat sensitivity are also important in maintaining the stability of cereal grain quality. This is true, especially in case of rice starch quality which is severely affected by higher night-time air temperature (NTAT) (Peng et al. 2004). The reduction in starch concentrations under elevated temperatures is mainly due to two factors; first, the shortening of the grain filling period, which reduces the duration of starch accumulation and second, impairment of starch metabolism. Changes have been observed in the amylose content under heat stress. In wheat and maize, the reported changes are non-significant, while in case of Japonica rice drastic reductions (20%) have been reported at temperatures above 30 °C. Indica rice amylose, however, is found to be more stable at higher temperatures with some minor or no changes (He et al. 1990; Inukai and Hirayama 2010; Zhong et al. 2005).

Even small changes in amylose content (~1%) may substantially affect the starch gelatinization and pasting properties. The chain length distribution of amylopectin glucan chains is also modified under high-temperature conditions. In case of rice and sorghum, the long glucan chain fraction is increased, while in wheat, accumulation of shorter glucan chains takes place. In maize, however, no changes have been observed in either longer or shorter chains when exposed to heat stress (Jiang et al. 2003; Matsuki et al. 2003; Li et al. 2003). Shape, size, and structure of starch granules are also altered by high growth temperatures. The extent of the change is determined by the severity and time of stress application. Changes in the proportions of A- and B-type granules have been reported in wheat and barley (Blumenthal et al. 1995; Wallwork et al. 1998a, b). Heat also reduced granule size in sorghum, rice, and normal maize, while in waxy maize granule size enlarged. Rice, wheat, maize, and barley starches under heat stress generally show higher gelatinization temperatures (Lanning et al. 2012; Lu and Lu 2013; Tester et al. 1991).

Compared with heat stress application during late grain development, high temperature during early grain development plays a more significant role in influencing the starch pasting properties, crystalline structure, and granule size of cereal starches. The severe loss of starch content upon application of heat stress during the early grain filling stage may be explained by high temperature at this stage disturbing endosperm cell division, reducing amyloplast numbers, deforming starch granules, restricting starch granule formation; high temperature at the late grain filling stage only moderately affect seed storage process (Lu et al. 2013).

The protein composition is the most important factor determining baking quality and is greatly influenced by heat stress. High-temperature stress occurring during the early or middle phases of grain filling defines the specific protein responses. The accumulation of the various protein fractions is not synchronized, suggesting that the protein composition of the kernels changes as they develop. High temperatures shorten the grain filling period, thus influencing the balance between the protein fractions (Jamieson et al. 2001). The protein changes that occur late in grain development are of particular interest, because they may impact the functional properties of the resultant flour. The baking quality of wheat is determined by the viscoelastic properties of the dough, which in turn is affected by the composition and quantity of the gluten proteins in the endosperm. A high correlation exists between the protein content and the volume and texture of bread (Hurkman et al. 2009).

This chapter will deal with the effect of heat stress on the grain quality parameters, physicochemical parameters, and end product quality in major cereals wheat, rice, maize, and barley.

10.2 Wheat Quality

Wheat is the staple food and is one of the main sources of calories and nutrition for most of the world population. The physical characteristics of grain shape, size, color, weight, and hardness are important as they are indicative of potential processing quality. Milling yield and quality of end products are very important from an industry and consumer point of view. The quality of raw materials for bakery products is not only governed by the protein content, protein polymers, and ratio of glutenin to gliadins, but also by the shape and size of starch granules. High-temperature exposures reduce the grain filling and early attainment of the harvest maturity leading to reduced kernel weight, changes in size distribution of starch granules, protein composition, sedimentation volume, and yellow pigment (Table 10.1).

10.2.1 Starch

High temperatures negatively affect the starch concentrations in the grains. The expression pattern of many genes and enzyme activity associated with the conversion of sucrose to starch under heat stress is significantly hampered leading to the reduction in starch content. The activity of ADP-Glupyrophosphorylase, starch synthases, granule-bound starch synthase (GBSS), and starch branching enzymes reduced significantly under heat stress leading to deformed grains (Lu et al. 2019). As a result, the starch synthesis either slows down or ceases completely depending on the stage and duration of heat stress.

Starch consists of 2 types of polymers, amylose and amylopectin, and occurs in predominantly larger A-type and smaller B-type granules. The starch/flour functional properties like water absorption and dough flexibility are determined by the amylose to amylopectin ratio and starch granule size distribution. B-type starch granules are found to be more sensitive to heat stress during grain filling period than the A-type. Temperatures above 30 °C during the first 14 days after anthesis produce grains with a high proportion of A-type granules. Thus, in response to high temperatures, the number of B-type granules decreases while that of A-type increases (Panozzo and Eagles 1998). This could be explained by the fact that the B-type granules started to develop during the stress period. The A-type granules (10–35 μm in diameter) start to develop 4–5 days after flowering and this process lasts to the end of the endosperm cell division phase. By contrast, B-type starch granules (1–10 μm) do not appear until 12–14 days after flowering and their growth continues until 21 days after anthesis (Bechtel et al. 1990; Hurkman et al. 2003). The two types of starch granules are utilized differently in processed foods and nonfood products. Large (A-type) granules lower the maximum viscosity as compared to small (B-type) granules. An inverse relation has been reported between the protein content and the B-type starch granules. Thus, the starch granule distribution in conjunction with the protein affects the physicochemical and baking properties of wheat flour (Park et al. 2009) (Table 10.1).

Table 10.1 Molecular studies in cereals to see the effect of heat stress on grain quality

Species	Quality parameters studied	Genomic regions identified	Molecular techniques applied	References
Wheat	Bread baking (grain quality and quality stability)	*Qsdscon.tam*-1B, *Qsdsheat.tam*-1D *Qsdscon.tam*-4A	QTL identification	Beecher et al. (2012)
Wheat	Grain filling	TaHsfC2a homoeologous genes on chromosome 7AL, 7BL and 7DL	Transgenic (overexpressing TaHsfC2a-B)	Hu et al. (2017)
Wheat	TGW	*HvSUT1* transgene	Transgenic	Weichert et al. (2017)
Wheat	Grain storage protein	HRPs	Proteomics and metabolomics	Wang et al. 2018
Wheat	Grain quality	GPC-1A, 1B, 2B, 3A, WGC-1A, 1B, 3A GSC-1B, 3A, WA-2A, 2B, 3A, 3D, 5A, 5D, DST-2B, 3B, 3D, 5A, 5D GH-2A, 2D, 3B, 5A, 5D *QNGPC.cau*-6A and *QNGH.cau*-5D harbored gene NAM-A1 for grain protein content and Pinb for grain hardness	GWAS	Lou et al. (2020)
Wheat	Grain storage protein	Gliadins, and LMW, glutenins proteins upregulation	Proteomics	Chunduri et al. (2021)
Durum wheat	Grain quality	Transgenerational impact and epigenetic regulation for quality traits	Proteomics	Liu et al. (2021)
Barley	Grain quality	HSR genes HvHSFA2d—endosperm-specific heat stress responses	Transcriptomics, Affymetrix 22 K Barley1 GeneChip microarray	Mangelsen et al. (2011)
Barley	Grain metabolites	SNPs identified on chromosome 1H (10), 2H (16), 3H (11), 4H (17), 5H (13), 6H (16), and 7H (5). GeneHORVU1Hr1G013450 on chromosome 1H	Candidate gene identification	Dawood et al. (2020)
Rice	Grain chalkiness	OsSUT1 and SuSy2, AGPS2b, BEIIb	Transcriptomics (Qrt-PCR)	Phan et al. (2013)
Rice	Grain protein	Transcriptome OsUP8, OsUP12, OsUP13, and OsUP14 related to glutelin, starch debranching enzymes (OsDP2 and OsDP6) and a GBSS (OsDP8) identified	Proteomics (MALDI TOF)	Liao et al. (2014)

(continued)

Table 10.1 (continued)

Species	Quality parameters studied	Genomic regions identified	Molecular techniques applied	References
Rice	Grain chalkiness	EN242, OsGSTZ2, Rice acyl carrier protein II, Rice *β*-expansin (EXPB3) downregulated. OsChib3H-h, Rice pathogenesis-related thaumatin-like protein, OsENOD93a early nodulin, Ramy1, Ose705 mRNA, OsENOD93a early nodulin, glutathione *S*-transferase OsGSTU3, and OsGSTF5, thionin Osthi1, OSE351, ERG3, rice hydrophobic LEA-like protein gene upregulated	Proteomics (using DNA microarray)	Yamakawa et al. (2007)

The reduced starch content during heat stress is also reflected by the fewer amyloplasts per endosperm. An increase in amylose content is observed under high-temperature stress by many researchers. Starches from wheat grown at 40 °C have shown increased proportions of unit chains with degrees of polymerization (DP) of 10–16 and reduced proportions of unit chains with DP 17–21 (Shi et al. 1994). Starch gelatinization temperature increases with increase in growing temperatures. Gelatinization enthalpy however remains more or less constant. Gelatinization properties have been correlated significantly with amylopectin chain length distribution. Swelling of such starches in hot water however decreased (Tester et al. 1995; Matsuki et al. 2003). High starch pasting peak viscosity is influenced by the waxy genes coding for granule-bound starch synthase, which are involved in amylose synthesis.

Heat stress is transgenerational in its impact and expresses itself in the form of epigenetic regulation. Transgenerational impact of post-anthesis heat stress on grain quality traits in durum wheat has been confirmed. When high temperature treatment is given to parents, a positive influence is observed on offspring's for traits like starch content, grain number and weight (Liu et al. 2021).

10.2.2 Protein

Under heat stress, flour protein concentration is the result of combined effects on protein as well as starch biosynthesis in the grain. Protein content is mostly significantly higher in late-sown wheat grains as compared to early (normal) sown, as heat shock proteins are synthesized at very high rates under high-temperature stress and are believed to have a protective role under environmental stress. The higher protein content in late conditions may be possibly due to low grain weight (Sial et al. 2005; Reynolds et al. 2001).

The processing properties and the end-use quality of wheat flour dough are strongly related to the presence and properties of very large glutenin protein aggregates named glutenin macropolymer (GMP). This fraction of glutenins are insoluble in 1.5% SDS and can be isolated from flour/dough by ultracentrifugation as a gel-like layer. The average size of particles in GMP can directly be related to dough mixing requirements, an important quality parameter. A high GMP content indicates a high quality of the flour. Prolonged exposure to high temperatures (up to 40 °C) significantly increased flour protein and GMP protein fraction, GMP quantity, as well as particle size. The HMW-GS/LMW-GS ratio of the glutenin macropolymer decreases, but larger glutenin particles occurred under heat stress (Don et al. 2003, 2005). Therefore, these differential changes in the amount of polymeric protein are likely the main factors responsible for the decrease in the gluten strength related parameters like swelling index of glutenin (SIG), alveograph area under the curve (W), mixograph peak time (MPT), and lactic acid retention capacity (LARC) under heat stress (Li et al. 2013a). An increase in the protein content under heat stress is related to the decreased SDS sedimentation values.

Durum wheat has higher protein content and yellow pigments as compared to the bread wheat. The durum wheat gluten is inextensible and is therefore usually milled into semolina for the production of pasta products. Heat stress studies in durum wheat showed a significant decrease in LARC and MPT. Overall, heat stress has a weakening effect on the gluten strength in durum wheat. This effect of heat stress on the gluten strength may be due to the changes in the composition and distribution of gliadins, soluble and insoluble glutenin (insoluble polymeric protein). Heat stress also enhances flour yellowness which may be due to the increased levels of various yellow pigments. This may be partly due to the reduced kernel weight under heat stress (Li et al. 2013b).

Dough rheological properties are mainly governed by the genotype, but the environment and the GxE interactions also play an important role in expressing the end-use quality of a genotype. Under heat stress, the alveograph parameters also change. P-value decreases while L increases leading to an overall reduction in P/L ratio. This indicates a decrease in gluten strength but an increase in gluten extensibility and bread loaf volume under heat stress (Li et al. 2013a) "Heat shock" is characterized by sudden, extremely high temperatures (>32 °C) for a short duration (3–5 days), while "chronic heat stress" consists of moderately high maximum temperatures (20–30 °C) for a longer duration. Chronic heat stress has a "strengthening" effect while heat shock has a "weakening" effect both in common and durum wheat. Moderately high (25–32 °C) temperatures, however, had a positive effect on dough properties and led to modifications in the composition of the gliadin fraction (Borghi et al. 1995; Wrigley et al. 1994).

High temperatures during grain filling may affect synthesis, accumulation, and/or assembly of gluten proteins. Heat shock produces grains with weaker than expected dough properties which may be due to lower glutenin: gliadin ratio. Under heat stress, this altered glutenin: gliadin ratio may either be because of higher rate of synthesis of monomeric proteins or reduced synthesis of gliadins. Genetic variation has been observed under heat stress with respect to this ratio (Castro et al. 2007). Cultivars with relatively stable grain characteristics and protein molecular weight distribution could be used as genetic sources for improving resistance to heat stress. The percentage of proteins and gliadins in the flour increases with the increase in temperature. The proportion of gliadins, α-, β-, and ω-gliadins increased with the temperature, and γ-gliadins decreased with the temperature (Daniel and Triboi 2000).

The observed changes in gliadin-to-glutenin ratio, in the distribution of glutenin macropolymers, and their relationship with decreased gluten strength parameters, have been frequently associated with plant protection mechanisms; the increase of gliadin-like heat shock elements (HSE), heat shock proteins (HSPs), and their chaperones due to heat stress (Maestri et al. 2002). Some of the proteins that increase in response to high temperature are present in dough liquor, a soluble fraction of wheat dough, or foams prepared from dough liquor and may have roles in gas bubble stabilization in dough and crumb structure of bread (Hurkman et al. 2009).

Albumins and globulins do not have much influence on the dough quality, but, they are important from the nutritional point of view, due to their high contents of essential amino acids. The ratio of albumins–globulins did not increase proportionately with the protein content in response to high temperatures (Triboi et al. 2003). The accumulation of albumins and globulins continues for approx. 20 days after flowering, after which a constant level is generally reached. Soil warming slightly increases concentrations of a number of amino acids in wheat grain. However, the level of essential amino acids is reported to be reduced under severe heat stress.

In wheat, QTLs for grain protein content (GPC) under heat stress have been identified on chromosomes 1A, 1B, 2B, and 3A. In the same study, five QTLs associated with wet gluten content (WGC) located on chromosomes 1A, 1B, 2B, and 3A, 6 QTL associated with water absorption, six QTLs associated with dough stability time (DST) on chromosomes 2D, 3B, 3D, 5A, and 5D and five QTLs associated with grain hardness on chromosomes 2A, 2D, 3B, 5A, and 5D have also been identified (Lou et al. 2020). Suliman et al. (2021) have identified significant and stable SNP markers *RAC875_c24504_119* and *BS00063624_51* on chromosome 3B for zeleny sedimentation test under heat stress.

The presence of the *Glu-Dd* allele (glutenin subunits *5* and 10), and increases in glutenin-to-gliadin ratio and in the percentage of very large glutenin polymers can be used as potential markers in breeding for heat tolerance. There is a very significant association between changes in dough properties and allelic constitution at the *Glu-DJ* locus, coding for the alternative HMW glutenin subunit combinations 5 + 10 (allele *Glu-Dld,* more tolerant) or 2 + 12 (allele *Glu-Dla,* more susceptible to change due to heat).

10.2.3 Other Grain Parameters

High-temperature stress also affects the mineral content of wheat with a general decline in the concentration of micronutrients, but the results strongly depend on varieties and environmental factors. In high zinc genotypes, the ratio of gliadins and polymeric protein is less affected by high temperatures. This may be due to a possible interaction between the zinc and the temperatures during grain filling, which in turn alters the protein composition (Wang and Liu 2021). The protein and zinc concentrations increased under heat-stressed conditions. The grain protein content gene Gpc-B1 is known to be associated with the accelerated senescence, and exhibited pleiotropic effect on GPC and increased grain Zn and Fe in field conditions (Uauy et al. 2006). In durum wheat, the concentrations of micronutrients and flour yellowness are reduced by heat stress except for Zn content. The increased zinc content under severe heat stress may be due to "concentration effect" induced by smaller grains (Guzmán et al. 2016).

Free amino acids and sugars are major determinants of processing quality and also food safety in some cases. They combine during baking, frying, and high-temperature processing to produce a host of compounds, including some imparting color, flavor, and aroma, and others that are potentially harmful to health (Halford et al. 2015). Heat stress during grain filling causes an increase in sucrose, reducing sugars and sugar phosphates. In one study, the stability of lysine was found greatly affected, while the level of threonine increases significantly under heat stress. Among nonessential amino acids, the levels of arginine and histidine decreased significantly. With decreased levels of amino acids, heat stress decreases the sedimentation index. However, the levels of K, Na, Cu, and Mn were not altered by high temperatures. Overall, the nutrient content was not significantly affected under high temperatures (Dias et al. 2008).

The primary effect of heat stress observed on grain quality is the reduced grain weight. This reduced grain weight leads to a reduction in test weight which in turn is associated with reduced flour extraction rates. Grain weight is more affected by the high temperatures in durum wheat than bread wheat (17 and 14%). Under high temperatures, the endosperm gets increasingly aggregated with starch granules embedded in protein matrix and an overall dense cellular structure (Dias et al. 2008). Grain hardness is a very important parameter affecting technological properties of wheat. It also alters the milling quality and will increase the starch damage. Under heat stress, hardness of the wheat grains increases. The grain hardness also increases at temperatures above 30 °C during the second 14 days after anthesis, and this increase in hardness is much greater in soft-grained cultivars than hard-grained cultivars (Panozzo and Eagles 1998).

Under heat stress, the β-glucan content is reduced while the arabinoxylans are increased (Rakszegi et al. 2014). Hagberg falling number, which is indicative of reduced starch and increased sugars is reduced under high-temperature stress (Gooding et al. 2003).

Under heat stress, the proportion of bound lipids increased significantly, but the changes in free lipids did not follow a significant trend. The changes in bound lipids correlated significantly with mix time and resistance breakdown (Blumenthal et al. 1995).

10.3 Rice Quality

Quality of rice is evaluated in terms of milling yield; appearance including translucency, shape, size, and color; cooking, aroma, and nutritional qualities. In addition to genetic factors, physical and biochemical traits are also influenced by the environmental factors. High temperatures during grain filling stage, reduces head rice yield (HRY), deteriorates transparency, increases chalkiness, reduces sensory attributes, and finally consumer acceptance. All these traits are interrelated as any change in rice grain appearance tends to affect the milling and cooking quality. Chalkiness of the rice endosperm is the principal factor which influences the market value of rice (Nevame et al. 2018).

10.3.1 Grain Chalkiness

Chalkiness is an undesirable trait that negatively affects milling, cooking, eating, and grain appearance and represents a major problem in many rice-producing areas of the world. The occurrence of chalkiness in rice is attributed to genetic and environmental factors, especially high temperature. The extent of damage caused by high-temperature stress depends on the time of exposure in relation to the stage of grain development. Chalk appears to be the result of poor filling of starch granules in the rice endosperm, primarily affecting amylose and amylopectin pathways and disrupting the spatiotemporal packing of starch granules. Exposure of the rice panicles to high-temperature stress reduces the cell division and ultimately the number of endosperm cells and starch granules which is responsible for increased spaces among the amyloplasts. Heat stress during grain filling stage downregulates the starch synthesizing enzymes and upregulates the starch degrading enzymes such as alpha-amylase, creating an imbalance in the synthesis and degradation of starch, resulting in a loosely packed starch granule deposition (Mitsui et al. 2016). Grain chalkiness is a result of air spaces trapped between loosely packed starch granules, translating into white or translucent portions in rice grains. Higher temperature reduces the amount of large mature amyloplasts in the endosperm and increases the number of small immature ones (Zakaria et al. 2002). Events leading to chalk formation are associated mostly with reduced sink strength and imbalances in carbon and nitrogen partitioning in a growing sink because of photoassimilate limitation under stress. The stored carbon is remobilized as sucrose across plasma membrane to the caryopses by a sucrose transporter, OsSUT1. Heat stress suppresses the expression of OsSUT1 gene and SuSy2, AGPS2b, BEIIb, and granule-bound starch synthase in seeds during early grain filling stage and the quality of rice is compromised (Phan et al. 2013). The rice grains ripening at high temperatures contain lower levels of amylose and long chain-enriched amylopectin due to the downregulation of GBSSI and BEIIb, respectively (Yamakawa et al. 2007). The genes for debranching enzymes (*OsDP2* and *OsDP6*) and a GBSS (*OsDP8*) are downregulated under heat stress (Liao et al. 2014).

Triggering of the chalky phenotype may also be due to imbalances in the finer readjustments with the starch degradation pathway during grain filling (Sreenivasulu et al. 2015). During ripening stage, high temperatures trigger grain chalkiness due to high α-amylase activity controlled by genes *Amy1A, Amy1C, Amy3A, Amy3D*, and *Amy3E* and also repress α-amylase repressing hormone, i.e., abscisic acid. So, the starch is degraded in developing grains under high temperatures. But RNAi-mediated suppression of α-amylase genes shows development of a few chalky grains under heat stress at grain development stage (Hakata et al. 2012).

Examinations by optical and scanning electron micrographs revealed that the starch granules of chalky endosperm cells are loosely packed, while those of normal translucent endosperm are tightly packed; the numerous air spaces between starch granules cause random light reflections to create a chalky appearance. It has been suggested that the occurrence of chalky grains caused by high-temperature exposure is due to a lack of starch substrates, the downregulation of some starch synthesis-related genes, and upregulation of starch degrading α-amylase-encoding genes (Morita et al. 2016).

The percentage of chalkiness in rice grain is an index that determines the appearance quality. Regarding appearance quality, the critical period of high-temperature effects in rice appearance quality is 12–16 days after flowering. If the rice encountered high temperatures during this period, the chalky grain ratio would increase significantly. When the temperature is above the threshold, grain yield and appearance quality would decrease significantly due to a large amount of chalky grains, which may be due to the regulations of gene expressions for storage molecules and stress-responsive proteins during the early grain filling stage (Tashiro and Wardlaw 1991a; Lin et al. 2010; Fig. 10.1).

The translucence conferred by starch accumulation in the developing rice endosperm moves outward from the center of the grains. As a result, when starch accumulation is disturbed by environmental stresses, the chalkiness often emerges at a site corresponding to the progression of translucence development in the endosperm (Nagato and Kobayashi 1959). The chalky grain defect is classified into several types based on the position of chalkiness in the grain. The major types induced by high-temperature exposure are milky-white, basal-white, and white-back grain (Nagato and Ebata 1965). Among the various types of chalky grains, white-back type of chalky grains (WBCG) are highly correlated with heat stress during grain filling. It is indicated that the ratio of white-back (WB) and basal-white (BW) grains increases if the average air temperature during ripening exceeds 27 °C (Wakamatsu et al. 2005; Wakamatsu et al. 2007). Depending on both the temperature level and duration, chalky endosperm tissue occurs in several forms: white-core kernels are evident at a temperature of 27/22 °C, and white-back kernels are most numerous at 36/31 °C when high-temperature stress occurs 16 days after heading (Tashiro and Wardlaw 1991b).

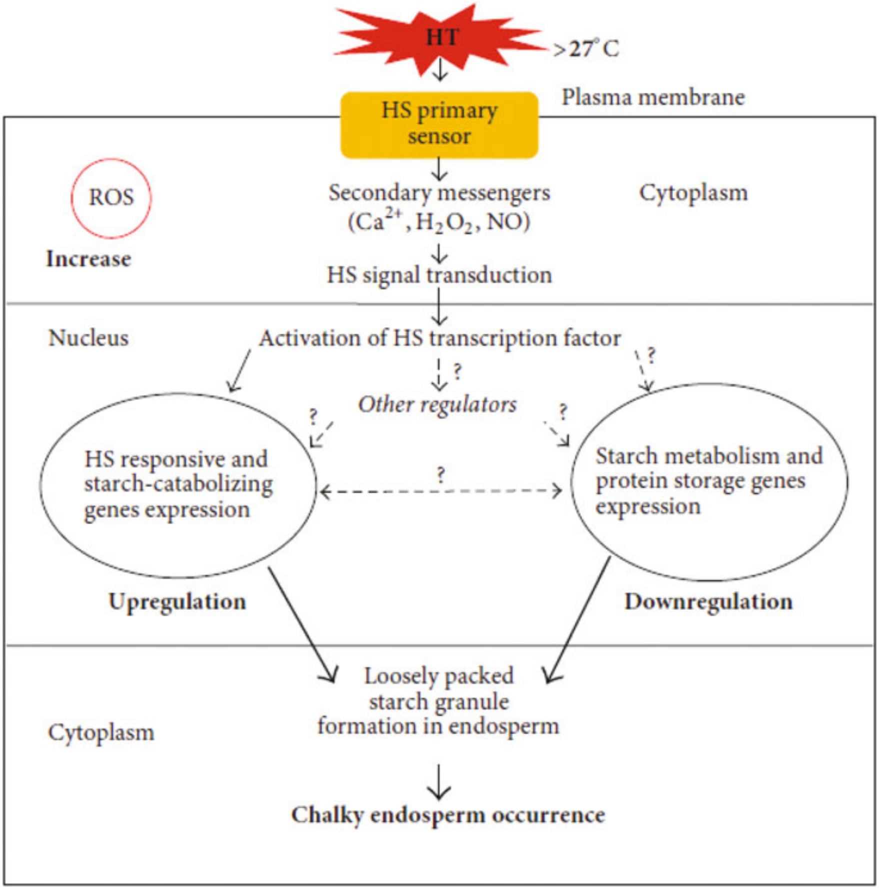

Fig. 10.1 Heat stress affects HS responsive and starch-metabolizing genes. Arrows in bold indicate the mechanism pathways. Dashed arrows are unclear regulation pathways or genetic interactions, which may be responsible for the chalkiness formation. Sample arrows represent HS effects transduction that leads to the expression of HS protein genes. HS: heat shock; ROS: reactive oxygen species (Adapted from Nevame et al. (2018))

Panicle type is directly correlated with rice quality, and the grain qualities of heavy-panicle rice varieties are more vulnerable to heat stress than normal panicle rice varieties. High temperature reduced head milled rice rate by 9.22% compared with normal temperature, but increased chalky grain rate and chalkiness degree by 69.6% and 4.10 times, respectively. These results indicate that exploitation of heavy-panicle rice germplasm resources in future rice breeding can not only enhance rice yield potential, but also improve their grain filling under high-temperature conditions, offsetting the partial detrimental impact of global warming. Exploitation of genetic traits characterized by stable qualities under high temperature and positive increase in 1000-grain weight is a key task for future super rice breeding (Chen et al. 2017).

Genetically, high-temperature effect on chalkiness is explained by the location of certain chalkiness genes in the vicinity of high-temperature responsive genes. A great variation in chalky grains ratio among *Japonica* rice cultivars under high-temperature conditions is reported. In addition, the formation of a chalky appearance might be related to the expressions of small heat shock proteins (sHSP) and stress regulatory proteins during the grain filling stage (Wu et al. 2016).

10.3.2 Side-Effects of Chalkiness

Synergistic or antagonistic effect of chalkiness has been reported on the accumulation of different macro and micronutrients providing useful clues on the relationship between chalkiness and grain mineral content. A close correlation is reported between P, K, Ca, and Mg contents and most plant morphological (plant height, tillers number, seed set, number of grains per panicle) and grain quality traits (protein content, amylose content, gel consistency). A significant impact of grain chalkiness on endosperm elemental composition has been reported highlighting complex associations between grain quality and mineral profile (Lin et al. 2016). Strong association of grain protein and chalkiness with different mineral elements suggests that breeding efforts to develop high-quality rice should also focus on improving essential mineral nutrients to further enhance quality, particularly under future [CO_2] enriched warmer climate (Chaturvedi et al. 2017).

The head rice yield is related to the cellular structure of the starch-containing molecules within rice grains, and this structure is temperature sensitive. The significant environmental effect on HRY is mostly due to high-temperature stress during the sensitive phase of gametogenesis. Chalk is one among many other variable parameters that influence MRY (Milled rice yield) and HRY. The gaps formed due to aborted starch granule formation are thought to be responsible for making chalky grains more brittle and for forming fissures along the grain. As a result, chalky grains crack easily during grain processing, which reduces HRY as a consequence of the elevated amount of broken grains (Lanning et al. 2011; Lisle et al. 2000). Negative effects to HRY were most strongly related to average NTAT rather than daily maximum temperature during particular reproductive stages. It is reported that increased NTAT during grain filling can explain 50% of HRY variability (Cooper et al. 2006). It was also reported that peak viscosities and gelatinization temperatures increased linearly as NTAT increased, whereas setback viscosities decreased (Lanning et al. 2012). The strong association of increased chalk formation with elevated NTATs suggests that NTAT effects are manifested as chalky sections of the kernels, with these sections producing very different physicochemical properties than translucent, chalk-free kernels (Siebenmorgen et al. 2013).

Although the variety is a major factor in rice palatability, air temperature during ripening would be most effective to the palatability among weather conditions. Regarding the relationship between the palatability and the grain appearance quality under heat stress, it was reported that the palatability decreases by chalkiness under heat stress (Chun et al. 2009; Ishizuki et al. 2013), especially with the addition of milky-white rice.

10.3.3 Effect on Physicochemical Properties of Starch

Up to 90% of the rice grain is starch (on a dry basis) and it is, therefore, a key contributor to many grain quality attributes. The cooking, sensory, and functional properties of rice are highly influenced by the quality and physicochemical properties of starch. High-temperature stress during the grain filling stage has deleterious effects on starch quality. High temperature affects the starch physicochemical properties, resulting in lower apparent amylose content and higher-order degree, gelatinization temperatures, and thereby increased peak, trough, and final viscosities in starch. High temperature during primordial differentiation stage inhibited cell development and starch deposition, thus leading to small starch granules and low retrogradation. However, temperature-stressed rice during pollen filling stage showed increased starch accumulation and larger granule size. Therefore, the effects of high temperature during the two stages on caryopsis development and starch properties were partly similar but also notably different (Sreenivasulu et al. 2015; Lin et al. 2020). Short-term heat stress at booting stage deteriorates most grain quality traits, posing a potential risk to rice quality. The impacts on grain quality could be well quantified by the combined effects of the intensity and duration of heat stress at booting stage (Zhen et al. 2019).

The physicochemical properties of starch are greatly affected by the relative proportions of amylose and amylopectin. The amylose content of rice starch is the major determinant of cooking and eating quality factors and correlates directly with volume expansion and water absorption during cooking and with hardness, whiteness, and dullness of cooked rice. Elevated growing temperatures increase the gelatinization temperature of starch by 5–6 °C and the resulting starches have reduced amylose and increased proportions of relatively longer glucan chains in amylopectin (affecting starch digestibility) (Asaoka et al. 1984; Ahmed et al. 2015). Changes in amylose/amylopectin ratio have been observed in plants grown at 32 °C were attributable to a reduction in the activity of GBSS, the sole enzyme responsible for amylose biosynthesis (Ahmed et al. 2015).

High night temperature (HNT) stress influences the formation process of amylose content and amylopectin structure, and then changed the crystalline and thermal properties of rice starch. Amylose content of HNT treatment decreased compared with that of ambient night temperature (ANT) at 35 d after anthesis. Amylopectin under ANT had a higher percentage of degree of polymerization (DP) 6–11, while amylopectin under HNT had a higher percentage of DP 12–23 (Song et al. 2015). The shorter amylopectin chains ($DP < 12$) had a negative influence, while longer amylopectin chains ($12 < DP < 24$) had a positive influence on the gelatinization temperatures of rice starch (Vandeputte et al. 2003).

RVA analysis of the rice flour grown under high temperature showed higher peak viscosity, higher breakdown, and lower setback. The changes in these characteristics may be due to the effect of heat stress on the biosynthesis of amylose, amylopectin, and proteins (Wu et al. 2016). The taste quality was positively correlated with peak viscosity and breakdown. Taste score is also reduced if the temperatures during grain filling rise above 26 °C by high temperatures (Matsue 2007). It was also reported that peak viscosities and gelatinization temperatures increased linearly as NTAT increased, whereas setback viscosities decreased (Lanning et al. 2012).

10.3.4 Cracked Grain and Immature Thin Grain with Deep Creases

Cracked grain, a serious damage causing the breakage of rice during milling, is induced by the physical stress built up in the endosperm by the unequal swelling resulting from the uneven absorption of water (Nagato et al. 1964). High temperatures at the earlier ripening stage, especially in the daytime, are a critical factor responsible for the formation of cracked grain. The formation of immature thin grain with deep creases is also a serious issue, because it can both lower grain quality by decreasing milling efficiency, and lower grain yield by reducing grain weight (Morita et al. 2005). Image analysis studies have confirmed that high temperatures during ripening stage promote creasing (Yonemaru and Morita 2012). Varietal differences have been reported in the occurrence of cracked grain in response to high air temperature was reported (Nagata et al. 2013). Across the RH range of 25–85%, high air temperature produces higher amounts of broken grains. At higher moisture content levels, milled rice sustains more extensive stress crack damage at low RH conditions and less stress crack damage at high RH conditions, relative to milled rice at lower moisture content levels (Siebenmorgen et al. 1998).

10.4 Maize Quality

Maize (*Zea mays* L.) is used for human food, animal feed, and also in biofuel production. Globally, maize contributes around 19.5% of caloric intake from all sources. It is utilized as raw material for many foods and feed-based industries and has attained a top rank among cereal crops. High-temperature stress at different growth stages has major influence on the growth and quality of maize (Sabagh et al. 2020). Grain filling period is the most sensitive to environmental stresses in maize, which strongly affects grain development quantitatively and qualitatively, especially in spring maize. Temperatures changes at different sowing dates can reduce the yield and deteriorates the quality of spring maize. Late planting of spring maize (March onward) faces a significant decline in yield (10–40%) and quality (10–50%) due to higher temperatures (Yasin et al. 2019).

Starch is the main component of maize grain, followed by protein, which determines the grain quality. The effects of heat stress are more severe when imposed at early grain filling stage. The change in grain components and contents is due to changes in enzymes involved in starch and protein synthesis. High-temperature stress reduces the starch content, enlarges starch granule size, and also increases the fraction of long chains in the amylopectin, which in turn increases the pasting temperature (Thitisaksaku et al. 2012). In one study, high-temperature stress increased the starch granule size and volume percentage of diameter larger than 17 μm. Other parameters like crystallinity, iodine binding capacity, and pasting and gelatinization temperatures also increased under heat stress at 11–20 DAP (Yang et al. 2015). As the size of the starch granules and the proportion of long-chain amylopectin increases, the thermal and pasting properties of starch are altered. High peak and breakdown viscosities and low setback viscosity are observed when high-temperature stress is introduced at 1–15 DAP (Huan et al. 2015). High temperatures inactivate important biosynthetic enzymes and restrain the production of small granules. Therefore, the available substrate is diverted toward preexisting large granules, allowing them to grow larger and leading to higher ratios of large to small granules by volume (Hurkman et al. 2003).

The percentage of large, pitted, and abnormally shaped granules is higher when the temperatures are high during the early grain filling stage than in the late grain filling stage. It indicates that the newly developed endosperm cells and starch granules are more prone to heat stress effects (Lu et al. 2013). Under high-temperature stress during early grain development, the increase in pasting temperatures and decrease in peak and breakdown viscosity of maize starch may be due to the high protein content, bigger granule size, high ratio of long chains, and low crystallinity. Such starch granules are hard to break down. The high protein content forms a matrix and restrains the starch swell, resulting in lower peak and breakdown viscosities. High-temperature stress increases the gelatinization temperatures, especially during the early grain filling stage. This is mainly due to high protein content and enhanced registration of amylopectin double helices and probably enhanced rigidity of amorphous regions (Tester and Karkalas 2001). High protein content results in high percent retrogradation as the denatured proteins lead to more aggregation (Lu et al. 2013).

The grain weight and starch deposition are reduced by high-temperature stress mainly due to the decreased activities of enzymes involved in starch synthesis, and the increased protein content is due to the enhanced activity of glutamate synthase (Yang et al. 2018). Duke and Doehlert (1996) observed a decrease in the activities of adenosine diphosphate (ADP)-glucose pyrophosphorylase (AGPase), aldolase, acid invertase, and acid phosphatase in normal maize grain by heat stress during grain filling. In normal maize, the activities of enzymes involved in starch metabolism, such as sucrose synthase, AGPase, glucokinase, soluble starch synthase, and starch branching enzyme decreased restricting the accumulation of starch. In sweet maize, heat stress during grain filling decreased the activities of sucrose phosphate synthase and sucrose synthase, leading to decreased sucrose content. In waxy maize, the starch is composed of 100% amylopectin with low hardness and high digestibility, clarity, and viscosity. High-temperature stress during grain filling period decreases the table quality of fresh waxy maize (Lu and Lu 2013).

10.5 Barley Quality

Barley (*Hordeum vulgare* L.) is the fourth major small grain cereal after rice, wheat, and maize both in terms of area and production. In recent times, approximately two-thirds of cultivated barley has been used for animal feed, one-third for malting and approximately 2–5% directly for food. Direct use of barley as food is limited to specific areas of the world. Barley and malt are now gaining renewed interest and are being used as ingredients of many functional foods because of the high content of soluble fiber β-glucan and many bioactive compounds with antioxidant activity. In this section, mainly the effect of heat stress on the barley malting quality is discussed.

The malting potential of barley depends on the biochemical attributes of the mature grain, which in turn are influenced by the interaction between genotype and the environment. A number of malting quality variables have been identified which are influenced by environmental conditions such as temperature, rainfall, and soil type. These variables include protein, enzyme levels (specifically, diastatic power), β-glucan, and malt β-glucanase potential. Heat stress during grain filling period in barley has a distinct and crucial influence not only on the grain yield but also deteriorates the malting quality. This reduction in malting quality is mainly attributed to the increase in protein concentrations and decreased accumulation of carbohydrates (Wallwork et al. 1998a, b). In barley, several genes involved in sucrose and starch biosynthesis are repressed by heat stress, along with downregulation of SUTs (HvSUT1 and HvSTP3) (Mangelsen et al. 2011) (Table 10.1).

In contrast to maize, wheat, and rice, no consistent changes are observed in amylose content in normal barley grains exposed to high temperatures. However, the amylose content in naturally high amylose barley is reduced by elevated temperatures (Fig. 10.2). This response may be due to the intrinsic properties of the crop (Beckles and Thitisaksakul 2014). Within barley starch, high temperatures also regulate the size of starch granules, the amylose-to-amylopectin ratio, and the amount of lipid complexed with the amylose. High growth temperatures may facilitate amylopectin crystallization and increase gelatinization temperatures, but may delay the onset and reduces the granule swelling in hot water (Tester 1997). Temperature stress studies with different barley mutants have shown lower temperature stability of starch mutants than of common barley cultivars. Waxy genotypes show higher temperature sensitivity for the yield parameters than the non-waxy ones. Short periods of high temperatures (>35 °C) result in decreased dry matter which is due to the reduction in the number rather than size of the starch granules. High temperatures reduce the amount of "maltable" grain by reducing size and increasing the screening percentage. Malt extraction reduces by 3–7%, which can be a big loss for the malting industry (Savin et al. 1996).

Final grain composition depended not only on the amount of endosperm storage component present in the grain (~85% of total grain dry matter), but also on the contribution of the non-endosperm components (including the embryo and husk) to final grain dry weight. Individual grain tissues may, however, respond differently to

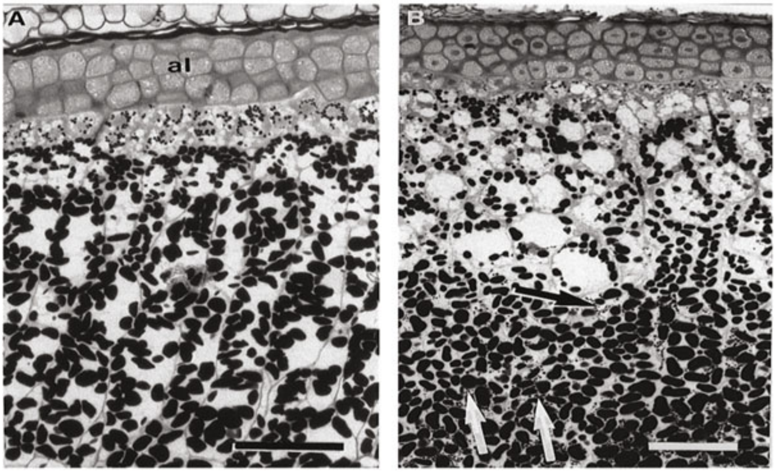

Fig. 10.2 Light micrograph of a longitudinal section through the dorsal endosperm of a control barley grain 24 DAA (**A**) and heat-treated barley grains 21 DAA (**B**) stained with PAS-TBO, Bar = 100 μm (Adapted from Wallwork et al. (1998a, b))

changing ambient conditions, so that minor changes in dry matter accumulation of the endosperm may be masked by opposing changes in other tissues of the developing grain. In some cases, changes in the contribution made by the non-endosperm components of the grain to final grain weight masked important high-temperature effects on key endosperm storage components. Although the non-endosperm grain component remains largely ill-defined with respect to malting quality (MBIBTC 1995), the role of these collective tissues may be important in determining the performance of barley during malting. The non-endosperm grain component may impose a limit on malting quality of a variety by reducing potential malt extract. Increased growth of the embryo, already observed to occur in heat-treated grains, suggests that the contribution of the non-endosperm component of the grain to total grain dry weight may be an important factor in determining the malting quality of grain which develops under high-temperature conditions (Wallwork et al. 1998a, b).

Although, protein and β-glucan content, as well as starch hydrolytic enzymes in barley grains are, to a different extent, controlled by genetic factors, they are all greatly affected by environmental conditions and agronomic practices. β-amylase and limit dextranase (LD) are the key enzymes involved in the conversion of starch into glucose and are therefore positively correlated with malt extract and wort viscosity. It is well known that in barley grains, β-amylase is a major protein consisting of about 2% of total proteins. Therefore, both the β-amylase and the limit dextrinase are positively correlated with the total protein content. Heat stress during grain filling leads to an increase in protein content and the activities of the starch hydrolysis enzymes, especially β-amylase in barley grains. Limit dextrinase (LD) is one of the limiting factors in complete starch breakdown, but few studies have reported the impact of heat stress on the LD activity during grain development.

The increased contents of grain protein under heat stress indicate that these accumulate earlier than starch and other carbohydrates during grain filling period (Shengjing et al. 2020).

Above ambient temperatures during grain filling of malting barley, the β-glucan content decreases. This reduction is attributed to the underdevelopment of the endosperm because of shorter duration for grain filling at higher temperatures and that β-glucan is mainly deposited during the late grain filling period (Aman et al. 1989). Increased β-glucan degradation is also observed in response to high temperatures, which may have been due to increased β-glucanase activities (Barber et al. 1994). But, this has not always been found to be the case. The enhanced degradation of β-glucan may have been due to heat-induced changes in the structure or organization of endosperm cell walls, making them more accessible to breakdown. The association between heat treatment and reduced viscosity and malt β-glucan represents a beneficial response associated with barley cultivation under adverse conditions. Low viscosity and low malt β-glucan reduce the likelihood of encountering processing problems, in particular filtration problems after extraction of malt and filtration or haze problems in beer production (Bathgate and Dalgleish 1975).

Exposure to high temperatures led to a reduction in hot water extract. Although protein has been correlated negatively with malt extract, total starch content does not consistently show a positive relationship with malt extract. Rather, associations have been found between malt extract and other starch qualities such as mean large granule diameter, starch pasting quality characteristics, and amylose content. Although, varietal differences have been found in the sensitivity of starch accumulation to increased ambient temperatures, it can be said that under some conditions environmental influences may also be greater than genotypic differences (Dunn et al. 1996).

10.6 Summary

Grain quality is strongly dependent on genotype and environment, and different quality attributes show diverse responses to abiotic stresses. However, the responses of plants to these stresses vary across the species, as well as at different developmental stages. The optimum temperatures for “normal” grain filling vary by species, and there may also be diurnal variations in heat sensitivity with higher nighttime air temperature having a larger effect on rice starch quality. General effects of high temperatures in developing cereal grains include a faster rate of grain development, a decrease in kernel weight, shriveled seeds, reduced starch accumulation, and alterations of polypeptide and lipid compositions. Starch and proteins are the most important components of cereal grains and their content and composition finally determine the quality of the end product. The biosynthetic processes involved in proteins, lipids, and minerals metabolism are all affected by adaptive responses. Due to these metabolic shifts, carbohydrate, protein, lipid, and mineral compositions are significantly affected. The ability to synthesize, store and remobilize starch at high temperatures is crucial to determination of grain sink strength. The activity of glutamate synthase is enhanced by heat stress, while sucrose phosphate synthase, sucrose synthase, and soluble starch synthase were significantly decreased during grain filling. Soluble starch synthase has been reported as the most thermolabile component of the starch synthesis pathway in rice, wheat, and maize.

In wheat, the quantitative and qualitative changes in the protein complement lead to the deterioration of dough quality. Moreover, the timing and duration of heat stress during grain filling have been shown to be important sources of variation in dough properties in wheat. In maize, waxy grain starch content decreased, whereas protein content increased, resulting in a change in grain quality. In rice, heat stress induces grain chalkiness due to impaired mechanism of starch formation and water distribution in caryopsis. The reduction in malting quality of barley under heat stress is mainly due to the increase in protein concentration and decreased accumulation of carbohydrates. Other malting variable like the beta-glucan, starch hydrolyzing enzymes, and malt glucanase are also influenced by heat stress. Thus, heat stress during grain filling leads to reduced milling rates and changes in flour or malt physicochemical and rheological properties, ultimately leading to the altered texture, color, and nutritional properties of the end product. In future, climate change will increase the number of heatwaves, which will result in more deterioration in the grain quality. Grain quality in cereals is a complex process involving many genes and regulatory pathways. Therefore, targeted breeding and molecular approaches are required to develop more heat-tolerant genotypes retaining their nutritional and processing quality intact.

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OMICS Tools and Techniques for Study of Defense Mechanism in Plants

11

Dwijesh Chandra Mishra, Sayanti Guha Majumdar,
Neeraj Budhlakoti, Anuj Kumar, and Krishna Kumar Chaturvedi

Abstract

In recent times, agriculturally important plants face increasing challenges in maintaining productivity, disease control, and welfare of farmers with changing climatic conditions. To accomplish this, the generation and analysis of large volumes of data, especially in the emerging "OMICS" areas of genomics, proteomics, and bioinformatics, is imperative for decision-making over large volumes of data with respect to various crops. Analysis of this large amount of diverged data needs specific tools and techniques. There are various tools and techniques available for the analysis of such data. In this chapter, a detailed discussion on omics data analysis related tools and techniques have been made. This chapter provides a single platform to help the various researchers working in different domains of omics research for analyzing the data.

Keywords

Genomics · Genomic selection · GWAS · OMICS · Phenomics · RNAseq · QTL

11.1 Introduction

Various environmental factors like heat, cold, salinity, and drought severely affects plants growth and development that affects its production and productivity significantly. To address the abiotic stresses, defense mechanisms are often triggered by the plant to mitigate these unfavorable conditions. Understanding the mechanisms of

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D. C. Mishra (✉) · S. Guha Majumdar · N. Budhlakoti · A. Kumar · K. K. Chaturvedi
Division of Agricultural Bioinformatics, ICAR-Indian Agricultural Statistics Research Institute, New Delhi, India
e-mail: dwijesh.mishra@icar.gov.in; neeraj.budhlakoti@icar.gov.in; kk.chaturvedi@icar.gov.in

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plant defense systems at molecular level, there is a need to conduct a comprehensive study to decode the molecular mechanisms using various bioinformatics tools and techniques. Advanced DNA sequencing technology has accelerated the pace of genomics and transcriptomic studies in plants and animals to understand the molecular mechanisms. With the progress in omics approaches (viz. genomics, transcriptomics, proteomics, metabolomics, and phonemics) and its use in agriculture, a huge amount of data has been generated in molecular and biotechnology labs which can be used to identify novel genetic and chemical elements controlling various physiological processes and pathways of plant defense system. However, using only one approach is not sufficient to understand the complexity of stress response in plants. Recent development in the field of next generation sequencing technology (i.e., high-throughput data generation with reduced cost) in OMICS era generated a huge volume of molecular data. The major omics approaches are composed of genomics, transcriptomics, proteomics, metabolomics, and phenomics. These approaches provide a holistic view of molecular pathways at the cellular, tissue, or organism level. The integration of different omics-based approaches provides many folds of biological information which resulted in the development of a new branch of life science known as system biology (Hong et al. 2016; Chaudhary et al. 2019). However, analysis of high-throughput data from various omics-based approaches is one of the biggest challenges to interpret the plant defense mechanism(s). There are several tools, techniques, and databases available in public domain for various omics-based analyses independently. To handle this challenge due to generation and availability of multi-omics data, one has to use these tools in a more judicial and integrated way for deeper and novel biological insights. This chapter discusses various omics techniques such as genomics, transcriptomics, proteomics, metabolomics, and phenomics which are used to explore and understand the defense mechanism of plants at the molecular level to address abiotic stresses. Moreover, this chapter also provides a list of some important and widely used tools which can further be used to integrate the results of these omics approaches to draw a meaningful inferential conclusion.

11.2 OMICS Approaches to Study Plant Defense Mechanism

11.2.1 Genomics

(i) Whole genome sequencing and resequencing: Genomics deals with the study of the complete genetic makeup of organisms or individuals. The field of genomics has grown exponentially in the past 20 years since the announcement of the first draft human genome in 2001. Further, the reduced sequencing costs and time accelerated the pace of whole genome sequencing due to the advancement of Next Generation Sequencing (NGS) technologies that have resulted in flooding of sequencing data (Fig. 11.1). This led to the development of advanced and efficient bioinformatics tools and techniques to handle such large-scale sequencing data for deeper and novel biological insights. We can

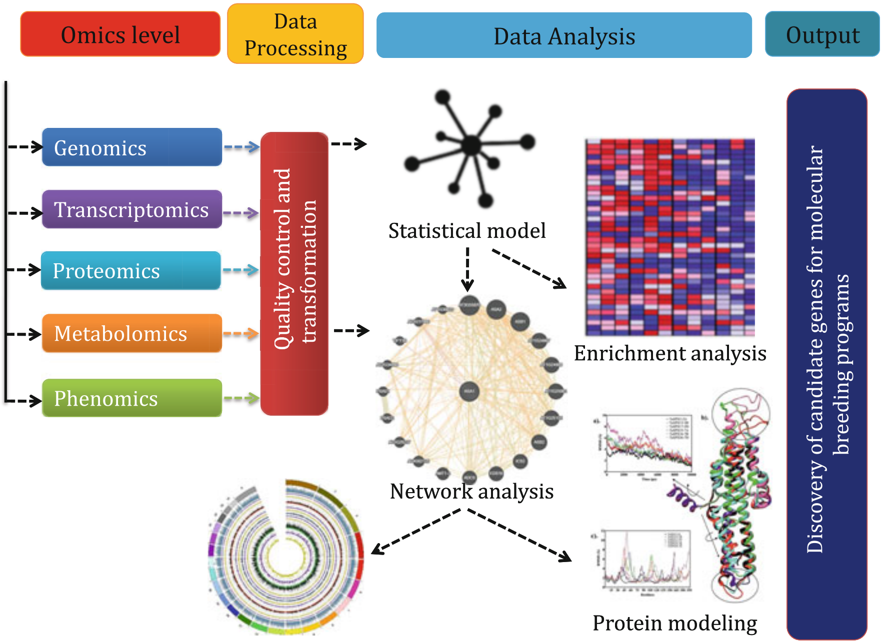

Fig. 11.1 Workflow diagram of omics approaches for study of plant defense mechanism

consider mainly two groups of genomics, i.e., structural genomics and functional genomics. Structural genomics deals with locating the mapped genes and markers to individual chromosomes which results in producing physical map of the genome whereas functional genomics focuses on relating genome sequences with its transcriptome and proteome (encoded proteins) to describe gene functions and interactions. The most efficient way to study molecular mechanisms in plants is to decode the whole genome sequence. In plants, *Arabidopsis* was the first genome to be sequenced by an international consortium (Berardini et al. 2015). Plant genome sequence helps to explain the organization, regulation, and evolution of studied genomes. The advent of next generation sequencing (NGS) technologies allows millions of molecules to be sequenced simultaneously and whole genome sequencing has become substantially cheaper and faster than traditional sequencing methods (Goodwin et al. 2016). Availability of high-quality whole genome sequence data and a well-annotated reference genome is very crucial for genomics and transcriptomic-based research. The catalogue of annotated gene models, genome organization, and synteny-based knowledge, repeats, and most notably the basis for distinguishing genetic variants are more apparent advantages acquired from genome sequencing. The reference genome is also used as the basis for the annotation of other genomes of closely related species. However, sequencing of the whole genome (i.e., resequencing) is faster and cost-effective for the species which have already sequenced high-quality reference genomes.

There are several Bioinformatics tools for assembling the reads of sequenced genome like Bowtie, Soap2, MIRA, Abyss, SOAPdenovo, and velvet (Wee et al. 2019).

(ii) *Identification of Molecular markers:* The whole genome sequences can be extensively studied in discovering the molecular markers. One of the promising marker systems suitable for laboratories is microsatellites or simple sequence repeats (SSRs). A valuable resource for upcoming breeding programs are being developed for genome-wide identification of microsatellites and subsequently helps in markers development. MISA and GMATA are two most popular and widely used bioinformatics tools for identification of SSRs in the genomic data. But nowadays, SNP genotyping approaches are gaining mainstream acceptance with the introduction of cost-efficient and high-throughput genotyping techniques. SAM tools, GATK, Picard, etc. are some variant calling tools that are used to identify SNPs from the whole genome sequence assembly. The genotyping by sequencing (GBS) approach is an extremely multiplexed framework for building RRL (reduced representation libraries), finding molecular markers, and genotyping for crop improvement among the various other SNP-based genotyping approaches (Eltaher et al. 2018; Elbasyoni et al. 2018). GBS has been applied to many crop varieties as a result of low cost and innovative technology (Poland and Rife 2012; Kim et al. 2016). For example, a tomato GBS study led to the discovery of 8784 SNPs based on an approach to NGS and 88 percent of these SNPs are commonly found in tomato germplasm, (Sim et al. 2012). GBS is simple and cost-effective solution but use is still limited because it requires specialized skills in computational and data analysis. In the future, it can be a commonly used approach with the availability of easy-to-use computational packages and pipelines.

(iii) *QTL mapping and GWAS:* Linkage mapping (LM) and association mapping (AM) by identifying marker–trait associations have contributed to the identification of QTL (Cockram and Mackay 2018). In many plant species, the importance has been given to mapping QTLs for many abiotic stresses, such as heat, salinity, drought, and cold. QTLs controlling seed germination under various stress conditions have been identified using QTL experiments. QTL mapping experiments are conducted to identify loci regulating stress resistance in particular, advancements in genomics have encouraged more complex approaches involving multi-parental populations such as nested association mapping (NAM) and Multi-parent advanced generation inter-cross (MAGIC). A Genome-wide association studies (GWAS) approach, on the other hand, has an advantage over linkage mapping (Linkage Disequilibrium, i.e., LD) as it examines the genetic variation and recombination events in germplasm collections and also offers higher precision mapping (Fukushima et al. 2009). This set is designed to capture the genetic variability for the trait of interest and represents the products of hundreds of historic recombination cycles, providing higher resolution during QTL mapping (Mackay et al. 2009). GWAS is systematically used to detect SNPs for agronomic characteristics in a germplasm collection (Pasam et al. 2012). However, associations detected in AM are often spurious because associations are based on LD, which not only depends on linkage but also on population stratification and relatedness among individuals. Nowadays, efforts have also been made to combine linkage-based QTL mapping with LD-based AM, and conduct joint linkage association mapping (JLAM) to overcome the limitations and exploit the benefits associated with each of the two approaches, i.e., linkage and LD.

(iv) *Genomic Selection:* The declining cost of SNP assays has made it possible to genotype vast numbers of experimental lines in stress-tolerant crop breeding programs to introduce the Genomic Selection (GS) method. The GS method is successful in simultaneously controlling all the loci that lead to the growth of the trait, regardless of the magnitude of their individual impact. The GS solution overcomes the disadvantage of QTL mapping-based breeding where it is difficult to track/identify small-effect QTLs. Importantly, the small effects of QTLs can collectively have greater effects on abiotic traits of economic significance. Due to epistatic interactions, the most economically significant traits are complex and influenced by unexpected trait expressions (Deshmukh et al. 2014). Therefore, by using all available molecular markers in conjunction with the phenotypic data of a training population, GS is the best way to predict genetic values for selection. A model has been developed to classify and analyze genotypic and phenotypic data to evaluate the phenotypic variation based on their genotypes of their whole genomes (genetic composition) (Yan et al. 2009). To estimate breeding values, different GS models like nonlinear regressions (RKHS and RF), Bayesian approaches (Bayes A and B), and penalized regressions (RR, LASSO, and EN) have been used in many studies.

11.2.2 Transcriptomics

For the efficient management of abiotic stress, understanding the gene regulatory cascades for stress responses is very important. The best strategy for investigating plant response regulation and identifying genes involved in mechanisms of stress tolerance is to collect and compare the transcriptome of different tissue types at various developmental stages. Thus, understanding the transcriptome of different tissues at developmental stages will lead to better understand the associated phenotypic variation. Several tools and techniques are available to obtain expression profiling for assessment of transcriptomic results both gene-by-gene and collectively for several genes at a time.

(i) *Microarray*
Microarray technology is based on hybridization between the target DNA and probe DNA designed with known sequences. It is capable of covering tens of thousands of genes at a time, it has made a significant contribution to research. It is well developed and is still being used as a major platform for transcriptome analysis of sequenced species, despite its shortcomings in the variety of target transcripts in the dynamic spectrum of quantification compared with

NGS-based RNA-seq technology (Wang et al. 2009; Jazayeri et al. 2014). Microarray is used to identify the differentially expressed genes in response to abiotic stresses, including salinity, heat, cold, drought, and oxidative stress. Numerous studies have been conducted in several plant species using microarray approaches to identify genes having significant roles in stress tolerance mechanisms as well as for the understanding of diverse molecular mechanisms (Kumar et al. 2018, 2019; Nagaraju et al. 2019, 2020).

(ii) *RNAseq*

This approach is based on high-throughput Next Generation Sequencing. RNAseq relies on high-speed sequencing of short cDNA fragments (typically 30–400 bp) reverse-transcribed from mRNAs. Further, number of cDNA fragments aligned to the reference sequence indicates the abundance of the mRNA. RNA sequencing (RNAseq) has become the most cost-effective, reliable, and high-throughput transcriptomic technology with the quick advancement of next generation sequencing. In contrary to microarray, the RNAseq approach is not only confined to comparing the transcripts levels, but also it is useful in discovery of novel genes and spliced forms, especially in non-model plants. Numerous reports on the application of RNAseq technology in case of plants are available (Ye et al. 2017; Xiong et al. 2017; Guan et al. 2019). RNAseq technology has also been applied to unsequenced organisms (Ekblom and Galindo 2010) as several computational tools enable de novo assembly of the reads without the availability of a reference genome (Oshlack et al. 2010; Grabherr et al. 2011). Although management of the huge data sets generated poses many challenges and this technology is becoming a mainstream of transcriptome analysis.

(iii) *HiCEP*

High-coverage gene expression profiling (HiCEP) is based on the amplified-fragment-length polymorphism technique. This approach can detect changes in transcript expression with high coverage (Fukumura et al. 2003). Amplified DNA fragments are first derived from mRNA followed by capillary electrophoresis. Their abundances are estimated by the peak observed through electropherogram. The relevant peaks are then fractionated and sequenced.

11.2.3 Proteomics

Proteomics is the large-scale study of proteins in a studied organism or system. The proteome represents a complete set of proteins that are produced by an underlying organism or system. Proteomics has enabled us to identify and validate the ever-increasing numbers of proteins. Proteins are important for living organisms as they produce a variety of functions. Modern proteomic technologies have made it possible to detect vast number of proteins in plant samples easily and simultaneously (Vanderschuren et al. 2013). Over the last few years in plant science, high-throughput quantitative proteomics studies gained considerable significance in characterizing proteomes and their differential regulation during plant growth, biotic and abiotic stresses. Proteomics experiments often found that many insect attack-responsive proteins were associated with the cycle of tricarboxylic acid (TCA) and also involved in carbon metabolism, which suggested that carbon metabolism was altered during insect attack for defense. High abundance of proteins such as ribulose-1,5-bisphosphate carboxylase oxygenase (Rubisco) creates considerable difficulties using shotgun plant proteomics for the whole proteome characterization. To understand the defense mechanisms during plant–insect interactions, an enhanced proteomic system, called Polyethyleneimine Assisted Rubisco Cleanup (PARC) was used (Zhang et al. 2013). George et al. (2011) reported the differential protein expression in maize (*Zea mays* L.) in response to infestation of a chewing (*Spodoptera littoralis*) and a boring insect (*Busseola fusca*).

(i) *Gel-Based Electrophoresis:*
In the first dimension, proteins are isolated either by an immobilized pH gradient strip or by an isoelectric focusing tube, and then followed by SDS polyacrylamide gel electrophoresis in the second dimension (Komatsu et al. 2007, 2012, 2013a, b, 2015). Protein spots are extracted from the gel after staining, reduced by dithiothreitol, alkylated with iodoacetamide, and digested with trypsin. A form of Mass Spectrometry (MS), such as nano-liquid chromatography (LC) tandem MS or nano-LC MS/MS, will then analyze the peptide mixtures. While 2D gel-based methods offer a visual description of proteins including intact protein profiles and they are not sufficient for the detection and identification of proteins with low abundance or with extreme molecular weights, isoelectric points, or hydrophobicity.

(ii) *Gel-free proteomics:*
Gel-free proteomics includes both label-free and labeling methods. In case of label-free method, protein samples are purified by chloroform-methanol extraction and reduced with dithiothreitol, alkylated with iodoacetamide, and digested with trypsin and lysyl endopeptidase. They are analyzed by nano-LC MS/MS (Komatsu et al. 2013b). Differentially expressed proteins are identified from the spectrum obtained by scanning with MASCOT Daemon client software against a peptide database. For identification and annotation of homologous proteins, positive matches are searched against protein databases available at NCBI (www.ncbi.nlm.nih.gov) through BLASTP. It is now a commonly used technology in proteomics, since its protocol is simple and helpful in identifying proteins in a large scale.

11.2.4 Metabolomics

Metabolomics is a promising approach that provides a biochemical snapshot of phenotype of an organism. Metabolomics makes it possible to systematically classify and measure low-molecular weight molecules which are closely related to essential toxicological and nutritional features. Information on genes, proteins, and transcriptomes are not adequate to thoroughly classify a cell but the broad variety of primary and secondary metabolites found in a cell must also be examined. Numerous studies have been done to explain the function of metabolites in plants under conditions of biotic and abiotic stresses. Plant chemical compounds that are not active in photosynthetic and core metabolic processes are linked to the evolution of the chemical defense mechanism against stress in plants (Mithöfer and Boland 2012; Gjindali et al. 2021). These compounds are classified as secondary metabolites that do not play any significant role in the plant's growth, development, or reproduction rather these compounds serve as signaling molecules or direct defense chemicals and include alkaloids, terpenoids, cyanogenic glycosides, glucosinolates, and phenolics (Bennett and Wallsgrove 1994; Zebelo and Maffei 2012). To study the chemicals involved in the interactions of living organisms, including the chemical defense system during plant–insect contact, a special area called "chemical ecology" is developed (Mithöfer and Boland 2012). Plants have to sacrifice some of the central metabolism by allocating energy to this defense while activating the defense response mechanism controlled by the secondary metabolites against insect attack. Along with secondary metabolism, during an insect or pathogen attack, the primary metabolism of a plant is often differentially influenced (Barah et al. 2013). It has been in use for the past decades to study the selective control of primary or secondary metabolites during plant–insect activity (Salem et al. 2020).

Recent developments in high-throughput metabolite profiling methods and advanced combinatorial protocols available in plant metabolomics are liquid chromatography–mass spectrometry (LC-MS), gas chromatography–mass spectrometry (GC-MS), Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS), flow-injection electrospray ionization mass spectrometry (FI-ESI-MS) and nuclear magnetic resonance (NMR). However, it is computationally difficult to analyze the enormously diversified plant metabolites produced using these methods (Allwood et al. 2008; Ernst et al. 2014). Hence in analyzing and processing highly complex biological data, the role of bioinformatics is very crucial.

11.2.5 Phenomics

Phenomics is the study of high-throughput phenotypic variation analysis, which is a complex web of genotype, phenotype, and environment interactions. Phenome represents a set of phenotypes. Studies of the genome and phenome with individuals or large populations are complementary to each other (Yasunori and Sinha 2014). Plants with stable phenotypes are strong genomic tools and are also a target to identify the alleles by high-throughput sequencing. Advances in sequencing technology have increased genotyping efficiencies, while phenotypic characterization has progressed more slowly over the past decade, restricting the characterization of quantitative characteristics, especially those associated with stress tolerance (White et al. 2012). There are recent developments in phenotyping methods which allow the identification of specific characteristics. Phenomics technology requires advanced imaging systems, sensors, automations, and computational resources for the phenotyping in plants. These make phenomics a high-throughput approach that is capable of handling thousands of genotypes for the evaluation of hundreds of phenotypic parameters simultaneously (White et al. 2012; Ubbens and Stavness 2017; Tardieu et al. 2017). There are various phenomics platforms available to investigate physiological parameters in plants under different stress conditions, e.g., one such tool is scan analyzer 3D. As phenomic data collection is an expensive and time-consuming method, integrated technological developments would help to minimize the associated costs and increase phenomic throughput.

11.3 Bioinformatics Tools and Techniques for Integration of Multi-OMICS Data

Due to availability of large-scale multi-omics data and their availability in public domain, e.g., in the form of various databases and repositories, poses a major challenge for bioinformatics community for integrating different tools and techniques so that one can draw biologically useful inferences because the use of only one approach at a time cannot lead to understand the defense mechanism robustly. Even after having lots of development in this area, integration of heterogeneous omics data to draw meaningful biological inferences is a major challenge (Keurentjes et al. 2011). However, to develop ultimate products like climate-smart cultivars, efficient integration of different tools, techniques, and approaches appears to be a promising strategy. For example, GWAS and QTL mapping both identify a genomic region or marker that is associated with underlying trait of interest and further in discovering the candidate genes. As the use of RNAseq data with gene expression profiles gives an idea about the functions of unknown genes. So, relating GWAS and QTL with their respective transcriptome will give the clue to identify differentially expressed candidate genes.

There are large number of user-friendly computational platforms are developed for the integration of multi-omics data (Table 11.1). Details of such few tools and software are given in Table 11.1.

11.4 Concluding Remarks

The recent developments in modern high-throughput sequencing technologies have flooded the web with the availability of biological data from various platforms. Recent efforts for development in integrating omics data are not sufficient in understanding such vast biological data. However, the integrative system-based approach, i.e., integrating multi-omics data generated from heterogeneous platforms, using various bioinformatics tools, techniques, and approaches, is the only solution to this problem of understanding and finding meaningful biological conclusions. Although efficient adaptation of bioinformatics tools and techniques depends on their availability and user-friendly manner. So, there is a need to develop more user-friendly and easy-to-use bioinformatics tools and pipelines for end users, such as

Table 11.1 Computational platforms used for the integration of multi-omics data

Tools/Software	Function	Availability	References
Weighted gene Coexpression network analysis (WGCNA): R Package	Inclusive of R functions for performing various aspects of weighted correlation network analysis	https://horvath.genetics.ucla.edu/html/CoexpressionNetwork/Rpackages/WGCNA/	Langfelder and Horvath (2008)
IntegrOmics: R Package	It implements integrative analyses of two types of omics variables measured on the same samples or conditions	http://math.univ-toulouse.fr/biostat	Lê Cao et al. (2009)
iCluster: R Package	Integrative clustering of multi-type genomic data	https://www.mskcc.org/departments/epidemiology-biostatistics/biostatistics/icluster	Shen et al. (2009)
VirtualPlant	Integrates genomic data and provides visualization and analysis tools to further explore and understand genomic data	http://virtualplant.bio.nyu.edu	Katari et al. (2010)
Babelomics	Platform for the integrative analysis of genomic, transcriptomic, and proteomic data with advanced functional profiling	http://www.babelomics.org	Medina et al. (2010)
PLAZA	Integrates comparative genomics data for both computational and experimental plant biologists	https://bioinformatics.psb.ugent.be/plaza	Van Bel et al. (2012)
TraitCapture	Platform for genomic and environment modelling of plant phenomic data	https://traitcapture.org	Brown et al. (2014)
MixOmics: R Package	Statistical multivariate methods for data exploration, integration, dimension reduction, and visualization	http://mixomics.org/	Rohart et al. (2017)
Miodin: R Package	Vertical and horizontal integration of multi-omics data	https://gitlab.com/algoromics/miodin	Ulfenborg (2019)
STATegra: R Bioconductor Package	Based on machine learning, non-parametric data combination, and a multi-omics exploratory analysis	https://bioconductor.org/packages/STATegRa/	Planell et al. (2021)

accessibility, easy to use tutorials and manuals, and interactive options to analyze multi-platform data. This will help the researchers to understand the biological system in a more realistic way, and will definitely help to translate this understanding to develop better crop varieties with improved defense mechanisms.

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Induced Mutagenesis for High-Temperature Tolerance in Crop Plants

12

Suman Bakshi, Sanjay J. Jambhulkar, Ranjeet Ranjan Kumar, Pradeep Bhati, and Uttam Kumar

Abstract

High temperature is one of the major abiotic stresses causing huge yield losses in all crop plants. The challenges posed by global warming are the major antagonistic factors to realize seed yield potential of a genotype. There is a need to generate allelic variation in the existing gene pool for high-temperature tolerance. Induced mutagenesis holds great potential to cause lesions ranged from single base pair to large deletions resulting into development of spectrum of new gene combinations for high temperature tolerance. Advances in scientific methods, especially related to quantifying existing thermotolerance at seedling and reproductive stages, understanding the function of each genetic loci and their position on a chromosome, and deciphering biochemical pathways to analyze the effect of these genetic loci made it possible to measure genetic value of the mutant genes. Substantial efforts have been directed to generate variability in cereal crops such as wheat, rice, maize, and barley in the coded fraction of genome for heat stress tolerance which was exploited to decipher functional characterization of genetic loci at morphological, physiological, biochemical, and molecular levels as well as direct improvement of crop cultivars for warm locations. In wheat; mutations for stay green, thousand kernel weight, small heat shock protein, and stable meiosis; in rice; spikelet fertility, characters at seedling and reproductive stage, chlorophyllide a oxygenase; in maize; EF-Tu factor; in tomato; MAPK gene and mutations for brassinosteroids in barley have been found useful to develop

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S. Bakshi (✉) · S. J. Jambhulkar
Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Mumbai, India

R. R. Kumar
Division of Biochemistry, ICAR-Indian Agricultural Research Institute, New Delhi, India

P. Bhati · U. Kumar
Borlaug Institute for South Asia (BISA)/CIMMYT, Ludhiana, Punjab, India

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heat-tolerant crop plants. A total of 14 heat-tolerant varieties have been developed through mutation breeding. Besides, precise mutagenesis techniques such as TILLING and CRISPR-cas9 have been found to be useful in developing heat-tolerant crop plants.

Keywords

High-temperature tolerance · Mutant · Mutation breeding · Stay green mutant · sHSP · EF-Tu factor · MAPK · Brassinosteroids · Wheat · Rice · Maize · Barley · Tomato · TILLING · CRISPR

12.1 Introduction

High-temperature stress is one of the penalties of a globally changing climate severely affecting agricultural productivity. The gradual rise in temperature is experienced all over the world leading to more warm days and nights with future prediction of mean temperature exceeding 2°C by the end of the twenty-first century (IPCC 2014). Under changing climate scenario, the increase in temperature can cause irreparable damage to plant growth and development resulting into major risk to food production and security (Christensen and Christensen 2007; Gitz et al. 2016). Climate change with higher heat stress events will disturb natural habitat of most agricultural crops across globe (Raza et al. 2019). The noteworthy studies to understand the effect of heat stress on vegetative and reproductive stage (Chaudhary et al. 2020; Cohen et al. 2021), potential genes for tolerance (Su et al. 2019), heat shock factors and proteins involved (Chandel et al. 2013; Guo et al. 2016) and strategies employed to antagonize the effect of heat stress (Tian et al. 2012) was carried out in various crop plants. Studies to analyze heat stress tolerance mechanisms in crop plants are further augmented by genomics, proteomics and metabolomics to recognize the gene networks involved in the tolerance of this complex trait. The functional characterization of several genes (*TaDmc1, OsCAO1 and SlMAPK3*) has been possible due to induced mutations which are either knock outs of a locus resulting in loss of trait in the derived mutant or disrupt or modify gene expression to the extent that it could be possible to discern it from its wild type. Conventional improvement which acts upon natural variability resulted in limited improvement in recent times due to lack of tolerant genes in usable germplasm of most crop species (Bhandari et al. 2017; Ahmar et al. 2020; Singer et al. 2021). However, mutation breeding with a fundamental objective to induce variability in a trait of interest and to enrich germplasm base of any crop species has been successfully used for improvement of almost all traits (Ahloowalia and Maluszynski 2001; Mba 2013; Oladosu et al. 2016; Holme et al. 2019) and especially traits essential to contribute adaptability in the era of climate change (Bakshi et al. 2020). Mutation breeding has proven its role in enriching germplasm of most crop species and their use in development of varieties with improved traits (Sikora et al. 2011; Wang et al. 2013). It has been documented in IAEA MVD database that a total 3406 mutant varieties developed in agricultural important crops across world which include induction of agronomic and botanic traits, nutrition and quality traits, resistance to biotic and abiotic stresses, yield and contributing traits (http://mvd.iaea.org/). Substantial efforts were carried out through induced mutagenesis for development of heat tolerant traits which were analyzed at morphological, biochemical and molecular levels and a total of 14 heat-tolerant varieties were developed in crop plants. Mutant varieties contributed to economic benefit to the growers and their economic impact is reviewed by Ahloowalia et al. (2004). Developments in field of genetics and molecular biology in understanding transcription factors, gene networks and biochemical pathways expanded the horizons from arbitrary to precise mutagenesis and resulted in development of techniques such as TILLING (Tadele 2016; Kumar et al. 2017) and CRISPR-CAS9 editing (Zaidi et al. 2020). These techniques supplemented mutation breeding efforts and made it possible to deal with complex crops like wheat and complex traits such as heat and drought stress governed by multi-gene families.

In this review, efforts directed and accomplished in area of induced mutagenesis for augmenting tolerance to high-temperature stress and its physiological, biochemical and molecular analysis along with advances in precision mutagenesis namely Targeting Induced Local Lesion in Genome (TILLING) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technologies have been mentioned.

12.2 Induction of Mutations in Crop Plants

Inducing mutations in plants is one of the proven methods for enhancing variability. Several morphological, physiological and biochemical traits related to heat stress have been modified with the objective to decipher their function and enrich working germplasm of crops such as wheat, rice, maize, barley, medicago and tomato using physical and chemical mutagens (Table 12.1) and crop-wise work has been mentioned below.

12.3 Wheat

High temperature has an impact on wheat productivity in tropical, subtropical, arid and semi-arid regions of the world. The high-temperature stress influences all wheat growth stages however, the effect at pre- and post-anthesis stages is more pronounced on grain filling and decides the grain yield of a genotype (Farooq et al. 2011; Prasad and Djanaguiraman 2014; Djanaguiraman et al. 2020). Heat-directed grain yield loss predictions on a regional or global basis revealed that sustaining wheat productivity and production needs concerted efforts particularly to breed for tolerant genotypes. Heat tolerance is a complex trait in plants and is likely to be under the control of multiple genes (Barnabás et al. 2008). Limited information is

Table 12.1 Induced mutagenesis for improvement of traits contributing to heat tolerance

Crop				
Triticum aestivum				
Variety	Mutagen	Traits improved	Mutant/ locus identified	References
WH147	Gamma rays	Higher contents of phospholipids, higher content of galactolipid-bound linolenic acid and especially Phospholipid-bound trans-c.-3-hexfldeeenoic acid	WH147M	Behl et al. (1997)
Guardian	Sodium azide and EMS	Higher light-saturated net photosynthetic rate (Pmax)	Heat-tolerant mutants	Mullarkey and Jones (2000)
Trinakria	Ethyl methane sulphonate	Delayed leaf senescence, higher seed weight and higher grain yield per plant	Stay green mutants	Spano et al. (2003)
Kronos & Cham1 (durum wheat)	EMS TILLING	Functional characterization of complex multi-gene family of sHsp26 and heat tolerant mutants	*Small hsps*	Comastri et al. (2018)
Shi4185	Gamma rays	Increased kernel weight	Fu4185 (*QTkw.cau-5D*)	Cheng et al. (2015)
Chinese Spring	Gamma rays	Chromosome pairing mutant at 30 °C	*TaDmc1*	Draeger et al. (2020)
Oryza sativa				
Hwacheongbyeo	N-methyl-N-nitrosourea	Slower rate of chlorophyll degradation	Hwacheong-wx (*sgr*(t)gene)	Cha et al. (2002)
IR64	Ethyl methane sulphonate	Expressed ribulose bisphosphate carboxylase large chain precursor, higher pollen viability and spikelet fertility, higher tiller number and yield per plant	NH219	Poli et al. (2013)
Jao Hom Nil	Fast neutrons	Higher spikelet fertility and grain yield	M9962, M3181 and M7988	Cheabu et al. (2019)
Super Basmati	Gamma rays	Higher grain yield, higher panicle fertility, higher cell membrane thermo-stability and antioxidant enzyme levels	HTT-121	Zafar et al. (2020)

(continued)

Table 12.1 (continued)

Crop				
Triticum aestivum				
Variety	Mutagen	Traits improved	Mutant/ locus identified	References
Dongjin	CRISPR	Play role in chlorophyll b synthesis and in regulating photosynthesis, short narrow flag leaves and pale green leaves, lower chlorophyll b and carotenoid content	*OsCAO1*	Jung et al. (2020)
KY131	Sodium azide	Increased thiolated tRNA level	SLG1	Xu et al. (2020)
Hordeum vulgare				
Delisa, Bowman	Sodium azide and *N*-methyl-*N*-nitrosourea (MNU)	Higher temperature tolerance in BR-deficient and BR-signalling mutants	522DK, BW084	Rudolphi-Szydło et al. (2020)
Other crops				
Medicago truncatula				
NF2089	T-DNA mutant	Maintained green leaves, green anthers, central carpels, mature pods, and seeds, mutants showed higher chlorophyll content during senescence and had increased crude protein content		Zhou et al. (2011)
Solanum lycopersicum				
Ailsa Craig	CRISPR/ Cas9-mediated mutagenesis	Knockout of *SlMAPK3* showed higher heat tolerance	*SlMAPK3* (Map kinase)	Yu et al. (2019)

attained regarding the role of individual genes controlling temperature tolerance in wheat (Mullarkey and Jones 2000).

12.3.1 Induced Mutations for Mitochondrial Functions

Acquired thermotolerance is an adaptive strategy used by crop plants in which exposure to mild temperature stress induces tolerance to high temperature. The cell viability assay is based on reduction of 2,3,5-triphenyltetrazolium chloride (TTC) by electrons from mitochondrial electron transport chain (Towill and Mazur

1974) and determines respiratory enzyme inactivation or mitochondrial dysfunction under heat stress. Acquired thermotolerance studies using electrolyte leakage and TTC cell viability assays for quantifying the membrane function temperature tolerance of wheat genotypes and their association with yield and yield parameters were extensively carried out in wheat (Ibrahim and Quick 2001; Blum et al. 2001; Fokar et al. 1998; Dhanda and Munjal 2006; Dias et al. 2010; Sud and Bhagwat 2010). In the same study, thermotolerance in the mutants was further confirmed by quantifying Pmax (light-saturated net photosynthetic rate) for which parents showed a 23% reduction in Pmax compared to stability of trait in mutants and chlorophyll content stability in mutants.

12.3.2 Induced Mutations for Stay Green Genotype

Stay green genotype is one of the key germplasm resources for breeding high-temperature stress genotypes. However, only functional stay green mutants those delayed the onset of senescence along with maintained photosynthetic capacity (Thomas and Howarth 2000) with improved translocation of photosynthates from source to sink are desirable (Rivero et al. 2007). Stay green traits in wheat has been found to contribute resistance to diseases by Joshi et al. 1997 and tolerance to heat, drought, cold stress in many other crops (Thomas and Smart 1993; Gregersen et al. 2013; Thomas and Ougham 2014). Stay green trait in the genotypes has been found significantly associated with high chlorophyll content, normalized difference vegetative index, grain yield, biological yield, kernel weight, and low canopy temperature in the heat stress compared to control (Latif et al. 2020). In a durum wheat mutagenesis initiative, delayed leaf senescence and functionally green mutants were isolated with higher net photosynthetic rate, efficient photosystem II and higher chlorophyll concentration in maturation phase and senescence of the flag leaves (Spano et al. 2003). These functionally "stay green" mutants had higher seed weight and grain yield per plant than the parental genotype. Molecular analysis of photosynthetic genes showed upregulation of rubisco activase, soluble starch synthase and a glycine decarboxylase in these mutants and this provides a novel source of characterized germplasm for improving the yield of bread and durum wheat genotypes, especially under unfavourable environmental conditions (Rampino et al. 2006). Stay green mutant (*tasg1*) has been developed through chemical mutagenesis of bread wheat variety HeSheng2 using ethyl methane sulfonate (Tian et al. 2012). The mutant *tasg1* has been found to have delayed leaf senescence under normal and drought stress conditions. The *tasg1* mutants maintained more integrated chloroplasts and thylakoid ultrastructure, lower malondialdehyde content and higher antioxidative enzyme activities. The *tasg1* has a competent antioxidant enzyme defence system as revealed by lower malondialdehyde and the hydrogen peroxide content during natural senescence and methyl viologen-induced oxidative stress (Hui et al. 2012). Tian et al. (2013) observed that *tasg1* plants could maintain higher hill activity, actual PSII efficiency (**Φ**PSII), maximal photochemical efficiency of PSII (*F*v/*F*m) and Ca^{2+}-ATPase and Mg^{2+}-ATPase activities, increased number of polypeptides in thylakoid membranes and upregulation of two genes encoding pigment-binding proteins *TaLhcb4* and *TaLhcb6* under drought stress. Wang et al. (2015) investigated the cytokinin metabolism (CK) of *tasg1* mutant and substantiated that stay green mutants had higher CK content, increased transcript levels of CK metabolic genes and higher sensitivity to the CK inhibitor lovastatin. This confirmed that stay green mutant showed delayed leaf senescence and stable thylakoid membrane and high antioxidant competence due to altered CK level. Wang et al. (2016a, b) showed that cytokinin metabolism appeared to be involved in the regulation of stay green phenotype through the invertase activity and resulted in sucrose remobilization and ineffective conversion of soluble sugars into starch ended in smaller grain size in *tasg1*. The molecular basis of stay green trait revealed differential expression of *cisZOGT1* (catalytic O-glucosylation in cis-zeatin) genes associated with CK and N metabolism for delayed flag leaf senescence in a feedback pattern in a durum wheat mutant (Wang et al. 2019a, b).

12.3.3 Induced Mutations for Thousand Kernel Weight

Inducing mutations is a proven and effective way to create novel genetic variations for agronomical traits in wheat. Grain yield in wheat is determined by productive spikes per unit area, grain number per spike and kernel weight. Among these yield traits, kernel weight is found to be highly heritable, however, quantitatively controlled trait (Peng et al. 2003). A thousand kernel weight (TKW) mutant Fu4185 has been developed through gamma irradiation of parent variety Shi4185. The mutant has been found highly stable and maintains a higher grain weight ranging from 36.6 to 42.8 g compared to parent 29.9–36.0 g across five locations experiencing heat stress. Polymorphism in the mutant and parent based on simple sequence repeats (SSRs) showed that 30% polymorphic marker loci located on D-genome and this indicated that gamma ray-induced mutations could augment genetic diversity of conserved genomes. In the same study, environmentally stable QTL (*QTkw.cau-5D*) at 5DL for grain weight is identified and this could serve as a potential source of favourable alleles for grain size in high-temperature stress.

12.3.4 Induced Mutations for Small Heat-Shock Proteins

The heat-shock proteins (HSPs) are pervasive molecules in plants that are rapidly induced by heat stress (Basha et al. 2012). Six types of HSPs namely HSP100, HSP90, HSP70, HSP60, HSP40 and small HSPs (sHSPs) have been found in higher plants. The small HSPs with a molecular mass ranging from 12 to 42 KDa showed ubiquitous occurrence in kingdoms of life (Haslbeck et al. 2005). The sHSPs act as molecular chaperones which negatively affect unwanted protein–protein interactions and assist in refolding of denatured proteins (Gupta et al. 2010). The sHSPs are found to be located in the cytosol or nucleus, mitochondria, plastids, endoplasmic reticulum and peroxisomes in the cell. The genes which encode for sHSP in wheat have been isolated (Rampino et al. 2012; Pandey et al. 2015) and especially genes for the chloroplast-localized sHSP26 proteins found ubiquitously in plants (Haq et al. 2013). sHSP26 is the most thermosensitive component of photosynthetic machinery (Joshi et al. 1997) and found to interact with photosystem II (Zhang et al. 2014a, b; Hu et al. 2015). TILLING was used to study the variation at sHSP26 loci and their effect on heat stress was confirmed in *Triticum durum* (Comastri et al. 2018). It has been found that TdHsp26-A1 showed highest upregulation after direct heat stress whereas TdHsp26-B1 showed the highest upregulation to heat stress applied after acclimation. This confirms different levels of thermotolerance of same sHSP family. A total of 50 mutants in these sHSP26 loci differing in their heat tolerance were identified and to follow these specific mutations competitive allele-specific PCR markers were developed to characterize these mutations and for marker-assisted selection in breeding programmes.

12.3.5 Induced Mutations for Stable Meiosis at High Temperature

Assessment of heat stress consequences on wheat confirmed that the heat stress has more pronounced negative effects on reproductive phase over vegetative phase (Fischer and Maurer 1976; Fischer 1985; Wardlaw et al. 1989) and exposure of 20–24 hours at 30 °C during meiosis can reduce grain number (Saini and Aspinall 1982; Draeger and Moore 2017). The process of meiosis for gamete formation is crucial for sexually reproducing organisms. Any disturbance in meiosis could lead to chromosomal aberrations like aneuploidy and result in loss of fertility. Temperatures lower or higher than optimum for a crop species can induce a variety of meiotic abnormalities including changes in the frequency of chiasma formation (Elliott 1955; Dowrick 1957; Bayliss and Riley 1972; Higgins et al. 2012). The meiotic irregularities such as reduction in chiasmata formation are due to the effect on chromosome synapsis and temperature at which meiosis fails varies in different crop species (Bomblies et al. 2015). Chinese Spring is a known heat-sensitive wheat cultivar (Qin et al. 2008) and reduced chiasma frequencies found in N5DT5B plants at low temperatures. This chromosome asynapsis is due to *low-temperature pairing* locus (*Ltp*) by Hayter and Riley (1967). *Ltp* was located on 5DL (Hayter 1969) and the locus was later known as *Ltp1* (Queiroz et al. 1991). Chiasma formation at 30 °C and above showed reduction in N5DT5B plants (Bayliss and Riley 1972) demonstrating that chromosome 5D has been associated with high-temperature tolerance. These 5D nullisomic plants showed a reduction in grain number after treatment at 30 °C during premeiosis and leptotene (Draeger and Moore 2017). Mutant lines developed in Chinese Spring using gamma ray treatment and used for deletion mapping of 5DL region using KASP markers (Draeger et al. 2020). The *ttmei1* mutant showed a deletion of 4-Mb of the long arm of 5D and this deletion segment has 41 genes of which 18 show expression at meiosis. Among these meiosis-specific genes, *TaDmc1-D1* is the strongest candidate for the low-temperature pairing phenotype and known to be involved in initiation of synapsis at early prophase I in wheat (Martín et al. 2017). The deletion of *Dmc1* locus in the diploid plant species namely, *Arabidopsis thaliana, Oryza sativa, Hordeum vulgare* with abnormal synapsis, multiple univalents and chromosome mis-segregation has been reported (Couteau et al. 1999; Wang et al. 2016a, b; Colas et al. 2019; Szurman-Zubrzycka et al. 2019). The functional understanding of *TaDmc1* as a candidate gene for stabilizing chromosome synapsis against extremes of temperature has supplemented information to wheat breeders which would assist in identification of wheat genotypes with temperature tolerant alleles at this locus.

12.4 Rice

Rice is the staple food for sustaining lives of more than half of the world's population. Optimum temperature range for growth and development ranged from 27 to 32 °C without economic loss of grain yields (Yin et al. 1996). However, further rise in temperature beyond 32 °C caused a substantial effect on plant growth and development at all stages (Aghamolki et al. 2014). Feng et al. (2019) reported that a temperature increase of about 35 °C or higher in the field has irreparable damage to growth and reproduction. Studies on thermotolerance in rice have primarily focused on the reproductive stage which is highly sensitive and showed association with grain yield under heat (Jagadish et al. 2012; Wang et al. 2019a, b; Takai et al. 2020; Xu et al. 2020).

12.4.1 Induced Mutations for Improved Spikelet Fertility

Cheabu et al. (2019) induced mutations with different levels of spikelet fertility at high temperature using fast neutron irradiation. In the wild type and sensitive mutants, high temperatures of 40–45 °C at the reproductive stage decreased rice yield by decreasing spikelet fertility, hundred grain weight and panicle weight. The reduction in spikelet fertility and the differential response of cultivars at high temperatures was mainly associated with impaired pollen viability and pollen germination. Heat tolerant mutant namely M9962 exhibited a minimum reduction in spikelet fertility and grain yield at higher temperatures. Whereas, susceptible cultivars namely; Sinlek, RD13 and RD33 had a heavy reduction in spikelet fertility and grain yield. Malumpong et al. (2020) developed four back cross derived high yielding and heat-tolerant lines exploiting higher spikelet fertility of fast neutron mutant M9962 as donor and higher yield of recurrent parent, Phisanulok 2 (PSL2).

12.4.2 Induced Mutations for Heat Tolerance at Seedling and Reproductive Stage

The reservoir of mutants with differential tolerance to heat is a valuable source for understanding the function of gene(s) governing susceptibility or tolerance to heat in any crop species. Zafar et al. (2020) evaluated 39 mutants of cv. Super Basmati along with IR-64 as a check under normal and heat-stress conditions to identify mutants with heat tolerance at seedling and reproductive stages based on agronomic, physiological and molecular indices. This study reported four heat-tolerant mutants namely HTT-121, HTT-112, HTT-101 and HTT-102. The most heat-tolerant mutant HTT121 had higher grain yield, panicle fertility, cell membrane thermo-stability (CMTS) and antioxidant enzyme levels under heat stress. On the contrary heat sensitive nutants have been found to ccumulate reactive oxygen species, reduced catalase activity and upregulated OsSRFP1 expression under heat stress.

Panigrahy et al. (2011) screened mutants of cultivar N22 developed from treatment of ethyl methane sulphonate (EMS) under prolonged drought and high-temperature stress conditions and found four mutants N22-H-*dgl56,* N22-H-*dgl101,* N22-H-*dgl162* and N22-H-*dgl219* with higher chlorophyll and carotenoid contents, improved photochemical efficiency of PSII and less accumulation of reactive oxygen. Heat resilience of mutant NH219 was revealed by improved morphological and physiological traits including plant height, tiller number, number of panicles, panicle length, yield/plant, pollen viability, spikelet fertility, chlorophyll a/b ratio (chl a/b), relative water content (RWC), electron transport rate (ETR) and photochemical efficiency (Fv/Fm). Leaf proteome analysis showed constitutive expression of ribulose bisphosphate carboxylase large chain precursor in mutant NH219 under ambient growth condition. Mutant genotype NH219 had a grain yield advantage over N22 parent under heat stress. Molecular marker analysis showed an association of RM1089, RM423, RM584 and RM229 with number of tillers and yield per plant, leaf senescence, leaf width and yield per plant, respectively, in F_2 mapping population of IR64 and NH219 (Poli et al. 2013).

12.4.3 Induced Mutations for Chlorophyllide a Oxygenase for Heat Stress

Photosynthesis is the key process for plant growth and provides the energy for synthesis of organic compounds (Krause and Weis 1991). Chlorophyll is the most important pigment for photosynthesis for harvesting light energy and converting it to chemical energy (Fromme et al. 2003). Improvement in chlorophyll content in rice is observed as an approach for better photosynthesis rate (Huang et al. 2013) contributing to higher yield. Chlorophyllide a oxygenase (*CAO1*) has a direct role in chlorophyll b synthesis as evident from T-DNA insertional mutants and RNAi mutated lines (Abe et al. 2012; Lee et al. 2005). CRISPR edited mutant lines of rice with knockout of *OsCAO1* demonstrated short narrow flag leaves and pale green leaves with a significant reduction of chlorophyll content and poor chloroplast development and photosynthesis (Jung et al. 2020). CRISPR/CAS9 edited lines exhibited poor photosynthetic efficiency, lesser biomass, lower grain yield and grain quality due to lack of chlorophyll b resulting in insufficient supply of photoassimilates at grain filling stage. Differential gene expression analysis of CRISPR edited knockouts established that *OsCAO1* controls the expression of genes related to responses to oxidation–reduction, protein phosphorylation, carbohydrate metabolic process, oxidoreductase activity and thus an important locus for response to environmental stress.

12.5 Mutation Breeding in Maize and Barley

12.5.1 Maize

Maize is the third most important cereal crop and is cultivated over diverse agroclimatic conditions world over (Tiwari and Yadav 2019). The optimum temperature for achieving maximum grain yield for maize is 33–38°C and temperatures more than 38 °C considerably influence economic yield (Koirala et al. 2017). Temperature over optimum limits have a constraint for both vegetative and reproductive growth, nevertheless, anthesis, silking, grain filling and seed set are the most sensitive to heat stress and may lead to total sterility and kernel loss (Lizaso et al. 2018). Induced mutations have contributed extensively to assigning function to individual loci coding for abiotic stress tolerance in many crop plants (Bahuguna et al. 2018; Singh et al. 2018) and similar strategies have been exploited (Ristic et al. 2004) to investigate the role of EF-Tu factor for heat tolerance in maize mutants using a reverse genetic approach. Chloroplast protein synthesis factor, EF-Tu is a member of a highly conserved, nuclear-encoded multigene family (Lee et al. 1997; Sugita et al. 1994) and has a molecular mass of 45–46 kD (Bhadula et al. 2001). EF-Tu factor is involved in GTP-dependent binding of aminoacyl-tRNA to the A site of the ribosome (Riis et al. 1990). Bhadula et al. (2001) reported in a heat-tolerant maize line, ZPBL 1304 that synthesizes and accumulates increased amounts of EF-Tu in response to heat stress. It was confirmed that Ef-Tu function as a molecular chaperone and guards chloroplast proteins from thermal aggregation and inactivation. The involvement of EF-Tu in heat tolerance in maize is confirmed by many studies (Momcilovic and Ristic 2004; Rao et al. 2004; Momcilovic and Ristic 2007). In a study by Ristic et al. (2004) involving maize mutants having MuDR insertion (4.94 kb) with reduced capacity to accumulate EF-Tu under heat stress was evaluated by analyzing heat stability of photosynthetic membranes (thylakoids), thermal aggregation of chloroplast stromal proteins, and plant growth at seedling stage after exposure to stress. The outcome from the analysis of mutant with reduced EF-Tu accumulation showed that EF-Tu factor function as a molecular chaperone and prevent thermal aggregation of stromal proteins in chloroplast.

12.6 Barley

Panicle development and pollen formation are most sensitive to high temperature and may cause complete sterility in Barley (Sakata et al. 2000). Short intervals of heat shock (≥35° C) in the post-anthesis period can significantly reduce grain weight in barley (Wardlaw and Wrigley 1994) and decrease grain quality (Savin et al. 1996). Therefore, heat stress is considered important abiotic stress that causes a significant reduction in yield. Developing mutant resources for heat stress contributing traits in barley would help to assign function to individual loci and advance their usage in barley improvement.

12.6.1 Induced Mutations for Brassinosteroids for Improved Heat Tolerance

Brassinosteroids (BR) play a major role as regulators of plant growth and development and have been found to guard plants against various types of stress including extreme temperatures (Tian et al. 2018; Tong et al. 2014; Small and Degenhardt 2018). BR antagonize the effect of both high and low temperatures at cellular and molecular levels which is translated to better growth, biomass synthesis and increased plant survival (Sadura and Janeczko 2018). Sadura et al. (2019) evaluated Barley mutants to understand the role of BR phytohormones in regulating their acclimatization to high temperature. The study included three mutants of which 522DK and BW084 are the BR-deficient mutant developed from Delisa and Bowman and a BR signalling mutant isolated from Bowman to study the effect on efficiency of photosystem II, membrane permeability and damage on leaf blade in high-temperature conditions and showed higher tolerance of mutants to high temperature. It has been reported that exposure to 38 °C showed no damage to leaf blade damage, membrane permeability and retained photosystem II efficiency in mutants. Further, exposure to higher temperature of 45 °C showed 74% lower leaf blade damage in mutant 522DK, no damage in BW084 and less damage in BW312 compared to parent. Membrane permeability values were ranged from 30 to 70% lesser at 45°C in mutant genotypes. Further, molecular studies revealed that mutant 522DK carried G > A substitution at position 1130 of the *HvDWARF* gene transcript (Gruszka et al. 2011) at position 3031 in the gene sequence (Gruszka et al. 2016b) and was responsible for the conversion of valine-341 residue into isoleucine. The *HvDWARF* locus is reported to translate brassinosteroid C6-oxidase and is involved in synthesis of castasterone in BR biosynthesis however, the mutant showed a reduced castasterone production (Gruszka et al. 2016a). Other BR-deficient mutant BW084 (*brh13.p*) carried a missense mutation in the *HvCPD* gene. The *HvCPD* codes the C-23α-hydroxylase cytochrome P45090A1 that has function in the early stages of BR biosynthesis. The C2562T transition causes a substitution of the highly conserved amino acid residue (Pro-445 to Leu) of heme-binding site in the C-terminal domain of the HvCPD enzyme (Dockter et al. 2014). The BW312 (*ert-ii.79*) has an anomaly in BR perception resulting from substitutions in the BR receptor kinase-BRI1. The mutant had two substitutions and substituted amino acid residue (Thr-573 to Lys) is positioned in the steroid-binding site of the BR receptor and accountable to prevent the binding of the BR molecules (Dockter et al. 2014). The presumed certainty based on existing evidences about BR pathway was that BR-deficient and BR-signalling mutants would be less tolerant to high temperatures compared to their wild type, however, the obtained results of higher temperature tolerance of BR-mutants in the study could be accounted for by their semi-dwarf habit which resulted in reduced aerial parts of mutants and hence, lesser transpiration which consequently resulted into better physiological performance. These heat-tolerant Barley mutants could be used to develop climate-resilient cultivars.

12.6.2 Regulation of Heat-Shock Protein in Brassinosteroids Mutants

Sadura et al. (2020) investigated the role of steroid hormones "brassinosteroids (BR)" in the regulation of heat-shock protein expression in BR-deficient (mutated *HvDWARF* or *HvCPD*) and BR signalling (mutated *HvBRI1*) mutants. Brassinosteroids have been found as positive regulators of heat shock expression from the response of BR signalling mutants which accumulated heat-shock protein transcripts and HS proteins irrespective of the considered plant growth and acclimation temperature. Whereas, BR-deficient mutants also confirm that BRs regulate the expression of HSPs, however, the relation of the level of BRs is not directly corresponding with HSPs expression rather genetic background of parent cultivars influence their expression.

12.7 Tomato (*Solanum lycopersicum*)

Tomato (*Solanum lycopersicum*) is highly sensitive to heat stress. Vegetative and reproductive growth of tomato occurs best at a temperature range of 18.5–29.5 °C (Jones 2008). A degree rise in temperature above the mean daily temperature of 25 °C (Peet et al. 1997) inhibits growth of reproductive organs, pollen viability and female fertility resulting in either severe decline or no fruit setting (Sato et al. 2000; Firon et al. 2006). Enhanced sensitivity to varied environmental stresses made tomato a model plant species to study effect of genes and transcription factors under stress conditions (Yu et al. 2019). The mitogen-activated protein kinase (MAPK) function has been studied in many plant species and reported to be involved in signal transduction for regulating expression of genes and protein functions and influencing plant development, hormone regulation, disease resistance and various abiotic stresses (Raja et al. 2017; Ding et al. 2018). The expression of MAPK genes has been found to be induced in response to heat treatment in *Arabidopsis thaliana*, maize, tobacco and tomato (Evrard et al. 2013; Wu et al. 2015; Mansour et al. 2008; Liu et al. 2017). Studies in tomato earlier evidenced that knockout mutants of *SlMAPK3* have reduced drought tolerance and decreased disease resistance to *Botrytis cinerea* (Wang et al. 2017a, b; Zhang et al. 2018a, b). Yu et al. (2019) found that CRISPR/Cas9-mediated simapk3 mutant lines are involved in elevating heat tolerance, reducing ROS accumulation and upregulating several heat-shock protein and heat-shock factor genes expression and substantiated that *SlMAPK3* served as a negative regulator of defence response to heat stress in tomato.

12.8 Heat-Tolerant Varieties Released Through Mutation Breeding

Mutation breeding provides twin opportunities either to use induced thermotolerant mutant directly as new variety or involve heat-tolerant mutant into crossing programme to augment trait lacking in the working germplasm. Mutation breeding has been used extensively for last nine decades since its inception in 1928 when Lewis Stadler first used X-rays on maize and barley plants and initiated a new era of inducing lesions in DNA of crop species. Since then, crop scientists adopted this technology across the world and resulted in release of 3402 improved mutant varieties with traits altered in all crop plants. Efforts in direction of development of climate-resilient varieties can be gauged well from release of 248 mutant varieties tolerant to all abiotic stresses in the world. However, progress in development of thermotolerant mutant varieties is limited to a few crops. A total of 14 heat-tolerant high-yielding varieties have been developed through mutation breeding programme (Table 12.2). Among them, four are in *Gossypium* spp., three in Oryza sativa, two each in *Solanum lycopersicum*, *Lathyrus sativus* and one each in *Lactuca sativa*, *Glycine max* and *Agrostis* sp. employing mostly physical mutagens except one variety of rice namely, José LP-20 which is developed through chemical mutagenesis. The thermotolerant mutant varieties developed in rice showed improvement in additional traits such as large panicle, better grain quality, high yield, short maturity duration, photo insensitive and drought tolerance. Other additional traits improved in cotton involved early maturity, higher yield, good fibre quality and salinity tolerance and virus disease resistance. Likewise, there are several traits other than high-temperature stress that is improved in crops such as tomato, soybean, lettuce, grass pea and creeping bent grass and benefit the improvement in specific crop species. The efforts for development of heat stress-tolerant mutant varieties may improve with availability of functionally characterized genetic loci through techniques of reverse genetics, especially for traits conferring heat tolerance.

12.9 Targeting Induced Local Lesions in Genome (TILLING) for Heat Tolerance

Targeting Induced Local Lesions in Genome (TILLING) is a reverse genetics technique that combines chemical mutagenesis with high-throughput screening of induced allelic variation in the gene of interest. Chemical mutagenesis creates a large number of non-sense, splice site and missense mutations throughout the genome and generate multiple alleles of a specific gene in small populations of crop species (Till et al. 2007; Sabetta et al. 2011). It is a non-transgenic method and can be applied to any crop species and ploidy level, however, the sequence of gene to be targeted for inducing lesions needs to be known. TILLING has been proved as one of the beneficial techniques to gain information about the function of structural genes and transcription factors, especially those involved in heat stress tolerance (Marko et al. 2019). Point mutants have been induced using ethyl methane sulphonate

Table 12.2 Thermotolerant mutant varieties developed in crop plants

Crop	Parent	Mutagen	Mutant	Year	Country	Direct or indirect	Traits improved
Oryza sativa	Zaoyeqing 8	Gamma rays	Zaoyeqing	1980	China	Direct mutant	Large panicle and tolerance to high temperature
	INCA LP-10 (mutant)/J112	Chemical	José LP-20	2012	Cuba	Indirect mutant	High temperature and drought tolerant, high yield and better grain quality
	Ashfal	Carbon beam	Binadhan-14	2013	Bangladesh	Direct mutant	High temperature tolerant, short duration and photo insensitive
Agrostis sp.	Pencross	Gamma rays	Springs	1983	Japan	Direct mutant	High-temperature tolerance
Gossypium spp.	NIAB-86 (Mutant)XW83–29	Gamma rays	NIAB Karishma	1996	Pakistan	Indirect mutant	Tolerance to high temperature and salinity
	F_1(H1 x LRA51 66)	Gamma rays	NIAB 999	2003	Pakistan	Direct mutant	Early maturity, resistance to high temperature and high yield
	F_1 (NIAW-313-12/ CIM1100)	Gamma rays	NIAB 111	2004	Pakistan	Direct mutant	Early maturity, resistance to high temperature and high yield
	Pollen irradiation	Gamma rays	NIAB 777	2009	Pakistan	Direct mutant	High yield, good quality, tolerance to high temperatures and virus diseases resistance
Glycine max	F_4 segregant	Gamma rays	DT99	2000	Viet Nam	Direct mutant	Early maturity, good adaptability, resistance to diseases and tolerance to high and low temperatures
Solanum lycopersicum	MST 32/1	Gamma rays (Cs137)	Summer Star	2019	Mauritius	Direct mutant	High temperature tolerant, improved yield and processing quality
	Amalia	Gamma rays	Domi	2015	Cuba	Direct mutant	Drought tolerance, high temperature tolerance, high yield disease resistance

(continued)

Table 12.2 (continued)

Crop	Parent	Mutagen	Mutant	Year	Country	Direct or indirect	Traits improved
Lathyrus sativus	Local variety	Gamma rays	Bogdan	2005	Moldova, Republic of	Direct mutant	High temperature and drought tolerant, high yield and high protein content
	Butter head	32P and gamma rays	Evergreen	1966	Japan	Direct mutant	Slow bolting and tolerance to high temperature
Lactuca sativa	Butter head	32P	Giant green	1966	Japan	Direct mutant	High-temperature tolerance

(EMS) and TILLING mutant population was developed in cultivar Red Setter of tomato. The binding protein namely HSBP in tomato is one of the negative regulators of heat stress response and represses the activity of HS transcription factors. Tomato mutant genotype carrying a missense mutation in *SlHSBP1* gene displayed increased thermotolerance. The methionine-to-isoleucine mutation in the central heptad repeats of HSBP1 caused partial loss of protein function, thereby reducing the inhibitory effect on *Hsf* activity. Tomato plants with a mutation in binding protein reported to have no inhibitory effect on development and therefore, this mutant line may serve as a potential donor source for contributing to heat tolerance in tomato improvement programme.

In durum wheat, it has been reported that small heat shock protein HSP26 played a significant function to prevent the irreparable aggregation of misfolded proteins and protect the photosynthetic machinery from heat-induced damage (Khurana et al. 2013). This protein family has four functional genes of which three are mapped on A genome and rest left single functional gene on B genome. Comastri et al. (2018) applied in vivo and in silico TILLING approaches for the identification of new alleles in HSP26 family and reported 50 TILLING mutant lines. These generated mutant lines have been characterized for their thermotolerance and KASP (Kompetitive Allele Specific PCR) markers which will be used to follow the specific mutations in marker-assisted selection.

In upland rice, mutant lines were induced by gamma rays and analyzed to discover alleles in heat-shock protein genes (Yona 2015). The rice mutant lines showed induced mutations with base pair substitutions and InDels included 50% and 41% in HSP90-1 gene and 23% and 35% in HSP17.9 gene, respectively. The developed TILLING mutant lines were evaluated for growth, yield and yield components and eight mutant lines produced higher yields under heat and drought stress.

12.10 CRISPR-Cas Technology for Development of Abiotic Stress-Tolerant Crop

Abiotic stresses like drought, heat and salinity are key threatening factors to food security (Pereira 2016). Development of crop varieties with improved tolerance to abiotic stresses is the only option left. Availability of desired variability and its utilization in cop improvement programme are the key factors to achieving the target. Conventional crop breeding methods are highly successful so far. In addition, induced mutagenesis has played a pivotal role to strengthen the desirable variability and development of high-yielding varieties. However, the process of induced mutagenesis is random but standard screening techniques assure the selection of desirable target trait (Bakshi et al. 2020). Development of high-yielding varieties resilient to climate change in shortest possible time needs precise manipulation in the genome (Osakabe et al. 2016; Osakabe and Osakabe 2017). This has led to the emergence of site-specific genome editing as an alternative to conventional plant breeding and transgenic strategies (Osakabe and Osakabe 2015).

Genome manipulation through CRISPR-Cas9 technology needs precise targets/genes. Various abiotic stresses disrupt plant growth and development by causing oxidative stress, osmotic stress, hormonal imbalance and alternation in nutrient uptake and accumulation. Targets to abiotic stresses are linked to various morphological, physiological (Fang and Xiong 2015; Shi et al. 2017; Lou et al. 2017) and metabolic imbalances by abolishing the activity of enzymes, protein metabolism and lead to the production of reactive oxygen species (ROS) resulting in programmed cell death (PCD) (Van Breusegem and Dat 2006; Huang et al. 2019). In plants, PCD is prevented by scavenging ROS by antioxidant enzymes like glutathione-S-transferase (GST), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT) and superoxide dismutase (SOD) (You and Chan 2015; Driedonks et al. 2015). Besides, various transcription factors like NAC, DREB, bZIP, MYB, TCP and WRKY confer abiotic stress tolerance, including drought in plants (Erpen et al. 2018). Heat stress tolerance, like other abiotic stresses, is controlled by complex molecular networks which include several transcription factors, heat-shock factors and HSPs genes and genes involved in the biosynthesis of complex metabolome. Nevertheless, the CRISPR edited knockout of *SlMAPK3*, a map kinase gene in tomato activated several pathways which included reduced contents of H_2O_2 and $O_2^{\bullet-}$ species, downregulation of *SlRBOH1* relative expression and upregulated expression of SOD, POD, APX and CAT genes and increased transcript levels of several HSPs namely SlHSP70, SlHSP90, SlHSP100 and SlHSFA1a, SlHSFA2 and SlHSFA3 and responsible for heat tolerance. In another study, CRISPR-Cas9 system has been used to knockout *OsNAC006* in rice and the mutant lines showed increased drought and heat sensitivity.

Development of abiotic stress-tolerant plants via CRISPR/Cas9- or Cas12-mediated genome editing has not only been reported in *Arabidopsis thaliana* (Liu et al. 2019) but also in *T. aestivum, O. sativa, Z. mays, Solanum lycopersicum, G. max, Hordeum vulgare and Sorghum bicolor* (Sánchez-León et al. 2018; Wang et al. 2017a, b; Liang et al. 2014; Tran et al. 2020; Li et al. 2020; Lawrenson and Harwood 2019; Gobena et al. 2017). Plants modified through CRISPR-cas9 technology with improved abiotic stress tolerance have been listed in Table 12.3.

12.11 Summary

Induced mutagenesis is one of the crop improvement methods which contributed enormously to world food security and economic benefit to the farmers. However, improving the tolerance of crops to heat stress is dauting task due to changing climate. Climate change needs enrichment of germplasm with the traits that provide buffering against rising temperatures and more dry spells or water shortages in future. Therefore, to develop climate-resilient crops need the knowledge of well-characterized genetic loci with their functions deciphered and induced mutagenesis is the most advantageous technology at hand. The mutants developed in wheat crops involved deciphering many complex traits such as oxidative damage to mitochondria and stability of meiosis in response to increased temperature. Besides, trait

Table 12.3 Improved crop plants for abiotic stress tolerance through CRISPR technology by editing specific gene

Crop	Edited gene	Improved trait	References
Arabidopsis	*OST2*	Stomatal response for drought tolerance	Oladosu et al. (2016)
	AREB1	ABA signalling-mediated drought tolerance	Roca Paixão et al. (2019)
	MIR169a	Drought tolerance	Zhao et al. (2016)
	S1HYPRP1	Salinity stress	Tran et al. (2020)
	SIMAPK3	ABA-dependent kinase signalling for drought tolerance	Wang et al. (2017a, b)
	SlNPR1	Drought tolerance	Li et al. (2019)
	SlCBF	Chilling tolerance	Li et al. (2018)
Rice	*OsRR22*	Salinity stress	Zhang et al. (2019)
	OsMPK5	Various abiotic stresses	Xie and Yang (2013)
	OsPDS, OsMPK2, OsBADH2	Various abiotic stress tolerance	Shan et al. (2013)
	OsDERF1, OsPMS3, OsEPSPS, OsMSH1, OsMYB5	Drought tolerance	Zhang et al. (2014a, b)
	OsAOX1a, OsAOX1b, OsAOX1c, OsBEL	Various abiotic stress tolerance	Xu et al. (2015)
	OsSAPK2	ABA signalling-mediated drought tolerance	Lou et al. (2017)
	OsSRL1, OsSRL2	Leaf rolling for drought tolerance	Liao et al. (2019)
	OsAnn3	Cold tolerance	Shen et al. (2017)
	OsRR22	Salt tolerance	Zhang et al. (2019)
	OsNAC006	Heat and drought tolerance	Wang et al. (2020)
	OsCAO1	Natural and induced senescence	Jung et al. (2020)
Wheat	*TaDREB2 and TaERF3*	Drought resistant	Kim et al. (2018)
Maize	*ARGOS8*	Drought tolerance	Shi et al. (2017)
Tomato	*SlMAPK3*	Heat stress	Yu et al. (2019)

discernible mutants assisted to understand the multi-gene controlled traits such as thousand kernel weight, senescence mechanism and heat-shock protein under high-temperature stress. Induced mutagenesis efforts in rice generated variation for both vegetative and reproductive traits which included plant height, tiller number, number of panicles, panicle length, pollen viability, panicle fertility and grain yield. Rice mutants with improved physiological traits such as chlorophyll a/b ratio, photosynthetic rate and improvement in biochemical traits, e.g. membrane thermo-stability, antioxidant enzyme activity, relative water content, electron transport rate and photochemical efficiency. In maize, mutation for Ef-Tu factor exposed its function as molecular chaperone and antagonize aggregation of stromal proteins in chloroplast in response to high temperature. BR-deficient and BR-signalling barley mutants exhibited unanticipated increased tolerance to heat stress and were used to discover brassinosteroids regulated heat shock protein synthesis in high-temperature stress. Advances in mutation breeding have led to the development of precision-indued mutagenesis like TILLING and CRISPR-cas 9 system which are found to be useful in the development of heat-tolerant crop plants. Mutations have been induced for heat-shock factors in tomato and heat-shock protein genes in durum wheat and rice. The variability induced and discovered through the use of mutations for several traits conferring high-temperature tolerance will serve as a buffer towards food insecurity threats posed by rising temperature.

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CRISPR/Cas-Based Genome Editing to Enhance Heat Stress Tolerance in Crop Plants

13

Gopika Mote, Pravin Jadhav, Sayali Magar, Pranita Thakur, Mangesh Moharil, and Rajkumar Biradar

Abstract

This chapter addresses the adverse effect of heat stress on plant growth, genes associated with heat stress tolerance and adaptive strategies that can be used to create heat-tolerant plants. CRISPR/Cas9 seems a promising approach regarding stress tolerance. The modified versions of CRISPR/Cas9 like CRISPRi, CRISPRa, base editing and CRISPR multiplexing offers more and more specificity and advanced editing options and minimizes the off-target effect. The versatility of CRISPR/Cas9 brings a new revolution in the field of plant science to alleviate abiotic stress like heat stress.

Keywords

Heat stress · CRISPR/Cas 9 · CRISPRi · CRISPRa · Base editing · CRISPR multiplexing

13.1 Introduction

In view of the ever-growing population, agricultural biotechnology has offered tremendous potential to overcome conventional methods of crop improvement, crop protection, quality management and improving other agronomical traits against several stresses. In recent year, biotic and abiotic stresses are being the limiting factors that significantly affect crop yield and quality. There is a need to enhance

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G. Mote · P. Jadhav (✉) · S. Magar · P. Thakur · M. Moharil
Biotechnology Centre, Post Graduate Institute, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola, Maharashtra, India

R. Biradar
Department of Botany, Sambhajirao Kendre Mahavidyalaya, Jalkot, Maharashtra, India

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food production with nutritional qualities that can possibly help to combat malnutrition in developing countries. This can be achieved by improving upon qualitative and quantitative traits of crop plants by adopting new analytical tools and technologies. Plant researcher are accountable to discover the frontiers of the natural biosphere and their fundamental mechanisms like genome editing which may improve lifestyle of human being.

Global agricultural production is facing unprecedented challenges due to climate changes. The world population by 2050 will reach 9.6 billion, aftermath the demand for staple food will have increased by 60%. Meanwhile, the swiftness of increasing yield caused by the revolution has been steadily declining. Unfavourable global climate change is foreseen to further limit plant production, thus cultivars with enhanced resilience to drastic environmental situations and with increased yield and improved quality need to be generated. However, the strategies used traditionally for crop breeding are very laborious, complicated and time-consuming methods of transferring desired traits into a superior cultivar. To tackle abiotic stress, different combinations of chemicals are used that may have adverse effects on human health and the environment, which may also develop chemical resistance in insects and weeds. Henceforth more-effective and time-saving plant breeding methods are required. Thus, breeding climate-smart crops that can tolerate various abiotic stresses such as heat stress, drought and salinity would be a sustainable approach to cope with such challenges. The most eco-friendly approach to cope with the challenge of abiotic stress is breeding tolerant cultivars. The use of recent advances in genome editing technologies assure new opportunities for improvement of crop by employing precision editing for target traits (Driedonks et al. 2016).

13.2 What Is Genome Editing and Why It Is Needed?

The recent few years have witnessed enormous excitement with the discovery of genome editing. Genome editing is a collection of advanced molecular biology techniques which allows precision, efficient and targeted modifications at genomic loci. The different approaches to editing involve use of site-specific nucleases (ZFN, TALEN, CRISPR/Cas), which create double-strand breaks (DSB) in DNA. All these genome editing techniques use a sequence-specific nuclease that allows to identify the target DNA sequence, once the target DNA sequence is identified a double-stranded break (DSB) is created. After the creation of a double-stranded break, the endogenous repair systems fix them by one of the two approaches that are Non-Homologous End Joining (NHEJ) and Homologous Direct Repair (HDR). In case of NHEJ, there is insertion or deletions of nucleotide causing gene knockouts while in case of HDR there is reconstruction of the cleaved DNA with the use of template DNA analogue to the break site sequence. However, out of the three nucleases, CRISPR/Cas9 attracted the maximum attention for developing several plant and animal products with desired genetic modifications through genome editing. Soon an alternative for Cas9 in the form of Cpf1 became available which paves the way to a superior system in the form of CRISPR/Cpf1, and has several advantages over CRISPR/Cas. ZFN/TALEN/CRISPR-mediated genome editing has been an approach that is preferred over transgenics, as no foreign gene is being introduced, and only an existing gene is altered or edited, using cell own machinery. Therefore, it has been largely debated point that products of genome editing technologies like CRISPR/Cas9 should not be subjected to the regulatory system, which is typically used in case of genetically modified organisms (GMOs). At least in some countries, this has made commercialization of genome-edited products easier. As an example, a strain of 'mushroom' with white buttons, which cannot turn brown (when stored) was developed using CRISPR and commercialized in the USA without being subjected to regulatory systems that are commonly applied to GMOs. In this, a gene PPO (polyphenol oxidase) which is responsible for browning was altered which results in reduction of PPO quantity by 30%. A mutant waxy corn that gave higher yield under drought conditions has also been developed through genome editing by DuPont, the same genome-edited waxy corn was also approved in the USA for commercial cultivation and may become available to the farmers for commercial cultivation within the next few years. CRISPR/Cas9 creates an opportunity to enhance productivity by creating genetic variability for breeding purpose, supply of disease-free and healthy planting material, improvement in different stress tolerance etc. CRISPR approach has been proved to give a number of options to provide resistance against different biotic and abiotic stresses and is used to create tolerant crops. This technique has been quite successfully used for creating resistant crop plants. Amidst all the issues raised against genetically modified crops, it is imperative to highlight the scientific principles involved so as to make full use of a technology that might solve the problem of food shortage.

CRISPR/Cas9 is a natural inspiration, one of the most powerful tools available which is derived from natural products. Originally, it is bacterial adaptive immune system used in their defence mechanism. In last few years, CRISPR/Cas9 captivated researchers globally by creating several plant and animal products with desirable genetic modifications through genome editing. The studies first recognized Cas9 as a large multifunctional protein which have two putative nuclease domains namely HNH and RuvC that are responsible for introducing DSBs into invading phages (Jinek et al. 2014) and plasmid enables in vivo targeting of temperate phages and plasmids in bacteria (Garneau et al. 2010). This defence system inserts or deletes bases, which turn DNA code into knockout situation of the targeted gene.

The popular CRISPR/Cas9 system for genome editing makes use of Cas9 having endonuclease activity for creating a double-strand break (DSB) at the target site of the target DNA strand. The target site is recognized with the help of a single-guide RNA (sgRNA), which is programmable and is designed using the target sequence that is intended to be edited. The sgRNA consists of a scaffold sequence that is of ~20 bp length and facilitates DNA binding to Cas9. CRISPRs thus have two main components such as nuclease Cas9 acting as a molecular scalpel and synthetic single-guide RNA (sgRNA). sgRNA is a complex of crRNA and trRNA in which trRNA is required for maturation of pre-crRNA to crRNA. Thus, sgRNA works in sense of a GPS system that guides the Cas9 to the exact target site of cleavage. Together, sgRNA–Cas9 complex creates site-specific double-strand break. Along with these components for genome editing PAM sequence is needed, in the absence of PAM, genome editing may not take place. The sgRNA is used to design in such a manner that it should lie upstream of a protospacer adjacent motif, 5′-NGG-3′ (PAM sequence). Cas9 and SgRNA together attach to a specific stretch of DNA bases due to complementary base pairing between one of the target strand and crRNA, endonuclease activity of Cas9 causes a cut in the double helix. Once DSB is formed the cellular repair mechanism tries to fix loss by rejoining the cut DNA ends, either by NHEJ or HDR. NHEJ competes with the preferred HDR-dependent genome editing, and creates a high frequency of indels and off-site alterations during genome editing. But HDR works poorly unless cells are dividing, which means this strategy does not function in cells such as brain and muscle cells that no longer copy themselves.

13.3 Food Security

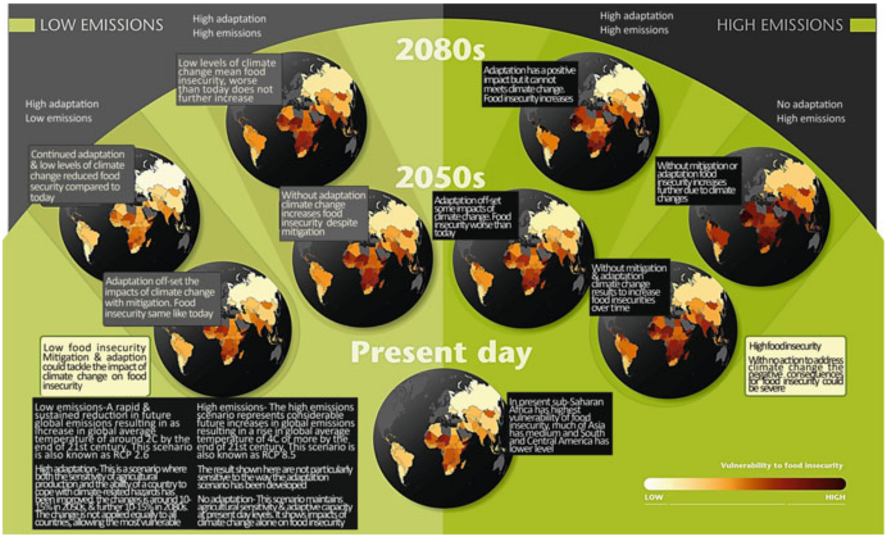
Climate change causes substantial risks to food production and global food security. There is an adverse impact on agricultural production due to extreme weather and the impact of extreme weather is likely to become more frequent in upcoming years which provides additional challenges to farmers to increase productivity for increasing population within the less available area of land. Climate change brings a Cascade of risks from physical impacts to ecosystems, agroecosystems, agriculture production, food chains, income and trade with economic and social impacts on livelihoods and food security and nutrition (FAO 2015). Food security exists when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life (World Food Summit 1996).

The above definition gives four dimensions of food security: availability of food, accessibility (economically and physically), utilization (the way it is used and assimilated by the human body) and stability of these three dimensions.

The key to increase crop productivity despite climate change is the ability to adapt to alternative climates, enabling expansion of cultivation and yield resilience using modern genome editing tools and technology. The relationship between climate-related events and vulnerability to food insecurity worldwide in developing and least-developed countries is shown in Fig. 13.1.

13.4 Engineered Crops Through Advanced Plant Breeding Approach

We know that in crop improvement programme, there are varieties of approaches used in plant breeding which has done an excellent job in the last 100 years for improvement of crop. Plant breeding arises with domestication and after that selection. In this, we were utilizing only those variable genotypes which are present in nature but to fulfil hunger of increasing population we need more variability to create



Low levels of climate change mean food insecurity, worse than today does not further increase

Continued adaptation & low levels of climate change reduced food security compared to today

Without adaptation climate change increases food insecurity despite mitigation

Adaptation off-set the impacts of climate change with mitigation. Food insecurity same like today

Low food insecurity Mitigation & adaption could tackle the impact of climate change on food insecurity

Adaptation has a positive impact but it cannot meets climate change. Food insecurity increases

Without mitigation or adaptation food insecurity increases further due to climate changes

Adaptation off-set some impacts of climate change. Food insecurity worse than today

Without mitigation & adaptation climate change results to increase food insecurities over time

High food insecurity With no action to address climate change the negative consequences for food insecurity could be severe

In present sub-Saharan Africa has highest vulnerability of food insecurity, much of Asia has medium and South and Central America has lower level

Low emissions-A rapid & sustained reduction in future global emissions resulting in as increase in global average temperature of around 2C by the end of 21st century. This scenario is also known as RCP 2.6

High emissions- The high emissions scenario represents considerable future increases in global emissions resulting in a rise in global average temperature of 4C of more by the end of 21st century. This scenario is also known as RCP 8.5

High adaptation- This is a scenario where both the sensitivity of agricultural production and the ability of a country to cope with climate-related hazards has been improved, the changes is around 10-15% in 2050s & further 10-15% in 2080s. The change is not applied equally to all countries, allowing the most vulnerable

The result shown here are not particularly sensitive to the way the adaptation scenario has been developed

No adaptation- This scenario maintains agricultural sensitivity & adaptive capacity at present day levels. It shows impacts of climate change alone on food insecurity

Fig. 13.1 The relationship between climate-related events and vulnerability to food insecurity worldwide in developing and least-developed countries. The vulnerability to food insecurity at present day is shown on the map at the bottom of the poster. Future projections are shown for a range of scenarios of different future global greenhouse gas emissions and adaptation levels. This shows that with both adaptation and mitigation, it is possible to successfully tackle the impact of climate change on future food insecurity. Source: United Nations World Food Programme 2015, Food Insecurity and Climate Change Map

a high-yielding resistant variety of crops. Then we came to hybridization breeding, in this we believe natural hybridization. We were using only those genotypes which are naturally compatible for hybridization. But that was not enough to fulfil increasing hunger. The researchers started studying artificial hybridization or wide hybridization but as we know that breeding approaches are time consuming. Then to overcome time barrier scientists moved towards mutation breeding. The results were really appreciating because a lot of variabilities we were getting using mutation breeding. But the drawback of mutation breeding method is sometimes mutation cannot be heritable or undesirable mutations can take place. Then to increase the efficiency scientists came to transgenic breeding. The beauty of transgenic breeding is that the desirable clone gene can transfer from any source to the host regardless of origin. But there are drawbacks to transgenic breeding also. Products of transgenic breeding called GMOs have to go through a lot of regulatory processes, in most countries GMOs are banned to prevent unpredictable risks to environment and food safety.

Therefore, the era of genome editing emerged massively. Genome editing is a type of engineering plants in which DNA is inserted, deleted or modified or replaced within plant genome. Spotlight came on genome editing in 2005, ZNF: Zinck Finger Nucleases. In 2010 TALEN: transcription activator-like effector nucleases and in 2013 CRISPR/Cas9 came. CRISPR/Cas9 is the most widely used genome editing tool to date. But some drawbacks are there in CRISPR/Cas9 too, to overcome these drawbacks of CRISPR the improved technology that is Base Editing has emerged in 2016.

Ethical and biosafety issues involving genetically modified organisms (GMOs) have been the subject of discussion for the last almost three decades. Consequently, GMOs have faced stringent regulations globally, thus restricting commercialization of many products that were developed. However, recently it has been argued that the products of gene editing following CRISPR technology should not be subjected to the same regulatory restrictions, which are used for GMOs. This argument has been accepted in America, so that the products of gene editing by CRISPR in America no longer require to undergo the regulations that are required for GMOs. Consequently, the issue has been examined globally, particularly in America (USA, Canada, Argentina) and Europe. In the USA, these products no longer require regulatory clearance like GMOs, but in Europe, the highest court recently decided that the gene-edited crops should be subject to the same stringent regulations that are used for GMOs. It is not surprising, since Europe has always been conservative in dealing with the subject of the release of GMOs. One would expect that the products of base editing (discussed in this chapter) would be treated like any other mutant product of conventional breeding, since only a single base is altered (as in base substitution mutants).

The cultivars with enhanced resilience to adverse environments and with increased yields and improved quality need to be generated. However, the traditional strategies used for crop breeding are laborious, time consuming and complicated, thus more effective and time-saving breeding methods are required. With the rapid progress in sequencing technologies, genomic information on an ever-increasing number of plant species is becoming available, and genome editing systems are offering the chance to edit genes with precision and creating new opportunities for crop improvement. Different strategies to generate improved crop varieties are shown in Fig. 13.2.

13.4.1 CRISPR-Mediated Genome Editing: The Evolution of Site-Specific Nucleases

Since the discovery of double-stranded DNA helix, the technologies for manipulating DNA have enabled advances in biology. Among these introducing site-specific modifications in the genomes of cells and organisms remain elusive. Recently, the site-directed Zinc Finger Nucleases (ZFNs) and TAL effector nucleases (TALENs) using the principles of DNA protein recognition were developed.

Difficulties in protein design, synthesis and validation have become a barrier to widespread adoption for routine use of these engineered nucleases. We have seen that field of biology is going through transformative phase with the arrival of facile genome engineering in animals and plants with the use of RNA-programmable CRISPR-Cas9. The CRISPR/CAS9 technology is originated from type II

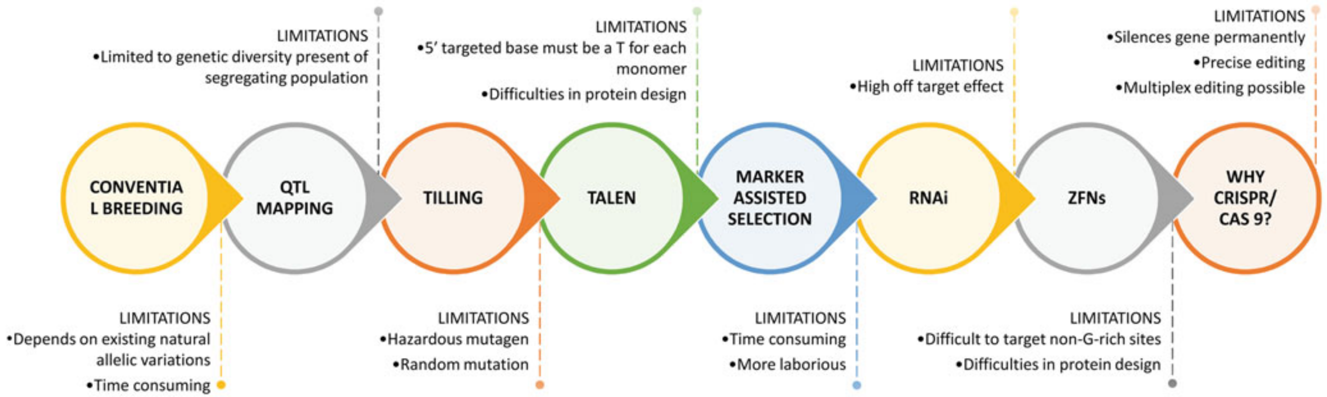


Fig. 13.2 Different strategies to generate improved crop varieties

CRISPR-Cas system, which provides an adaptive immune system to bacteria against viruses and plasmids. Cas9 is CRISPR-associated protein having endonuclease activity. Cas 9 uses a guide sequence within an RNA duplex, tracrRNA: crRNA, which forms complementary base pairing with the target DNA sequence, enabling Cas9 to introduce a double-stranded break. Single-guide RNA (sgRNA) has two critical features: one is the sequence at the 5′ end—determines the target DNA site by complementary base pairing and the second is the sequence at 3′ side which binds to Cas9 (Doudna and Charpentier 2014).

CRISPR was first described in 1987 by Japanese researchers as a series of short direct repeats interspaced with short sequences in the genome of *Escherichia coli* (Ishino et al. 1987). Later CRISPRs were detected in numerous bacteria and archaea and predictions were made on the role of CRISPRs in DNA repair or gene regulation (Makarova et al. 2002). In the year 2005, observations were made that many spacer sequences within the CRISPRs are derived from plasmid and viral origin. Along with the findings that CRISPR loci are transcribed and with the observation that Cas (CRISPR-associated) genes encode proteins having putative nuclease and helicase domains, it was proposed that CRISPR-Cas is an adaptive defence system that might use antisense RNA as memory signatures of past invasions (Makarova et al. 2006). The experiment conducted in 2007 in which the infection of lactic acid bacterium *Streptococcus thermophilus* with lytic phages provided the first evidence of CRISPR/Cas-mediated adaptive immunity (Barrangou et al. 2007). The finding of CRISPR/Cas-mediated adaptive immune system led an idea that CRISPR/Cas systems which exist naturally in cultured bacteria used in dairy industry can be utilized for immunization against phages this is the first successful application of CRISPR/Cas for biotechnological purposes (Barrangou and Horvath 2012). In the year 2008, it was noted that mature CRISPR RNAs (crRNAs) acts as guide in a complex with Cas proteins to interfere with virus proliferation in *E. coli* (Brouns et al. 2008). In the same year, the DNA targeting activity of the CRISPR/Cas system was found in *Staphylococcus epidermidis* (Marraffini and Sontheimer 2008). Functional CRISPR/Cas loci consist of a CRISPR array which are identical repeats intercalated between invader DNA targeting spacers which later encode the crRNA components and also an operon of Cas genes encoding the Cas protein components. Naturally, viruses can be matched to their bacterial or archaeal hosts by detecting CRISPR spacers (Andersson and Banfield 2008). Different studies depicted that viruses are evolving constantly to overcome CRISPR-mediated attenuation.

CRISPR/Cas immune response is usually divided into three distinct groups, i.e. Adaptation, Expression and Interference. CRISPR/CAS systems are extremely diverse and are classified into 2 classes, 6 types, and 19 subtypes (Makaroya et al. 2015). Despite this diversity, there is a common feature in all systems and that is a CRISPR locus with alternative repeats and spacer structures and a set of associated Cas genes. There are two proteins that are present almost in all CRISPR/Cas systems and they are Cas 1 and Cas 2. Cas 1 and Cas 2 are functions in the adaptation phase.

There are two CRISPR/Cas classes which are divided into three types each. Class 1 consist of type I, III and IV, and Class 2 consist of types II, V and VI. Each type of

CRISPR system has distinct architectures of the effector modules that include unique signature proteins. Each type is further divided into multiple subtypes. There is a difference in the mechanism of pre-crRNA processing in Class 1 and Class 2 CRISPR/Cas systems. In case of Class 1 system, the maturation of crRNA is catalyzed by a dedicated complex of multiple Cas proteins which was first identified in subtype I-E and designated cascade (CRISPR-associated complex for antiviral defence). Binding takes place between cascade complex and pre-crRNA and additional Cas protein, Cas 6 is recruited, which have nuclease activity and is responsible directly for processing. In case of type II systems, an external bacterial enzyme, RNAse III catalyzed the prototype of Class 2, with the help of an additional RNA species, the trans-acting CRISPR RNA (trRNA), encoded within the CRISPR/CAS locus. trRNAs were also found in subtype V-B systems, but the cleavage, in this case, remains uncharacterized. In case of types V and VI, incompletely characterized nuclease activity of the same large effector protein that is involved in target cleavage catalyzed the pre-crRNA processing (Koonin and Makarova 2019).

13.5 Strategies to Design Abiotic Stress-Tolerant Plants with CRISPR Technologies

Nowadays, CRISPR/Cas9 is used in developing abiotic stress-tolerant plants. It is possible to target the abiotic stress tolerance mechanisms by CRISPR/Cas9 system as it allows CRISPRi (CRISPR interference) and CRISPRa (CRISPR activation) of genes (Zafar et al. 2020). So, CRISPR/CAS9 can be used for activating tolerant genes as well as suppressing sensitivity genes. CRISPR multiplexing and base editing can also be implemented to design abiotic stress-tolerant plants (Fig. 13.3).

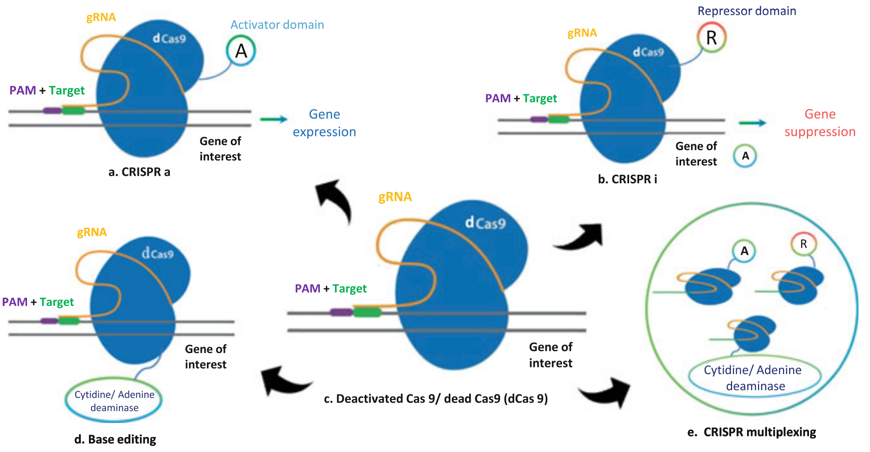


Fig. 13.3 Strategies to design abiotic stress-tolerant plants with CRISPR technologies

CRISPRi

The unique property of Cas9 to bind DNA at sites recognized by the sgRNA sequence and the PAM permits its application beyond permanent modifications of DNA. A catalytically inactivated version of Cas9 called as dCAS9 can be utilized for targeted gene regulation on a genome-wide scale. This strategy is well known as CRISPR interference (CRISPRi). CRISPRi functions by directly blocking transcription and thus causing gene silencing or suppressing the expression of a particular gene. Thus, the CRISPRi system can also be used to knockdown various gene expressions. In CRISPRi some effector domain such as KRAB/SID functions as repressor which binds to dCAS9. CRISPRi is one of the promising platform for modulating gene expression in a broad range of host cells. Presence of complex host factor is not required for CRISPRi instead of that it only depends on dCAS9 protein and guide RNAs and due to this reason, CRISPRi is flexible and highly designable. sgRNA-guided targeting is specific due to specificity dictated by its sequence identity and is not affected by the presence of other sgRNAs this enables regulation of multiple genes simultaneously by CRISPRi. The silencing by CRISPRi is very specific with no detectable off-target effects. CRISPRi can be used efficiently to suppress the susceptible gene for different abiotic stress (Qi et al. 2013).

CRISPRa

In CRISPR/Cas9-based transcriptional activation, i.e. CRISPRa the catalytically inactivated version of Cas9 called as dCAS9 is used which is genetically fused to an activator domain such as VP16/VP64. Dead Cas9 (dCas9) also called as nuclease null Cas9 fuses with an effector domain which is an activator and allows users to precisely direct a given functional activity to any random locus within the genome (Chavez et al. 2012).

Base Editing

Base editing offers precision-targeted nucleotide editing without requiring double-stranded break or donor DNA template and does not rely on HDR. Due to CRISPR repair mechanism, Liu's and co-workers in 2016 made changes in CRISPR's tool kit by modifying Cas enzyme known as base editors, they fused sgRNA with a dead Cas9 (dCas9). Now disable dead Cas9 (dCas9) is unable to cut whole double helix but still unzip at appropriate spot. Cas9 to dCas9 (Asp10Ala, His840Ala) repair mechanism is Base excision Repair (BER) that undo the change which means reverting U to the original G base due to U glycolase enzyme due to this lacuna. The disabled Cas9 with nickase activity together with cytosine/adenine deaminases for the event of four generations of cytosine base editors (BE1–BE4) for C → U conversion and a minimum of seven generations of adenine base editors (ABE1–ABE7) for A → I conversion. These base editors exhibited improved efficiency and reduced frequency of deletions among the products (Gupta 2019). The base editing technology will bring precision to gene editing technology for crop improvement. Further dCas9 is modified into nCas9, the nCas9 also known as NICKASE snips the unedited strand which gooses the cell DNA Mismatch Repair (MMR) mechanism and converts C:G to U:G to UA to T:A (Yan et al. 2018; Gupta 2019).

13.6 Heat Stress: Impact on Crop Production

Heat stress causes adverse effects on the performance of yield of the crop. High-temperature shocks at the reproductive phase can cause a drastic reduction in yield of cereals in temperate regions. There is an adverse effect on the quality of final produce like oil, starch and protein in cereals and oilseed crops due to heat stress. Reduction in grain weight and total number of grain was reported due to elevated temperatures. There is a significant decline in the yield of rice due to temperature stress as it causes a reduction in different rice growth and yield traits. Researchers have reported that tillering stage in rice is very sensitive to elevated temperature. The grain weight of rice is not affected under stress-free environment but in contrast at high night temperature leads to significant reduction in yield of rice per unit area. Drastic yield reduction due to heat stress is reported in case of common bean (*Phaseolus vulgaris* L.) and peanut (*Arachis hypogea* L.). Drastic effects of heat stress on the yield performance of tomato (*Solanum lycopersicum*) are reported as it affects meiosis, fertilization and growth of fertilized embryo (Fahad et al. 2017).

Thus, heat stress affects adversely crops and there is a significant reduction in growth and yield of several important crops. The extent of damage due to heat stress depends on crop stage and severity of stress. Basically, it is found that the reproductive stage is more sensitive to the stresses and causes a drastic reduction in the yield.

13.6.1 Plant Response to Heat Stress and Adaptive Strategies

The Global agricultural production is facing challenges and in future will have to face unescapable demand due to unpredictable environments, specially heat stress. Heat stress is among one of the major abiotic factors that affect plant growth, development and yield. Rise in temperature persistently above optimal for plant growth can induce heat stress and which results in low yield. At some threshold, the effect of heat stress may be lethal. When plants encounter heat stress, the percentage of seed germination, photosynthetic efficiency and yield declines. Under heat stress, during the reproductive growth period, the function of tapetal cells is lost and therefore the anther is dysplastic. In general, heat stress have a negative effect on crop physiology which causes decrease in rate of photosynthesis and increase in the rate of respiration and ultimately affects the plant growth and yield. Plant root system affects negatively due to heat stress which in turn causes adverse effects on nutrient and water uptake and its transfer to various plant parts leading to disrupted pollination, flowering, root development and growth stages. Seed size and quality also have negative impact due to heat stress (Janni et al. 2020).

The excessive emission of greenhouse gases leads to rise in global temperature and it is predicted to be responsible for reducing food grain yield which threats global food security. The negative impact of abiotic stresses is much severe in regions like Africa and South Asia as these regions are already experiencing food insufficiency. Therefore, making climate smart crops is a need of hour to tackle food insecurity and this can be achieved with use of advanced genome editing approach (Zafar et al. 2020).

13.6.2 Strategies for Heat Stress Management

The different molecular-biotechnological approaches are used for the development of heat stress tolerance in plants. Along with biochemical and physiological mechanisms, molecular approaches are advancing to understand the concept of heat tolerance. Plant acquired stress tolerance by modulating multiple genes and by co-ordinating the expression of gene in different pathways. In general, heat stress triggers the upregulation of several heat-inducible genes, commonly referred to as 'heat shock genes' (HSGs), these are master players in heat stress tolerance. HSGs encode HSPs and these products are significantly necessary for plant's survival under fatal high temperatures. The high temperature induces most of those proteins' constitutive expression to protect intracellular proteins from being denaturation hence, preserve their stability with high performance through protein folding, thus act as chaperones. Though plant produces HSPs in certain developmental stages the expression of these proteins is restricted such as in embryogenesis, seed germination and fruit maturation hence it can be the reason for heavy losses to plants in heat stress. Plants give response to heat stress by enhanced expression of heat-shock protein (HSP), other stress-related proteins and production of reactive oxygen species. There are various mechanisms that plant implement to cope with the heat stress some of them are maintenance of membrane stability, scavenging of ROS, production of antioxidants, accumulation and adjustment of compatible solutes, induction of mitogen-activated protein kinase (MAPK) and calcium-dependent kinase (CDPK) cascade, chaperon signalling and transcriptional activation. These are the different mechanism that enables the plants to thrive under heat stress (Wahid et al. 2007).

Thermotolerance counter to heat stress is accomplished in plants transferred with heat-shock regulatory proteins. In most of the plants, HSFs are expressed constitutively; in ordinary conditions, these HSPs proteins exist as a monomer bound to one of the HSP70 within the cytoplasm. Once the plant has sensed a heat stress, nucleus activity for tolerance starts, the HSP70 dissociates from cytoplasmic monomeric HSFs then it enters into the nucleus and forms a trimer that can bind with the HSEs. Upon binding of heat-shock factor, it recruits other transcriptional components, resulting in natural phenomenon within minutes in increased temperature. Since all HSGs contain HSE conserved sequences, overexpression of HSF gene intern turned on most HSGs and consequently provides protection against heat stress. Although this basic system is universal to eukaryotic cells, it is highly complicated in plants. Unlike animals and yeasts, which may have four or fewer HSFs, plants are shown to possess multiple copies of these genes: tomato features a minimum of 17 and Arabidopsis has 21 different HSF genes. These genes are classified into three groups (classes A, B and C), which are discriminated by features of their flexible linkers and oligomerization domains. Many of the HSFs are heat inducible, suggesting that the precise HSF involved in transcription of a selected gene may vary relying on the timing and intensity of the strain. Generally, overexpression of plant HSFs can increase plant's thermotolerance, but gene knockouts of individual HSFs tested thus far have had little effect on survival at HT. Thus, plants appear to possess a stimulating ability to finely control the expression of heat-induced genes through the HSF system. Some studies also support that there is an immediate correlation between the HSP level within the cell and respective stress tolerance.

13.6.3 Genes Associated with Heat Stress Tolerance

In light of global warming production of plants that are tolerant to heat stress is of immense importance. Plant cells show response to heat stress by the use of genetic machinery present in themselves for survival and reproduction. By altering expression of heat shock protein genes/ factors high temperature tolerance in transgenic plants had been largely achieved. Overexpression of transcription factors such as DREB2A, bZIP28 and WRKY proteins have the potential to impart heat stress tolerance (Table 13.1). Several transcription factors other than HSFs, DREBs and WRKYs have been reported their significant role in high temperature tolerance. Nuclear transcription factor X-box binding 1 gene promotes acquired high-temperature tolerance.

13.6.4 CRISPR-Mediated Approach to Enhance Heat Stress Tolerance

The CRISPR/Cas 9 have the potential to edit gene essential for the development of heat-tolerant crops. Tomato seems an ideal model for testing editing by CRISPR/Cas9 due to its ability to undergo efficient transformation for achieving quality improvements (Pan et al. 2016). CRIPSPR/Cas-mediated editing of the slagamous-like 6 (SLAGL6) gene gives parthenocarpic fruits additional heat tolerance (Klap et al. 2017). With the use of CRISPR/Cas editing tool mutation of the thermosensitive genic male-sterile maize plants had been done (Li et al. 2017).

Table 13.1 Examples of some potential heat-tolerant genes

Plant	Gene
Arabidopsis thaliana	*HSF 1, HSF 3, DREB2ACA,* Hsp70, *ATHSF1* (*HSF*), *FAD7, HSP101*
Rice (*Oryza sativa)*	*Spl 7, Athsp101*
Wheat (*Triticum aestivum*)	*TamiR159, TaGASR1*
Chilli pepper (*Capsicum annuum*)	*CabZIP63, CaWRKY40, Hsa32, MT-Shsp, ATG5, ATG7, NBR1, WRKY33*
Tomato (*Solanum lycopersicum*)	*2-CP1, 2-CP2, 2-CP1/2, ATG5, RBOH1, MPK1, MPK2, ATG7, Hsc70.1, SlLrgB*
Barley *(Hordeum vulgare*)	*APX 1*
Carrot *(Daucus carota)*	*Hsp17.7*

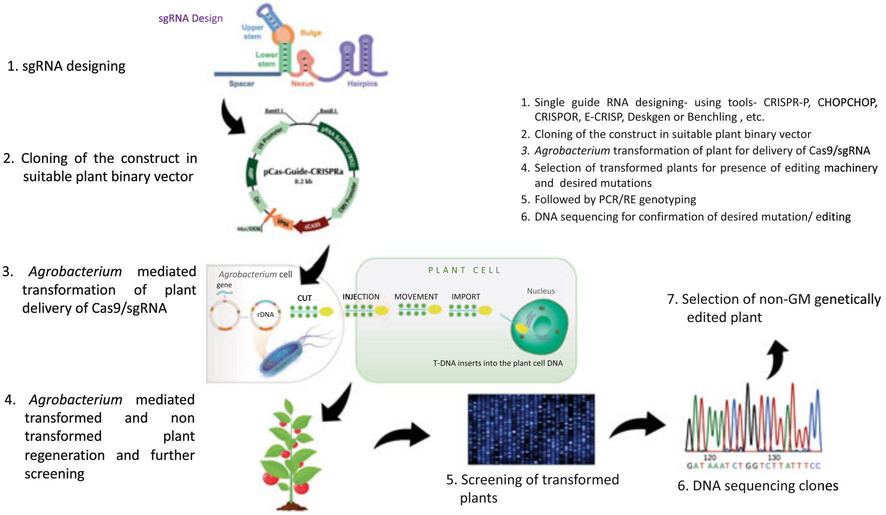


Fig. 13.4 Steps involved in plant genome editing using CRISPR/Cas9

BZR1 overexpressing and CRISPR-bzr1-mutant tomato lines depicted the involvement of BZR1 in thermo-tolerance due to regulation of the Feronia (Fer) homologs (Yin et al. 2018). Due to CRISPR-bzr 1-mutant, there is impaired production of H_2O_2 in apoplast, reduction in induction of Respiratory Burst Oxidative Homolog 1 (RBOH1), and heat tolerance, in contrast, its overexpression enhances H_2O_2 production and recovery of thermo-tolerance. The rationale steps that are involved in CRISPR are depicted in Fig. 13.4.

13.7 Limitations and Future Prospects of CRISPR

13.7.1 Limitations

In actual program of editing, it has been noticed that to get desirable product, a variety of products need to be exercised selection process vigorously, which generates only a frequency of not more than 5% of the desirable one. Being such a magical tool for genome editing CRISPR-Cas9 introduces random insertions, translocations, deletions and unwanted base to base conversions, off-target editing which is one of the major limitations associated with this tool.

Off-target Effects

Off-target effect is the most debated criticism in case of CRISPR editing tool. There is a risk of accidentally mutating non-target genes of target organism which can lead to unintended effects on ecosystem. There may be chances of activation of unwanted genes such as disease susceptibility genes due to mutation at unexpected sites. Several strategies have been adopted to reduce off-target risks from Cas9 by optimizing the sgRNA or proper design of sgRNA. Truncated sgRNA use is found effective to reduce the undesired mutations at some of the off-target sites without the sacrifice of on-target genome editing efficiencies (Ding et al. 2016). Improving specificity by minimizing the off-target effects of CRISPR-Cas system have been achieved by adopting the strategies like Cas9 nickase, Cas9n and dCas9, along with careful design and gRNA truncated at 50 ends (trugRNAs) (Osakabe et al. 2016).

13.7.2 Future Prospects

With the use of CRISPR/Cas9 technique precise editing of target gene is possible. Utilization of CRISPR/Cas 9 technology widens the scope of crop improvement for different aspects through genetic manipulation. Site-specific cleavage or site-directed mutagenesis seems to be a boon for crop improvement as it provides more specificity and efficiency. CRISPRs have potential to act on exons or coding sequences and thus can create null alleles, it can also act on regulatory sequences and ORFs which thus leads to enhanced expression. CRISPRs have potential ability to create single or multiple mutations either in homologous or non-homologous regions. Hiring these potential applications of CRISPR researchers have focused on improvement of biotic and abiotic stress tolerance. One of the other important advantages is non-transfer of transgenes to the next generation as they can be excluded with the use of process of segregation leading to the production of transgene-free plants which can be used in further study. The use of CRISPRi, CRISPRa, base editing and CRISPR multiplexing offers more and more specificity and advanced editing options and minimizes the off-target effect. Thus, the versatile technology CRISPR/Cas 9 promises to bring a new revolution in the field of plant science to alleviate abiotic and biotic stresses.

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Genomics-Enabled Breeding for Heat and Drought Stress Tolerance in Crop Plants

14

Ved Prakash Rai and Manish Kumar Vishwakarma

Abstract

Among abiotic stresses, heat and drought stresses present a considerable threat to the global food production. Cereals and grain legumes provide major dietary demands worldwide but in changing climate scenario enhancing food production for feeding evergrowing population is very challenging. Therefore, breeding cultivars with drought and heat stress tolerance traits are going to be a challenging task in the coming decades. Plants respond to heat or drought stress by triggering a cascade of molecular events and counter the stress by switching on various stress-responsive genes. However, drought and heat tolerance traits are very complex and poorly understood mechanisms and substantial G × E and epistatic effects offer major bottlenecks to the genetic gains against both stresses. Therefore, in current situation attention should be given to the rapid and precise introgression of heat and drought tolerance-related gene(s)/QTLs to the susceptible cultivars through integration of molecular breeding tools. Innovative techniques like genomic selection (GS) and genome wide association study (GWAS) are gaining importance as they capture the quantitative variation underlying abiotic stress tolerance and provide a great impetus to the progress of breeding for heat and drought tolerance.

Keywords

Tolerance · Molecular markers · QTL · Genomics · Genomic selection

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V. P. Rai
Agricultural Research Station, Navsari Agricultural University, Tanchha, Bharuch, Gujarat, India

M. K. Vishwakarma (✉)
Borlaug Institute for South Asia-CIMMYT, New Delhi, India

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14.1 Introduction

The main aim and desirable feature of any crop is yield, which is worldwide, threatened by the changing climate that apparently poses abiotic stresses (Mir et al. 2012). Abiotic stresses are reciprocally connected to each other either independently or collectively. Among abiotic stresses, heat and drought are a menace to food production globally. Rising temperatures, heatwaves, and insufficient or sudden unpredictable rainfall patterns cause significant yield losses with great risks for future global food security. The Inter-governmental Panel on Climate Change (IPCC) report warned that greenhouse gas concentrations have increased as well as air and ocean temperatures also increased that will be resulted in 2–4 °C rise in temperature at the end of twenty-first century (IPCC 2014). During crop growth period, drought stress cause approximately 30–70% loss of productivity of field crops (Kumaraswamy and Shetty 2016). Both of these factors have direct influences on plant growth and crop yields (Bita and Gerats 2013). Most of the undernourished human populations live in arid or semiarid regions that are one-third of the world's land area (Malagnoux et al. 2007), in addition to that 80% of food production comes from rainfed agriculture that grows about 60% of the world's cereal grains (Molden et al. 2011) providing livelihood for rural areas and producing food for the urban population. Drought stress is mainly caused by lack of rainfall or irrigation for a time period enough to exhaust soil moisture, and affect plant water supply that is high enough to cause injury to the plant (Blum 2005). When a plant suffers from drought stress, it shows different responses, depending on the intensity and duration of stress, physiological stage of the plant and environmental conditions (Fig. 14.1). Responses of plants against drought stress are categorized as: drought avoidance, drought

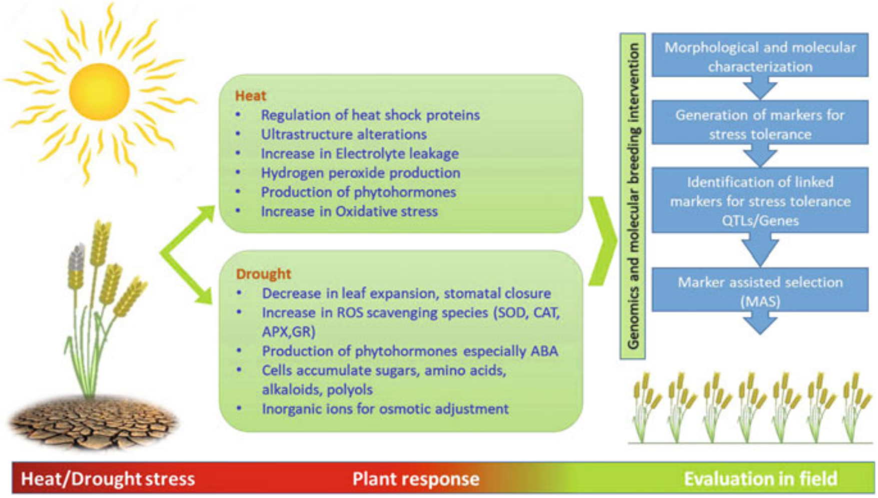

Fig. 14.1 Genomics intervention to improve drought and heat tolerance traits

escape, drought tolerance, and drought recovery, all together working as drought resistance mechanisms.

Traditionally, crop breeding has not been overtly bred for improving abiotic stress tolerance but has instead merely focused on maximizing the yield of the crop. The reasons for this include an incomplete understanding of the mechanisms of stress tolerance and genetic elements behind them, obstruction in direct selection due to low heritability, genetic interactions, viz. epistasis, genotype × environmental interactions, and polygenic effects. While the technical limitations of marker-assisted breeding are linkage drag (undesirable QTLs/genes) related to introduction of genes that help to improve stress tolerance. In recent years, technological advances and the never-ending agricultural challenges have led to the emergence of high-throughput tools to explore and exploit plant genomes for crop improvement. Compared to the last decades, breeders now have new views for plant improvement for abiotic stresses because of improvements in genomics and next generation sequencing technologies. They are equipped with new tools, such as diagnostic markers for QTLs and single genes for plant modulation that will ease the selection from phenotype to genotype. This is headed toward marker-assisted approach to integrating knowledge of the traits maintaining yield under abiotic stresses as well as gathering randomly dispersed QTLs and/or transgenes into superior genotypes. This will lead to new high-yielding varieties with improved performance in both high- and low-yielding environments is named as “breeding by design” strategy (Peleman and van der Voort 2003). In this chapter, marker-assisted breeding approaches, genomic selection, and some other promising genomics approaches for crop improvement against heat and drought stresses will be discussed.

14.2 Molecular Markers

The advent of molecular markers has speeded up crop improvement by accelerating the genetic gain and reducing the breeding cycles in many crop species (Bohra et al. 2020). The first generation of molecular markers, restriction fragment length polymorphism (RFLP; Botstein et al. (1980), random amplified polymorphic DNA (RAPD; Williams et al. 1990), and amplified fragment length polymorphism (AFLP; Vos et al. 1995) have been developed and employed mainly for diversity studies in many crop species. Whereas, second-generation markers were developed for harboring the variations in repeat sequences among individuals which mainly includes SSR markers from the genome sequence (gSSRs), expressed sequence tags (ESTs-SSRs) and bacterial artificial chromosome (BAC)-end sequences (BES-SSRs) (Litt and Luty 1989; Saxena et al. 2010; Upadhyaya et al. 2011). SSRs consist of tandem repeated nucleotides with a core sequence ranging from 1 to 6 bases. Chromosomal locations of these markers are frequently known, thus providing additional information in genetic mapping and genetic diversity studies. SSR markers have been vastly used in genetic mapping of gene(s)/QTLs for biotic and abiotic stress tolerances in many crop species (See Gupta et al. 2017; Jha et al.

2020). With the advancements in next generation sequencing (NGS) technologies, the third-generation marker systems like SNPs and InDel markers (Vishwakarma et al. 2017), which were high-throughput efficient and more cost effective came into existence. In brief, molecular breeding has witnessed a transition from the gel-based (RAPD) and hybridization-based (RFLP, DArT, SFP) to sequencing-based Allele specific, CAPS, dCAPS, and SNP markers (Pandey et al. 2017).

Recently, genotyping-by-sequencing (GBS) has become a very popular approach (Elshire et al. 2011) for characterizing the individual plants in segregating populations this can lead to the identification of haplotypes and recombination maps by identifying a significant number of SNPs in segregating populations. The identified haplotype blocks can then be employed as markers for genetic mapping of important agronomic traits and allele mining for genetic improvement of various crops (Deschamps et al. 2012). Soon, plant breeders may conduct genomic selection on a novel germplasm or species without prior knowledge of the genetic map, genome, or diversity in the species. These techniques open up new avenues for applying GBS to breeding crops for biotic and abiotic tolerances and have become an indispensable component of future biology.

14.3 Genomics-Enabled Breeding

In heat or drought prone environments genetic improvement of a crop for stress tolerance is a prerequisite and an economically feasible option (Blum 2005). For breeding heat/drought-tolerant varieties, crop species have great potential with the available genetic variability that contributes directly or indirectly toward heat/drought tolerance. Albeit, inspite of the large resources committed to heat or drought tolerance breeding, there are several reasons due to that progress to improve tolerance against these stresses is hampered. (i) Small yield differences between high- and low-yielding genotypes are main hinderance for selecting high-yielding genotypes in optimum moisture conditions as it is a basic requirement. (ii) Emphasis in crop breeding has always been given to biotic stress due to ease in evaluation in comparison to abiotic stresses, due to that achieving a broad genetic base for improving drought tolerance is difficult. (iii) Drought tolerance is a complex character that is governed by several complex factors, viz. genotype, environment, and their interaction (Carter Jr et al. 1999). Notwithstanding, with the information of genotyping data genomic-assisted selection could be performed to assist selection for rapid development of drought tolerant cultivars.

Genomics-enabled breeding has two main contents: marker-assisted selection (MAS) and genomic selection (GS). In MAS, strong association of molecular markers with target traits are prerequisite, which further assists in the selection of lines/individual plants inbreeding populations. Whereas, genomic selection (GS) uses the genome-wide marker data to predict the genetic value of a set of untested lines. In GS, genome-wide marker data and phenotypic data of a training population are used to develop selection models to select lines (untested lines) with high genome-estimated breeding values (GEBVs) in the breeding population

(Shikha et al. 2017). In the last few decades, MAS has been enormously used and played important role in breeding many crop species, while GS is recently employed in the crops, and has to do more with high precision.

14.4 Marker-Assisted Selection for Drought Tolerance

MAS is a breeding procedure where selection of lines/individual plants for a desirable trait is done with molecular markers that are linked with the QTL/gene for that particular trait (Collard and MacKill 2008). QTL mapping or association mapping is the procedure to identify marker–trait associations through correlation between phenotyping (accurate, reliable, and high-throughput trait evaluation) and genotyping with the dense molecular markers across the genome. There are several QTLs studies have been done for drought tolerance in major crops like rice (Huang et al. 2010), wheat (Kollers et al. 2013), maize (Brown et al. 2011), soybean (Hwang et al. 2014), sorghum (Morris et al. 2013), pearl millet (Bidinger et al. 2007), and foxtail millet (Jia et al. 2013). Due to strong genotype-by-environment interaction (G × E) and drought conditions, a QTL may have positive or negative additive effects (Collins et al. 2008). Nevertheless, these QTLs are not stable in different environments. Thus, QTL identification for drought tolerance has to be more realistic and could be based on the target drought scenario. In case of maize, four tolerant and susceptible recombinant inbred lines (RILs) were utilized in a microarray experiment and 22 differentially expressed genes were identified which were co-located on the genetic map with QTLs for drought tolerance (Marino et al. 2009). Eventually, these QTLs could be pyramided in elite background through marker-assisted backcrossing (MABC). In chickpea, many large effect QTLs for 13 drought tolerance associated traits, which showed phenotypic variation ranging 10–58% have been identified (Varshney et al. 2014a, b). These identified QTLs could be fine-mapped to identify drought-responsive genes. The high-density genotyping data and extensive phenotyping data enabled scientists to further narrow down candidate genomic regions associated with drought tolerance. In the genomic region containing 13 main effect QTLs for 12 drought tolerance traits in chickpea, a "QTL hotspot" region was identified, which later on introgressed successfully into the background of elite varieties using MABC (Varshney et al. 2014a, b). Further this "QTL hotspot" was splitted into "QTL-hotspot_a" and "QTL-hotspot_b" covering about ~300 Kb size genomic regions (Kale et al. 2015). In rice, mega variety IR64 was improved for drought tolerance by introgression of several QTLs through MABC (Swamy et al. 2013; Kumar et al. 2014; Henry et al. 2015). Similarly, in wheat introgression of QTLs for several drought-related traits through MABC (canopy temperature, chlorophyll content, days to anthesis, NDVI values, stay-green habit, grain yield, and its related traits) into two elite Indian wheat varieties under the Generation Challenge Programme funded by CIMMYT, Mexico, and the National Initiative on Climate Resilient Agriculture Project supported by ICAR, India (Jain et al. 2014) for details of QTLs and introgression of drought tolerance through MABC (see Kirigwi et al. 2007; Gupta et al. 2017).

In many crops, marker-aided recurrent selection (MARS) has been employed to develop many novel cultivars (Tayade et al. 2018), viz. maize, wheat, rice, soybean, sorghum, and sunflower primarily by the private industries (Thudi et al. 2014). Precision in phenotypic characterization during marker-trait discovery stage is important for success in MARS. In crops like sorghum, four stay-green (Stg) QTLs Stg1, Stg2, Stg3, and Stg4 governing canopy development, leaf anatomy, root growth, and water uptake, resulting in developmental plasticity were located on key chromosomal regions. Later on, these stable QTLs located on "B35" has been introgressed in various elite genetic backgrounds through MAS (Kamal et al. 2017). In many reports, QTLs for stay-green were found to be co-localized with QTLs for drought and temperature stress tolerances (Xu et al. 2000; Vadez et al. 2011). CGIAR also launched an initiative to improve heat/drought tolerance in wheat through the MARS approach. The Generation Challenge Programme (GCP) of CGIAR system launched a program to improve heat/drought tolerance in wheat through the MARS (CGIAR 2016) through the involvement of ICAR-IARI, New Delhi, India, Chinese Academy of Agricultural Sciences (CAAS), China and partners from Australia. ICAR-IARI, New Delhi, made an effort through MARS to combine several QTLs for stress adaptive traits such as SPAD values at vegetative and reproductive stages, early vigor, chlorophyll fluorescence, NDVI, and flag leaf area under the GCP program (Jain et al. 2014). In recent years, differential gene expression analysis of plant tissues of contrasting parental genotypes in response to a specific level of stress have become very popular to identify group of genes related to physiological processes involved in drought tolerance. Several drought-responsive genes were identified in several studies to explain molecular mechanisms of drought stress tolerance and could be validated with quantitative PCR (Schafleitner et al. 2010).

14.5 Marker-Assisted Selection for Heat Tolerance

Conventional breeding approaches based mainly on phenotypic selection have shown limited success in mitigating the effects of heat stress on crop productivity (Lamaoui et al. 2018). However, advances in the discovery of DNA markers and genotyping platforms have made it possible to accurately determine the chromosomal positions harboring QTLs for heat tolerance in several crops (Paliwal et al. 2012; Bonneau et al. 2013). Several major and minor QTLs responsible for heat tolerance have been identified in various crops such as rice, wheat, and maize (Table 14.1). More information about the various abiotic stresses related to QTLs controlling tolerance in different crops can be seen at the PLANTSTRESS site (http://www.plantstress.com/biotech/index.asp?Flag=1). In rice, two loci associated with heat tolerance were identified at chromosomes 4 and 3, through bulk segregant analysis (BSA) using a set of SSR markers in 279 F_2, that controlled 17% and 3% proportions, respectively, of the total PV (Gui-lian et al. 2009). Jagadish et al. (2010) identified eight QTLs governing spike fertility under high temperatures on different chromosomes, such as 1, 2, 3, 8, 10, and 11. A major dominant locus OsHTAS

Table 14.1 List of major and minor QTLs responsible for heat tolerance in cereals

Crop	Markers linked to the QTLs	Name/No. of the QTLs/loci	Range of PV (%)	References
Arabidopsis	SNP	5 THERM QTLS (*THERM1e, THERM3.e, THER4.1.e,* THERM4.2.e and THERM5.e)	7	Li et al. (2014a, b)
	SNP	15 QTLs (developmental stage specific)	–	Bac-Molenaar et al. (2015)
Azuki bean	–	HQTL1 and HQTL2 pollen viability under HS	–	Kaga et al. (2003), Vaughan et al. (2005), Tomooka et al. (2011)
Barley	SSR	34 putative QTLs	–	Mohammed (2004)
	SNP	6 QTLs	–	Gous et al. (2016)
	SNP	Associated with variation for PC, TKW, PC, GYPS, CCF, and TKW	–	Dawood et al. (2020)
Brassica	AFLP, RAPD	5 QTLs	–	Shuancang et al. (2003)
	SNP	5 QTLs	62.1	Branham et al. (2017)
Cowpea	SNP	*Cht-3*	11.5–18.1	Lucas et al. (2013)
Chickpea	SNP	qfpod02_5, TS qts02_5,GY qgy02_5,q% podset06_5,qvs05_6, qfpod03_6,qgy03_6, andq%podset08_6	3.92–16.56	Paul et al. (2018)
	SNP	*Hbs1, Hbs2 and Hbs3*	6.2–77.3	Pottorff et al. (2014)
	SNPs	*77 QTLs (37 major and 40 minor)*	5.88–43.49	Jha et al. (2021)
Maize	RFLP	6 QTLs (cellular membrane stability)	–	Ottaviano et al. (1991)
	RFLP	3–8 QTLs, heat-shock protein (HSP) expression		Frova and Sari-Gorla (1993)
	RFLP	5 QTLs for IPGG and six QTLs for IPTG.	–	Frova and Sari-Gorla (1994)
Potato	AFLP, SSR	QTLs resistance to internal heat necrosis (IHN)	–	McCord et al. (2011)

(continued)

Table 14.1 (continued)

Crop	Markers linked to the QTLs	Name/No. of the QTLs/loci	Range of PV (%)	References
Rice	AFLP, SSR	*qhr1, qhr3–1, qhr4–3, qhr8–1, qhr11–1* and *qhr11–2*	1.3–22.8	Cao et al. (2003)
	RFLP	3 QTLs	8.94–17.25	Zhu et al. (2005)
	–	9 QTLs thermotolerance for amylose content and gel consistency	–	Zhu et al. (2006)
	SSR	WBK–*qWK1–1*, *qWK1–2*, *qWK2* and *qWK8*	8.8–15	Tabata et al. (2007)
	SSR	2 putative QTLs associated with white-back kernels	15.2–59.6	Kobayashi et al. (2007)
	SSR	*qHt3, qHt9a*	7.6–11.4	Chen et al. (2008)
	SSR	3 QTLs (*qhts-2, qhts-3* and *qhts-5)*	6.59–10.72	Zhang et al. (2008)
	SSR	*RM3735* and *RM3586* loci	3–17	Gui-lian et al. (2009)
	SSR	*qtl_2.3,qtl_4.1, qtl_1.1,qtl_2.2, qtl_8.2,qtl_1.1, qtl_8.1, qtl_10.1, qtl_1.1,qtl_3.4, qtl_8.3,qtl_10.1, qtl_1.1,qtl_11.1, qtl_10.1,qtl_1.1, qtl_10.1 and qtl_11.1*	7–17.6	Jagadish et al. (2010)
	SSR	2 QTLs	21.3–25.8	Xiao et al. (2011)
	SSR	*qPF4, qPF6*	15.1–9.31	Ying-hui et al. (2011)
	SNP	*qHTSF1.1, qHTSF4.1*	12.6–17.6	Ye et al. (2012)
	SNP	*OsHTAS* locus	–	Wei et al. (2013)
	SSR	*qHTS1–1, qHTS1–2, qHTS2, qHTS3 and qHTS8*	6.83–14.63	Lei et al. (2013)
	SSR	*(qWB3*, *qWB4*, *qWB6* and *qWB9)* QTLs for WBK	(31.5–36.8) WBK	Kobayashi et al. (2013)
		(qKW3–1, qKW3–2, qKW6, qKW7 and *KW10)* QTLs *for KW, (qDH1, qDH3 and qDH6) QTLs for DTH*	(8.4–12.1) DTH	

(continued)

Table 14.1 (continued)

Crop	Markers linked to the QTLs	Name/No. of the QTLs/loci	Range of PV (%)	References
	SSR SNP	QTL for white-back grains	–	Shirasawa et al. (2013)
	SSR	9 QTLs	17.1–36.2	Buu et al. (2014)
	SNP	5 QTLs, *qSTIPSS9.1, qSSIPSS12.1, qSTIY5.1, qSSIY3.1, qSSIY5.1*	6.27–21.29	Shanmugavadivel et al. (2017)
	SSR	qNS1, qNS4, qNS5 and qNS6, qHTS1a, qHTS1b, qHTS3, qHTS4, qRRS1 and qRRS3, qRRS4	4.75–13.41	Li et al. (2018)
Sorghum	RFLP	*Stg 1, Stg 2, Stg 3, Stg 1, Stg 2 responsible for heat-shock protein expression*	46	Xu et al. (2000)
Tomato	AFLP	QTLs related to fruit set under HS	32.8	Grilli et al. (2007)
	RAPD and SSR	2 QTLs	–	Xiang-yang et al. (2008)
	ISSR, RAPD, and AFLP	*21 significant QTLs correlated to the heat-tolerant traits*	10.5–30.2	Lin et al. (2010)
	–	29 putative QTLs, 2 QTLs contributed to the viability of pollen under HS	–	Schafleitner (2014)
	SNPs	*qREC-1-1,qREC-1-2, qREC-1-3,qREC-2-1, qREC-3-1,qREC-9-1, qREC-12–1,qCC-1-4, qCC-1-5,qCC-2-2, qFv/Fm − 5-1, andqFv/Fm − 12–2*	1.04–16.48	Wen et al. (2019)
Wheat	SSR	1.4 genes	–	Yang et al. (2002)
	SSR	2 QTLs	11–12.	Yang et al. (2002)
	AFLP and SSR	3 QTLs	27.3–44.3	Mohammadi et al. (2008)
	SSR	Common QTL for drought and heat stress traits, 17 (yield QTL) and 28 (CT)	–	Pinto et al. (2010)
	AFLP, SSR	(Q75%Gh.ksu-2A, Q75%Gh.ksu-2A,	53 (75%Q), 28 (25%G),	Vijayalakshmi et al. (2010)

(continued)

Table 14.1 (continued)

Crop	Markers linked to the QTLs	Name/No. of the QTLs/loci	Range of PV (%)	References
		Q75%Gh.ksu- 3B) 75%G, (Q25%Gh. ksu-2A, Q25%Gh. ksu-2A) 25%G, (Q50%Gh.ksu-2A, Q50%Gh.ksu-6A) 50%G, (QMrsh.ksu-2A) (QTmrsh.ksu-2A, QTmrsh.ksu-6A, QTmrsh.ksu-6B) TMRS, (QPgmsh. ksu-3A, QPgmsh. ksu-6B) PGMS, (QFv/Fmh.ksu-7A) Fv/Fm	63 (50%G), 40 (MRS), 55 (TMRS), 36.4 (PGMS), 11.2 (Fv/Fm)	
	SSR	5 QTLs	–	Mason et al. (2010)
	SSR	3 QTLs (*QSg. bhu-1A, QSg.bhu-3B* and *QSg.bhu-7D*)	38.7	Kumar et al. (2010)
	SSR	14 QTLs for heat susceptibility index (HIS)	Individual QTL	Mason et al. (2011)
	SSR	Xgwm132-linked QTL, *Xgwm577-linked QTL* and Xgwm617-*linked QTL*	3–25.	Barakat et al. (2011)
	SSR	12 QTLs	22–64	Barakat et al. (2012)
	SSR	QHthsitgw.bhu-2B, QHthsitgw.bhu-7B, QHthsitgw.bhu- 7D, (TGW), QHthsiYLD. bhu-7B, QlsYLD. bhu-7B, (YLD), QHthsigfd.bhu-2B (GFD), QHtctd.bhu-7B (CTD), Qls-dm. bhu-7D (DM)	9.78–20.34 (TGW), 13.21 (YLD), 20.15 (GFD), 19.81 (CTD) and 7.42 (DM)	Paliwal et al. (2012)
	DArT and SSR	2 QTLs, (*Q.Yld.aww-3B-2* and *Q.Yld.aww-3D)*	22	Bennett et al. (2012)
	SSR	4 QTLs (*Qsdscon. tam-1B, Qsdsheat. tam-1D,*Qsdscon. tam-4A and Qsdssta. tam-7A)	18–30	Beecher et al. (2012)

(continued)

Table 14.1 (continued)

Crop	Markers linked to the QTLs	Name/No. of the QTLs/loci	Range of PV (%)	References
	SSR	Marker locus *gwm299*	–	Bonneau et al. (2013)
	AFLP, SSR, DArT	TKW QTLs linked or pleiotropic to DH and DM	39 (TKW)	Lopes et al. (2013)
	AFLP, SSR, DArT	Few QTLs associated with ECG	17.4 (ECG)	Lopes et al. (2013)
	AFLP, SSR, DArT	Consistent QTLs were detected for CTvg and CTgf	16 (Ctgf)	Lopes et al. (2013)
	SSR	QChlc.tamu-1B (chlorophyll content), QFlt.tamu-2B (FLTD), QIkw.tamu-5A (IKW)	–	Ali et al. (2013)
	SSR	7 QTLs associated with HSI traits, GFD, TGW, GY and CT	–	Tiwari et al. (2013)
	SSR	QTL for leaf and spike temperature depression and leaf wax	8–12	Mondal et al. (2014)
	SNP/ DArTseq	*QHst.cph-3B.1, QHst.cph-3B.2, QHst.cph-3B.3 and QHst.cph-1D*	13–35	Sharma et al. (2017)
	SSR	24 QTLs	11.2–30.60	Bhusal et al. (2017)
Lentil	SSR	*QTLs, qHt_ss and qHt_ps*	12.1 and 9.23	Singh et al. (2017)

(*Oryza sativa* heat tolerance at seedling stage) associated with high-temperature tolerance up to 48 °C, especially during seedling and grain-filling stages were identified in the genotype HT54 (Wei et al. 2013).

The near isogenic lines (NILs) developed by introgressing desirable alleles into the heat-sensitive lines showed a considerable reduction in the heat-induced injuries (Kobayashi et al. 2013). Recently, a major QTL controlling better grain quality under heat stress has been transferred from “Kokoromachi” to “Tohoku 168” using marker-assisted backcrossing (Shirasawa et al. 2013). The developed NILs had better grain quality with better heat tolerance over the susceptible parent.

MAS for complex traits like heat tolerance is not effective mainly due to the genotype × environment and epistatic interactions, which result in a low breeding efficiency (Collins et al. 2008). On the other hand, introgressed QTLs often do not translate well across different genetic backgrounds, resulting in smaller stress tolerance than expected which makes the exploitation of QTL strategy very challenging (Collins et al. 2008). In this scenario, recurrent selection seems to be one of the most efficient methods for improving quantitative traits like heat and drought, although the probability of obtaining superior genotypes that reunite all the favorable alleles is very small, especially in case of multiple crosses (Govindaraj et al. 2018). That can be overcome by using a large number of plants in a population. This approach has led to the development of heat stress-tolerant genotypes in potato with increased tuber production and quality (Benites and Pinto 2011). To mitigate the challenges posed by heat stress, "physiological" or "developmental" trait breeding through recurrent selection is being currently utilized by scientists and has been successfully used to improve heat tolerance in wheat (Gororo et al. 2002; Machado et al. 2010) and potato (Benites and Pinto 2011). The QTLs associated with high levels of heat-shock proteins (HSPs) in anthers, spikelets, and flag leaves have been identified in rice variety N22 (*aus* ssp.) that helps in the maintenance of yield under high night temperatures (Ye et al. 2012; Shi et al. 2013).

14.6 Potential of Genomic Selection for Heat and Drought Tolerances

Since the last two decennia, molecular marker technology has done a tremendous job in plant breeding programs and accelerated selection, albeit, MAS did not fulfill desirous achievements to improve quantitative traits (Bernardo 2008; Xu and Crouch 2008). Application of MAS has proven itself for incorporating large effect QTLs/genes, but it is failing when small effect QTLs are to handle (Moreau et al. 2004). Therefore for the improvement of complex inherited traits such as grain yield and abiotic stresses MAS is not the perfect choice. Last decade has witnessed that there have been enormous developments in genome sequencing technologies, especially, throughput and sequence read lengths have increased tremendously which offered increased options for de novo sequencing of new genomes, and re-sequencing genotypes within a species. New kinds of marker systems, viz. hybridization-based marker systems with thousands of markers such as Axiom array, Infinium SNP assays, InDels (Vishwakarma et al. 2017), QTLseq, and InDelseq (Pandey et al. 2017; Singh et al. 2017).

Genomic Selection (GS) is a technique which utilizes a genome-wide marker profile for the prediction of the genetic value of untested genotypes. The basic difference between GS and marker-assisted selection (MAS) is the concurrent use of whole genome marker data, in place of a few markers linked to QTL (Heffner et al. 2009). The main aim of GS is to ascertain the genetic potential of an individual rather than to identify the targeted QTL in segregating populations. The first step of GS is to develop a prediction model to predict the "genetic merit" on the basis of genotyping and phenotyping data of individuals of "training population," while in the second step genotypic data is generated from untested individuals of a "candidate population" to develop genomic estimated breeding values (GEBV, Jannink et al. 2010). These GEBVs do not render information pertain to genes but are the perfect selection measure. The great potential of GS is as it can exploit minor effects alleles to use in selection while MAS/MARS miss this feature (Cairns and Prasanna 2018). Several factors, influence the prediction accuracy of GS models, viz. relationship between the training and the breeding population, the number of generations that distinguish the training and the breeding populations, genetic marker type and marker density, the precision of the phenotyping of training population, and the heritability of the trait (Nakaya and Isobe 2012).

In recent past, GS is being employed for drought resistance breeding in maize and wheat by the international maize and wheat improvement center, CIMMYT. In maize, out of seven models, the best model was used in drought-phenotyped genotypes and compared the GS results with GWAS results (Shikha et al. 2017). In another study of GS about 7.3% higher grain yield was obtained in comparison to conventional selection under drought stress (Beyene et al. 2015). In wheat and soybean some successful examples with the more accurate prediction model can be seen (for more detail see Crossa et al. 2017). GS was originally incepted in animal breeding programs, but it has started gaining interest in crop improvement of complex quantitative traits. For drought tolerance breeding, GS has been applied to rice (Huang et al. 2019; Xu et al. 2018), wheat (Rutkoski et al. 2011; Norman et al. 2018; Belamkar et al. 2018), maize (Shikha et al. 2017; Liu et al. 2020), sugarcane (Gouy et al. 2013), chickpea (Li et al. 2018; Roorkiwal et al. 2016), pigeon pea, and groundnut (Varshney et al. 2013), white spruce (Beaulieu et al. 2014), cassava (Oliveira et al. 2012; Andrade et al. 2019), apple (Muranty et al. 2015), etc. The Diversity Arrays Technology (DArT) emerged as a promising technology to cover genome-wide marker resolution and speed, necessary for plant molecular breeding with the aid of genomics (Jaccoud et al. 2001). DArT is known for its high throughput, genome coverage, and transferability for a large number of distinct crops including rice (Jaccoud et al. 2001), barley (Wenzl et al. 2004), wheat (Akbari et al. 2006), and groundnut (Vishwakarma et al. 2016). In recent past, availability of next generation sequencing (NGS) technologies enabled researchers to integrate genotyping-by-sequencing (GBS) in genotyping large populations which is now the most accepted technique used for a genotyping for crop genomics studies based on genome complexity reduction by using restriction enzymes followed by ligation of barcode adapter, PCR amplification, and sequencing (Elshire et al. 2011). Abundant genome-wide SNP markers can be achieved through this genotyping plateform in a very short time that can be exploited in the identification of marker–trait association, and crop improvement programs based on GEBVs (Varshney et al. 2014a, b).

14.7 Challenges of Genomics-Enabled Breeding in Crops

Since the last decades NGS has become a powerful affordable, cost saving, time saving with more precision result oriented technology, enforcing more availability of bioinformatic tools to carry out genomic analyses, that fasten genetic dissection and molecular marker discovery (Bolger et al. 2014). There are several tools for analysis of genomic data and applications for marker development or genomic selection, but majority of them were deployed in diploid crops. Albeit, polyploidy crop faces many challenges due to the complexity of genome like genotyping, genome assembly and mapping of DNA sequence reads, linkage mapping, association studies, and genomic selection. Due to sequence variability between haplotypes genome assemblies are much more broken (Tang 2017). The available genotyping platforms are good for diploid crops as they easily distinguish homozygotes from heterozygotes, but that is not perfect for polyploid (Vos et al. 2015). Perfect tools for identification of allelic dosage should be available because a number of allelic options are more in polyploidy crops. During the meiotic pairing, double reduction takes place which leads to nonrandom chromosomal segregation, which leads to a number of genotype classes (Luo et al. 2004). Genotyping approach should be efficient to distinguish homologues and homologues (different subgenomes) in Allopolyploids because different ancestors have contributed to their subgenomes.

14.8 Novel Executive Tools of Genomics to Improve Drought Tolerance

New generation breeding tools including multi-parent mapping populations (nested association mapping [NAM], multi-parent advanced generation intercross [MAGIC]), and speed breeding have paved the new way to a better understanding of the improvement of drought-tolerant trait in crops (Rasheed et al. 2017). Meanwhile, an attractive gene editing technology CRISPR/Cas9 (Pennisi 2013) has been used to improve abiotic stress tolerance like drought tolerance traits (Wang et al. 2017; Shen et al. 2017; Shi et al. 2017). Heretofore, there are so many successful examples available like editing of root and nodule traits (Cai et al. 2015; Du et al. 2016), GmDrb2a and GmDrb2b genes in soybean (Curtin et al. 2018), GmFT2a gene related to photoperiod flowering pathway (Cai et al. 2018), *M. truncatula* Huaenhancer1 (MtHen1) gene in *Medicago truncatula* (Meng et al. 2017) and SPL9 gene in *Medicago sativa* (Gao et al. 2018), to improve soybean adaptability to climate change (Li et al. 2018). Recently, a new rapid generation advancement platform generated in the name of Speed breeding which is flexible and cost-efficient breeding protocols that is based on controlling photoperiod and temperature conditions during crop growth in artificial convolular and glass house condition. Availability of such a platform can accelerate the process of trait discovery and introgression many generations ahead (Hickey et al. 2019; Watson et al. 2018).

14.9 Conclusion and Future Prospects

Among all abiotic stresses drought is a complex mechanism involving multi-traits that hamper crop productivity and menace food security globally. A number of physiological, morphological, biochemical traits, involve genes and its pathways, and the QTLs/genes with minor effects made breeding for heat/drought tolerance complicated. Precision in phenotyping and high-throughput genotyping of the plant population are the center point to finding the actual allelic combinations to increase the resistance level through MAS or GS. Genomics-enabled methodologies made it possible. Heat/Drought is governed by quantitative genes or alleles, hence MAS needs to be very precise for combining small effects QTLs/genes. GS is commencing and breeding for heat/drought tolerance has been started in many crops and near future shortly it will gain center position for crop improvement. Due to high throughput sequencing, advanced marker identification, and genomic data analysis it become now possible to identify and introgress many minor alleles involved in drought tolerance. In near future, GEB approaches, MAS, and GS can be a boon for the plant breeders for improvement of heat and drought tolerance in crop plants.

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