HEAT STRESS IN FOOD GRAIN CROPS: PLANT BREEDING AND OMICS RESEARCH

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CONTENTS

PREFACE

High temperature during the growth period of most crop plants causes negative impact from crop germination, vegetative and reproductive stage to grain development, thus causing serious crop yield penalty. Exposure to high temperature can change different metabolic functions. Research on heat sensitive cultivars showed that heat stress for longer duration inhibits Rubisco activity and reproductive functions and thus produces an array of changes in plants. The insights on various mechanisms in plants for adaptation is crucial for developing resilience to high temperature.

The present book is an excellent review of recent advances in research on analyzing negative impacts of heat stress challenging crop yield and intervention of various studies to overcome the challenge of heat stress. To overcome the challenges of heat stress, the authors have elaborated on the various approaches, including conventional plant breeding, physiological trait-based breeding approach, various 'omics' based approaches covering genomics, transcriptomics, proteomics, metabolomics and ionomics. Efforts have been made to highlight the scope of emerging novel breeding schemes *viz*., genomic selection, and genome editing tools for improving genetic gain in crop plant.

The book contains chapters authored by scientists/researchers who are actively involved in improving the yield of agricultural crops by mitigating heat stress. Their contribution is enormous in presenting up-to-date information on the subject. The book will be beneficial to plant breeders, molecular biologist and plant physiologist as it gives insights into advanced breeding schemes, discovery of novel candidate gene(s)/QTLs related to heat stress tolerance and various adaptive mechanisms working at physiological and biochemical level mediating heat tolerance in plant. Thus, the information contained in this book will enrich our understanding of various pathways, genes rendering heat tolerance in plants and also helps us to develop various strategies to ensure global food security against heat stress.

We editors are thankful to our parent organization, Indian Council of Agricultural Research (ICAR), New Delhi for supporting our scientific pursuit in the form of a book "Heat Stress In Food Grain Crops: Plant Breeding and Omics Research". We are highly thankful to Dr. T. Mohapatra, Director General, ICAR and Secretary, DARE, Ministry of Agriculture and Farmers' Welfare, Government of India and Dr. T.R. Sharma, Deputy Director General (Crop Science), ICAR for their constant support and encouragement in this endeavor.

We thank our families for being patient and supportive in this long journey, without their moral support, it would not be possible. The entire team at Bentham, especially the Publishing Editor, and Production Editor who have always been cooperative to make this publication a reality. They have been very generous in accommodating even last minutes changes and deserve our genuine appreciation. We hope that this book will absolutely serve its purpose and will provide a latest and comprehensive treatise to the readers in furthering their academic and research pursuits.

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CHAPTER 1

Mitigating Heat Stress in Wheat: Integrating Omics Tools With Plant Breeding

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Abstract: Wheat crop is adapted to cooler climatic conditions and has an optimal daytime growing temperature of 15 °C during the reproductive stage. Heat stress is becoming a major constraint to wheat production as it affects every stage of the crop but the anthesis and reproductive stages are more sensitive. The situation will be aggravated due to climate change as predicted by the Intergovernmental Panel on Climate Change, for every degree rise in temperature above this optimum leads to a 6% yield reduction. Being quantitative in nature, heat stress is a complex trait and is strongly influenced by genotype x environment interaction. The new omics approaches like transcriptomics, proteomics, metabolomics and ionomics will be useful in understanding the underlying mechanism of heat tolerance. In this chapter, we will summarize the impact of heat stress on wheat production, physiological traits contributing to heat tolerance and how to integrate new omics tools such as transcriptomics, proteomics, metabolomics and ionomics with plant breeding.

Keywords: Chromosome substitution lines, Conventional plant breeding, Multi-pronged approach, Osmoprotectant molecules, Temperature stress, Transcriptomics.

INTRODUCTION

Wheat being cultivated as a major staple crop from the prehistoric times, caters to the energy requirement of the human population in India and across the globe (Sharma *et al.* 2015). Wheat improvement efforts in the form of conventional breeding aimed at yield enhancement in the past have led to significant growth in productivity and production.

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However, there is an increased demand for wheat due to changes in consumption patterns in the form of increased demand for wheat based end products such as biscuits, noodles, pasta, *etc.* According to FAO estimates, globally around 840 million tons of wheat must be produced by 2050 from the current levels. Further climate change scenarios in the form of increased heat and drought stress events would pose serious constraints for the achievement of 2050 targets (Reynolds *et al.* 2009). The global climate change in the form of elevated CO_2 concentration, warming temperatures, and changes in rainfall patterns is becoming a major threat to crop production (IPCC 2007). The increased events of temperature rise in both the ocean and on the earth until 2012 has been reported (Team *et al.* 2014). The severe and more abrupt rise in temperatures in several parts of the world led to severely reduced crop yields (Kaushal *et al.* 2016). The adverse effect of increased temperatures on plant growth mechanisms is higher especially in the arid and semi-arid regions of the world (Cooper *et al.* 2009). The vulnerability of especially heading and grain filling period in wheat to high temperature stress has been reported (Liu *et al.* 2016; Yang *et al.* 2017; Priya *et al.* 2018).

The emergence and development of automated sequencing methods started the era of omics in the form of genomics and led to the sequencing of the whole genome of *Arabidopsis thaliana* in 2000 (Initiative and others 2000). Later on, several other organisms and crop plant genomes such as rice (Goff *et al.* 2002), soybean (Schmutz *et al.* 2010), maize (Schnable *et al.* 2009) and even the most complicated polyploidy species such as wheat (Consortium and others 2018) were sequenced and made the latest omics tools amenable to crop improvement. The word “omics” formally refers to a study related to genome, proteome, or metabolome, and aims at the characterization of a large family of cellular molecules and exploring their roles, and their interactive effects in an organism. These omics approaches are mainly performed through the application of several high-throughput technologies that mainly involve qualitative and/or quantitative detection of novel or previously identified genes, transcripts, proteins, and metabolites and other molecular species through genomics, transcriptomics, proteomics, and metabolomics, respectively (Ebeed 2019). Application of various omics approaches in understanding the abiotic stress responses in general (Kole *et al.* 2015; Meena *et al.* 2017; Lamaoui *et al.* 2018; Ebeed 2019; Wani 2019), drought stress (Hasanuzzaman *et al.* 2018; Ding *et al.* 2018) and heat stress (Xu *et al.* 2011; Jacob *et al.* 2017; Salman *et al.* 2019) particularly in crop plants and their mitigation has been reported by earlier researchers. It is therefore suggested that a multi-disciplinary and multi-pronged approach integrating the conventional plant breeding with the latest omics tools will be useful in mitigating the adverse effects of heat stress on wheat production. This chapter briefly deals with the latest reports of the application of omics approaches in improving wheat tolerance for heat stress.

IMPACT OF HEAT STRESS ON WHEAT PRODUCTION

Heat Stress, Extent of Damage/Threat to Wheat Area and Mechanisms Affected

The climate predictions by the Intergovernmental Panel on Climate Change (IPCC) indicated that the mean atmospheric temperatures are expected to increase between 1.8 to 5.8°C by the end of this century (IPCC 2007). The increase in the frequency of hot days and greater variability in temperatures in the future is also predicted as an effect of climate change (Pittock *et al.* 2003; Team *et al.* 2014). Extreme temperatures directly influence crop production by specifically affecting plant growth and yield realization posing a serious threat to food production (Team *et al.* 2014). Higher temperatures are likely to affect around seven million hectares of wheat area in developing countries and around 36 million hectares in temperate wheat production countries (Reynolds 2001). Warmer temperatures resulted in an annual wheat yield reduction to the tune of 19 million tons amounting to a monetary loss of $2.6 billion was observed between 1981-2002 (Lobell and Field 2007). In India, it has been predicted that with every rise in 1°C temperature, the wheat production will be decreased by 4–6 million tonnes (Ramadas *et al.* 2019). Approximately, 3 million ha wheat area in northeastern and northwest plain zones is exposed to terminal/reproductive heat stress (Gupta *et al.* 2013). Another report by Joshi *et al.*, (2007) stated that around 13.5 million ha wheat area in India is vulnerable to heat stress. Temperatures above 34°C in northern Indian plains leading to significant yield loss was reported (Lobell *et al.* 2012).

High temperature stress when occurred at germination and early establishment stages is known to decrease germination and seedling emergence leading to abnormal seedlings, poor vigour, reduced overall growth of developing seedlings (Essemine *et al.*, 2010; Kumar *et al.*, 2011; Piramila *et al.*, 2012). Further high temperature stress is found to severely impact dry matter partitioning, reproductive organ development and reproductive processes in crop plants (Prasad *et al.* 2011). Intermittent spells of temperature above 30 °C during the reproductive stage causes high temperature stress leading to decreased seed set and low grain number (Prasad and Djanaguiraman 2014; Sehgal *et al.* 2018; Qaseem *et al.* 2019). There are reports which also indicate deterioration of grain quality parameters under high temperature stress (Britz *et al.* 2007).

Grain filling is an essential growth stage involving mobilization and transport processes involving many biochemical processes regulating the synthesis of proteins, carbohydrates and lipids and their transport into the developing grains (Awasthi *et al.* 2014; Farooq *et al.* 2017). Processes leading to grain filling and

CHAPTER 2

Genetic Enhancement of Heat Tolerance in Maize Through Conventional and Modern Strategies

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Abstract: Globally, maize is an important crop and serves as a livelihood for millions of marginal farmers across South Asia and sub-Saharan Africa. However, heat stress has become a globally prominent and growing concern for maize farmers, owing to its adverse impact on maize growth and productivity. In addition, the mean maximum temperature may increase by 2.1–2.6°C in 2050, with significant temporal and spatial variations, across South Asia. Further, the heat-stressed areas would increase to 21% from the current baseline. Heat stress is known to induce a series of morpho-physiological, anatomical and molecular changes in maize, thereby affecting growth and development, which ultimately leads to a drastic reduction in the grain yield. Regulation of osmoprotectants, detoxification of excess reactive oxygen species (ROS), expression of heat-responsive/shock genes, and change of plant phenology help in the development of heat tolerance. The molecular basis of heat stress tolerance mechanisms has been appreciably understood and updated using the innovative physiological and molecular tools. Further, functional genomics strategies resulted in the identification of genes and regulatory pathways involved in heat stress tolerance in maize. Several attempts have been made in breeding heat-tolerant maize cultivars. The availability of genomic resources, accessibility to sequence information and millions of SNP markers in maize facilitated the selection for heat tolerance at the genome-level. Genomics-assisted mapping revealed several QTL and interactions for heat stress functional adaptive traits. The new breeding approaches like doubled haploid inducers, genome editing tools and high throughput phenomics at the breeders' disposal are opening up a new era in maize breeding for development of heat resilient maize hybrids.

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Keywords: Climate Change, Functional Genomics, Genomics-Assisted Breeding, Heat, Maize, Resilience.

INTRODUCTION

Anthropogenic climate change is rapidly changing the cropping pattern, and agronomic practices developed over thousands of years due to changing abiotic and biotic stress dynamics. During this climate change era, global warming is becoming a major concern to ensure sustainable food production (Ainsworth & Ort, 2010). An increase in mean annual temperature beyond the optimum level also results in greater yield penalty in crops, which in turn affect food and feed availability (Tigchelaar, Battisti, Naylor, & Ray, 2018). The projections estimated 31-50% losses of grain yield in major food crops *viz*., wheat (Balla *et al*., 2011), rice (Peng *et al*., 2004), maize (Bassu *et al*., 2014), soybean (Schlenker & Roberts, 2009), Brassica (Angadi *et al*., 2000); sorghum (Tack, Lingenfelser, & Jagadish, 2017), and groundnut (Cooper *et al*., 2009). Globally, maize is the widely grown crop and leading the cereals in terms of production. In the developing countries of Africa, Asia and Latin America, maize is one of the major contributors towards food and nutritional security (Agrawal, Mallikarjuna, & Gupta, 2018; Mallikarjuna *et al*., 2014; Prasanna, 2012). The percent share of maize as a source of food ranges from 61% in the Mesoamerica region to 4% in South Asia (Shiferaw, Prasanna, Hellin, & Bänziger, 2011). Along with rice and wheat, maize provides at least 30% of the food calories to more than 4.5 billion people in 94 developing countries, including 900 million poor consumers (Shiferaw *et al*., 2011). In addition, maize also has a diversified usage *viz*., feed for poultry and livestock, bioenergy production and raw material for various industries (Agrawal *et al*., 2018; Cairns *et al*., 2012a; Mallikarjuna *et al*., 2014, 2015; Prasanna, 2012).

Presently, maize growing area is showing an annual growth rate of 2.7%, 3.1%, and 4.6% in Africa, Asia and Latin America, respectively (http://www.fao.org/faostat). Further, the demand for maize in the developing world will double by 2050 to meet the requirement of ~10 billion people (Mickelbart, Hasegawa, & Bailey-Serres, 2015; Rosegrant, Ringler, & Zhu, 2009). However, maize is affected by various abiotic and biotic production constraints *viz*., drought, heat, waterlogging, pests and diseases. Among the various stresses, heat is difficult to manage through cultural operations, unlike pests and diseases. Heat stress is becoming a regular phenomenon owing to global warming and changing climate. In rainfed maize, each degree spent above 30°C reduces the final yield by 1% and 1.7% per day under optimum irrigation and drought conditions, respectively (Lobell, Bänziger, Magorokosho, & Vivek, 2011). Based on the growth stage, the overall extent of heat-induced grain yield losses in maize goes up to >50% (Bassu *et al*., 2014). Recently, (Tigchelaar *et al*. 2018) predicted the maize yield reduction in response to 2°C and 4°C increase in mean temperature. Hence, to meet the maize requirement of the growing population, it is imperative to enhance the maize yield sustainably in the era of climate change and global warming. Among the various approaches available, genetic manipulation of maize could be the most sustainable and economical approach to address the adverse effects of heat stress on maize. Genetic and molecular mechanisms play a crucial role in assigning of heat tolerance and survival of plants. Therefore, the successful development of heat-tolerant maize hybrids necessitates the understanding of genetics, physiological and molecular mechanisms associated with heat tolerance. In this chapter, we have summarised the updates on the genetic and molecular basis and breeding approaches of heat tolerance in maize.

THE RESPONSES OF MAIZE TO HEAT STRESS

The sensitivity of maize to heat stress varies with crop duration, plant architecture, genotypes, and degree and intensity of heat stress. Heat stress affects the plants by inducing a cascade of morpho-physiological and molecular changes (Fahad *et al*., 2017). The important phenological and physio-molecular responses are briefly discussed under the following broad headings (Fig. **1**).

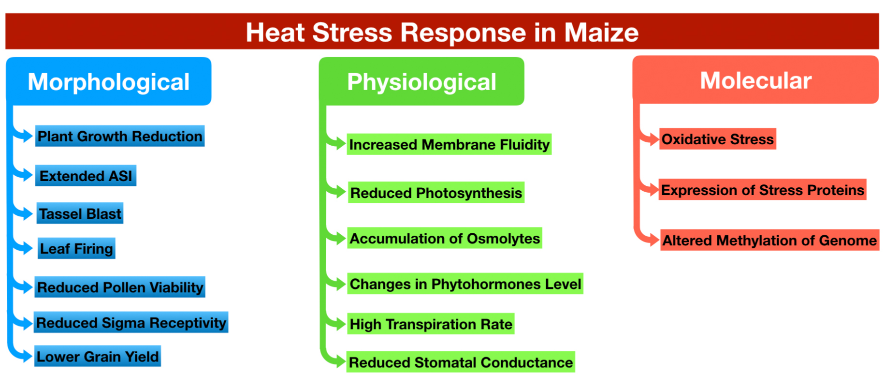

Fig. (1). Major morphological, physiological and molecular effects of heat stress on maize.

Morphological Responses

The heat stress is known to affect the various vegetative and reproductive traits, which are directly and indirectly associated with grain yield. The exposure of the maize seeds to 45°C reduced the germination percentage and shoot dry mass from 67 to 55%, and 6.13 to 4.16g, respectively (Ashraf & Hafeez, 2004). One of the

CHAPTER 3

Breeding Pearl Millet for Heat Stress Tolerance

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Abstract: Pearl millet (*Pennisetum glaucum* L. R. Br.) is an important cereal crop grown by resource poor farmers of semi-arid and arid tropics. It is grown for food in Asia, Africa and Latin America and for fodder in the USA, Australia and Brazil. Due to its inherent ability for tolerance to temperature and drought, salinity and nutrient-poor soils, it is grown in harsh environments. Owing to the changing climatic conditions, the crop holds promise for food and nutrition security for the increasing world population. However, high temperature stress is one of the main reasons for low productivity in pearl millet under semi-arid and arid environments. Further improvement for thermo tolerance is needed for the economization of agriculture.

Heat stress (HS) is a complex function of intensity, duration, and rate of increase in temperature. Tolerance mechanisms to HS are exhibited in all stages of crops such as seedling emergence, vegetative stage, flowering/ reproductive, and grain filling stages. For surviving under HS, crop plants show short-term (avoidance) and long-term (adaptation) strategies. A wide range of plant developmental and physiological processes are negatively affected by HS. Heat tolerance (HT) has been linked to increased tolerance of the photosynthetic apparatus and correlated with increased capacity of scavenging and detoxifying of reactive oxygen species (ROS). Induction of thermotolerance may be ascribed to the maintenance of a better membrane thermostability (MTS) and low ROS accumulation due to improved antioxidant capacity, osmo-regulation of solutes and synthesis of heat shock proteins (HSPs). Heat tolerance can be evaluated by a field screening, lab cum field screening or laboratory screening protocols and testing under hotspot locations. In pearl millet, the studies on heat tolerance are limited and the few studies made to date suggest seedling thermo-tolerance index (STI), seed to seedling thermo-tolerance index (SSTI) in pearl millet, and heat tolerance index (HTI) are indicative of heat tolerance. However, these are not indicative of maturity stage traits wherein membrane thermo stability holds promise. For breeding for heat tolerance, information on genetic variability, gene action (additive and non-additive), heritability, stability and correlation are available. Landraces are adapted to their native environment and could be the potential sources of HT. Gene interaction on heat tolerance showed its complex nature of inheritance. Plants are relatively more sensitive to HT during reproductive than vegetative stages. Breeders should consider and devise tools for heat tolerance screening which directly links to the productivity of a crop. Different breeding strategies such as conventional

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breeding methods, physiological trait-based breeding, molecular or transgenic approach can be applied individually or in combination for genetic improvement for heat tolerance.

Keywords: Breeding strategies, Growth stage, Gene action, Membrane thermostability, Screening, Thermo-tolerance index.

INTRODUCTION

The arid and semiarid zones constitute about 41% of the land area of the world that are characterized by unpredictable and challenging environmental conditions that are detrimental to the optimum crop production (Safriel *et al.,* 2005). For every degree centigrade increase in average growing season temperature, the crop yields are estimated to reduce up to 17% (Lobell and Asner, 2003). Due to global climatic changes, a 1-4°C on an average is expected to increase by the end of the 21st century (Driedonks *et al.,* 2016). The climate change in terms of amount and distribution of rainfall and rise in temperatures will be detrimental to crop yield. Crops that are resilient to adverse climatic conditions would play an important role in sustainable food availability to the ever-increasing world population. Pearl millet is grown in hot semi-arid areas where it is better adapted than other crops and thereby has a great potential as an excellent genomic resource for isolation of candidate genes for tolerance to drought and heat stresses. Among the millets grown in India, 75% of the total area is occupied with pearl millet. During 2017-18, the crop was grown on about 7.4 million ha with an average production of 9.13 million tons and a yield of 1237 kg/ha (Directorate of Millets Development, 2019). Though pearl millet is a climate resilient crop, owing to its cultivation in harsh conditions, the abiotic stresses such as the low and erratic distribution of rainfall, high temperatures especially during seed germination, poor soil fertility limit the crop production to subsistence level. These abiotic stresses force the crop to complete its lifecycle thereby reducing the length of the growing period (LGP) thereby resulting in a reduction in productivity (Cooper *et al.,*2009).

Heat stress tolerance is the ability of the plant to evade the negative impact of heat stress and attain economic yields near to that of normal conditions (Wahid *et al.,* 2007). Tolerance varies from species to species and between genotypes within a species. At the cellular level, high temperatures trigger certain genes and production of metabolites which enhance the plant's ability to tolerate heat stress (Hasanuzzaman *et al.,* 2013). Understanding the response of plant leaf tissues to climate change would be helpful in order to be able to predict plant performance under various stress factors. The development of heat-tolerant hybrids/varieties and the generation of improved pre-breeding materials for any breeding program is crucial in meeting food security (Ortiz *et al.,* 2008).

HEAT STRESS AT DIFFERENT PHENOLOGICAL STAGES

The effect of heat stress depends upon the phenological stage of the crop exposed to heat stress. The impact on the grain yield is more when these critical stages are exposed to heat stress. In pearl millet, the seedling stage is more vulnerable in areas where it encounters high soil temperatures during germination (Howarth *et al.,* 1997). Heat stress during flowering and seed development also affect the grain yield (Hall, 1992) especially in areas where pearl millet is grown during the summer season.

Seed Germination: Soil temperature determines both germination percentage and rate of germination. The percentage of final germination, rate of germination, seedling survival and growth increased with an increase in temperature (Pearson, 1975) and at around 42°C, the percentage and rate of germination decreased. Millet germination rate and plumule emergence had optima at 37–38°C (Ashraf and Hafeez, 2004). Seed size and density also affected seedling thermo tolerance (Gardner and Vanderlip, 1989). Small seed affects adequate plant stand and establishment of pearl millet in dry areas. Seedling emergence increased from 40% with small and low-density seed, to 62% with large and high-density seed. Germination, seedling height and proportion of vitreous starch in seed endosperm were positively related to seed density. Seedling respiration rate, on a per-seed basis, was associated positively with seed density and size (Lawan *et al.,* 1985). Days from seeding to anthesis decreased from 70 with small, low-density seed, to 62 with large, high-density seed. Seed density has a positive linear relationship with seedling emergence percentages but not with increased yield. Seed size has a major influence on seedling and plant vigour (Gardner and Vanderlip, 1989). Medium-sized millet seed showed higher germination over a wider temperature range than small or large seed. At seven days after planting, seedlings from large and medium-sized high-density seeds were taller than from small or low-density seeds (Mortlock and Vanderlip, 1989).

***Seedling Stage*:** In the arid regions, pearl millet is sown with the first onset of monsoon wherein the emerging seedling experiences very high temperatures that affect seed germination, seedling growth and development that ultimately determines the forage and grain yield. The heat stress experienced during the seedling stage affects the photosynthesis, reduces the chlorophyll content, increases the respiration rate thereby causing the death of the seedlings due to excessive transpiration of leaves magnified when accompanied with water stress (Ristic *et al.*, 2007, Cossani and Reynolds, 2012). The earlier studies on photosynthesis indicated that heat stress causes swelling of the thylakoid membrane and malfunction of photosystem II involved in the photosynthetic activity (Ristic *et al.*, 2007; Talukder *et al.,* 2014). Chlorophyll pigment present in

CHAPTER 4

Advances In Breeding For Heat Stress Tolerance In Chickpea

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Abstract: High temperature stress is one of the important abiotic stresses hindering in achieving potential yield in crop plants, particularly cool-season grain legumes. Chickpea is one of the important cool-season grain legume crops. It experiences high temperature stress at different growth stages. Prevalence of heat stress during the reproductive stage reduces the crop yield drastically. Although genetic resource is available for heat stress tolerance in chickpea, studies on inheritance and its utilization in breeding program remain very limited. Research efforts through conventional breeding have been targeted to identify the traits for indirect selection. Advancement of molecular breeding approaches has led to the identification of markers linked to traits contributing to heat stress tolerance. Despite the availability of large scale genomic resources, most of the studies were limited to identify the molecular markers linked to quantitative trait loci (QTL). The functional genomics provides better insight into the molecular pathways and functions of the genes involved in heat stress tolerance. Limited information is available on the genes and pathways of gene activation controlling effective stress resistance in chickpea. Genome-wide analysis of *Hsfs* gene family resulted in the identification of *Hsf* genes which belong to four major groups with several paralogous and orthologous genes, and are unevenly distributed across all of the eight chromosomes. The next-generation sequencing and genome-editing techniques will greatly contribute in designing abiotic stress tolerant crop plants including chickpea.

Keywords: Chickpea, Genetic variability, Genomics, Heat stress.

INTRODUCTION

Chickpea is a cool-season pulse crop, mainly cultivated in the rainfed ecology of

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the Indian subcontinent, the Mediterranean region, the West Asian and North American region, East-African region and Latin America (Rani *et al*., 2020). Change in climatic conditions and changes in cropping systems are pushing chickpea cultivation to relatively warmer growing conditions. For example, in India, there has been a major shift in the chickpea area (about 3.0 million ha) from northern India (cooler, long season environment) to southern India (warmer, short season environment) during the past four decades. The major factor contributing to this shift is the change in cropping system replacing chickpea with wheat in Northern India. The cultivation of chickpea in warmer climatic conditions is facing new biotic and abiotic stresses. Among the abiotic stresses, high temperature stress is becoming a major challenge.

Globally, India is the largest producer of chickpea, accounting for 65% (9.075 million tonnes) of the total production. Australia is the second leading country with a 14% share in chickpea production (Merga and Haji, 2019). In both countries, chickpea is exposed to high temperature stress in the growing season, mainly during the reproductive phase (Devasirvatham, 2012a). Brief exposure of plants to high temperature stress during the reproductive phase can accelerate senescence, diminish seed set and seed weight, and ultimately, reduce yield (Siddique *et al*., 1999).

The United Nations Inter Governmental Panel on Climate Change (IPCC) has projected an increase in global average temperature by 1.5 to 2.8 ºC over the next century (Jones *et al*., 1999). The Indian subcontinent and South Asian regions are believed to experience an increase in temperature of 0.5 ºC and a warming of 2-4ºC by the end of this century. The report also projects that by the end of 21st century, rainfall in India will increase by 15.40 percent, warming will be more pronounced in parts of North India and there will be an increase of 10 percent rainfall during *kharif*, while there is an uncertain prediction of rainfall and rise in temperature during *rabi*.

High temperature stress is defined as the rise in environmental temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. Base threshold temperatures vary with plant species, but for cool-season crops, 0°C is often the best-predicted base temperature (Miller *et al*., 2001).

EFFECT OF HEAT STRESS AT VARIOUS PLANT STAGES

The different developmental stages of chickpea ranging from seedling to grain filling stage are significantly affected by high temperature stress (Rani *et al*., 2020). The crop growth stage and duration of occurrence of heat stress affect the crop duration and grain yield. The prevalence of hot and dry conditions (>30ºC) results in forced maturity by reducing the crop duration (Summerfield *et al*., 1984). Physiologically this can be interpreted as a reduction in the source as well as sink size and ultimately affects the grain yield. The temperature at seed germination, seedling establishment and reproductive stage are very critical for chickpea (Table **1**).

Table 1. Optimum temperature and traits affected by heat stress during different crop stages of chickpea.

Growth Stage	Optimum Temperature	Heat Stress	Traits Affected	Reference
Germination	31°C - 33°C	>42.5°C	Membrane injury Lack of embryo growth	Ibrahim, (2011) Covell *et al*., (1986)
Vegetative growth	20°C - 27°C	>30°C	Photosynthesis, Transpiration Forced maturity	Singh and Dhaliwal, (1972) Summerfield *et al*., (1984)
Reproductive period	20°C – 26°C	>35°C	Pollen viability Stigma receptivity Number of pods per plant Seed size	Devasirvatham *et al*., (2013) Kaushal *et al*., (2013) Summerfield *et al*., (1984) Munier-Jolain and Ney, (1998)

High temperature stress at the time of sowing affects seed germination and seedling establishment. The seed germination is affected mainly due to hindrance in mobilization of cotyledon reserves required for embryo growth (Covell *et al*., 1986). Genotypic variation was observed in chickpea affecting rate of germination under various temperature (Ellis *et al*., 1986). Chickpea seed germination completely ceased when the temperature ranged between 45 to 48ºC (Singh and Dhaliwal, 1972). The seedling growth and development is affected due to reduction of photosynthetic rates and increased transpiration rates under high temperature stress, resulting in reduced plant establishment in chickpea (Singh and Dhaliwal, 1972).

The reproductive phase in chickpea is known to be very sensitive to changes in environmental conditions, and exposure to heat stress at this stage leads to a reduction in seed yield (Summerfield *et al*., 1984). The male (pollen, anthers) and female (stigma, style, ovary) reproductive parts of the flower are most sensitive to heat stress (Fig. **1**). The pollen viability, stigma receptivity and ovule viability are useful indicators of sensitivity to heat stress in chickpea. At the time of flower initiation, high temperature stress affects pollen development. The small, shrunken and empty pollen produced under high temperature stress affects their

CHAPTER 5

Genetic Improvement of Groundnut for Adaptation to Heat Stress Environments

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Abstract: Groundnut or peanut (*Arachis hypogaea* L.), an annual legume, is an important oil, food, fodder and feed crop grown in more than 100 countries. Heat and drought stress, and their combination are important abiotic constraints of groundnut production in Asia and Africa, which together accounts over 90% of global groundnut area. An increase in mean air temperature of 2-3 °C is predicted to reduce groundnut yields in India by 23-36% as heat stress during critical stages affects the pod yield. Moreover, heat stress worsens the burden of moisture stress aggravating the pod yield losses. Although groundnut genotypes continue to produce photosynthates under heat stress, only tolerant genotypes possibly have coping mechanisms to partition photosynthates to pods. Understanding the physiological, biochemical, molecular and genetic mechanism of heat-stress tolerance in groundnut is useful to devise breeding strategies to improve adaptation to heat stress. Intense phenotyping of plants grown in the field and glasshouses distinguishes sensitive and tolerant genotypes for heat stress, and to study the associated physiological and morphological differences between such genotypes. This chapter elaborates on the effects of heat stress on different life stages in groundnut, mechanisms contributing to adaptation to heat stress and recent developments in phenotyping, genetics and genomic tools to improve adaptation to heat stress.

Keywords: Climate change, Genetics, Groundnut, Heat stress, Mapping, Phenotyping.

INTRODUCTION

Climate is an important contributing factor to agriculture production and productivity. All living organisms, including microorganisms, plants, animals,

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human beings, *etc.* are exposed to different kinds of stresses. However, plants, due to their sessile nature, are more likely to be affected by abiotic and biotic stresses prevalent in their surrounding environment. Among the abiotic factors, drought, salt, cold, and heat stresses are predominant that individually or in combination can affect crops. Global climate changes have become an important concern in several agro-ecologies necessitating changes in crop and cropping preferences to sustain food security. Depending upon the geographic location, crop yields will be either increased or decreased by climate change. The climate change prediction effects for the semi-arid tropics (SAT) regions indicate a negative trend for yield, thereby threatening food security in these regions (Fischer *et al.*, 2005; Howden *et al.*, 2007). Changes in climatic factors such as temperature, rainfall, day length, light intensity, *etc.* will have a significant influence on plant growth, development and reproductive processes. All plant processes, including germination, seedling emergence, vegetative growth, floral development, pollination, *etc.* have a minimum, maximum and optimum temperature range beyond which these processes are severely impacted. The minimum/maximum temperature below/beyond which irreversible damage can occur is referred to as threshold temperature. If the temperature goes beyond the threshold by even a single degree, then the plant is said to be exposed to heat stress. Reduction in crop yields by 15-35% in Africa and Asia, and by 25-35% in the Middle East is predicted due to increase in temperature by 3-4 ^{o}C (Ortiz *et al.*, 2008).

Groundnut (*Arachis hypogaea* L.) or peanut is an economically important oilseed and food crop mainly cultivated by resource-poor small and marginal farmers in the SAT regions of Asia and Africa (Dixon *et al.*, 2001). In most of these regions, the temperatures have already reached the critical limits beyond which the growth processes and productivity of crops are likely to be adversely affected. Groundnut is mainly cultivated in these regions as a rainfed crop with minimal inputs. Nonetheless, rainfall distribution during crop production is critical to ensure higher yields. During the peg development, it is vital that sufficient moisture is present in the soil to allow the pegs to penetrate the soil. The increasing temperatures have already altered the rainfall patterns in many places and this is expected to become more erratic and extreme as the globe warms up (Kumar *et al.*, 2012). The mean temperature in future climates is expected to be 1.5-6.0 ^{o}C higher than normal due to global warming, which along with unpredictable day-to-day weather trends would further accentuate the problems of groundnut farmers in these areas (Wheeler *et al.*, 2000; Houghton *et al.*, 2001). The fourth assessment report of the Inter-Governmental Panel on Climate Change (Solomon *et al.*, 2007) has found that the increase in greenhouse gases (GHGs) caused by human anthropogenic activities has resulted in the warming of the climate system by 0.7 ^{o}C over the past 100 years; and is projected to rise about 1.8-4.0 ^{o}C by 2100. For the South Asia region, the predictions indicate temperature rise of 0.5-

1.2 ºC by 2020, 0.9-3.2 ºC by 2050 and 1.6-5.4 ºC by 2080, depending on future development scenarios.

As in other crops, heat stress can affect all groundnut growth stages, with profound effects on reproduction and seed filling stages (Hamidou *et al.*, 2013), resulting in a significant reduction in yield and/or quality in some growing regions (Akbar *et al.*, 2017). Reproductive stage vulnerability to heat stress can be due to damage to male components, resulting from developmental as well as functional disturbance, such as sucrose and starch accumulation in pollen grains (Sita *et al.*, 2017). Groundnut is geotropic in nature, *i.e.*, flowering, pollination and hybridization occur aerially, but subsequently, the peg containing the developing embryo moves down into the soil and the following processes such as the development of pods and kernels and accumulation of nutrients happen underground. Therefore, both high soil and air temperatures can impact the potential yield as well as kernel quality in susceptible groundnut genotypes. The optimum diurnal temperature requirement for photosynthesis and vegetative growth is between 30 and 35 ºC whereas it is much lower (~23 ºC) for pod and kernel yield (Cox, 1979). Reduced fruit set and subsequently reduced number of pods and kernel yield were observed when the day temperatures during the reproductive process rose above 35 ºC (Ketring, 1984; Prasad *et al.*, 1999a). Studies on the effect of soil temperature on groundnut have revealed that processes such as dry matter accumulation, flower production, the proportion of pegs forming pods, and individual seed mass are affected when the soil temperature exceeds 35 ºC (Golombek and Johansen, 1997; Prasad *et al.*, 2000a).

Under such conditions, heat-stress tolerant groundnut genotypes will be needed to counteract the high-temperature stress and sustain productivity in these environments. Screening techniques are available to identify tolerant and susceptible genotypes. Once we identify the stress tolerant genotypes, the next step is to elucidate the mechanism behind heat-stress tolerance. The mechanism by which plants counteract heat stress is a complex process involving many biochemical, molecular and physiological aspects of the plants or their interactions. Depending upon the extremity, duration of stress, plant type, growth stage and other environmental factors in the surroundings, the response mechanism of plants can vary. In this chapter, we have tried to review the effects of heat stress on important life cycle events in groundnut, screening tools that are being deployed to select tolerant genotypes, key traits that are used as a measure of tolerance during screening and elucidate the physiological, biochemical and molecular mechanism behind heat-stress tolerance/avoidance.

CHAPTER 6

Mungbean And High-Temperature Stress: Responses And Strategies To Improve Heat Tolerance

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Abstract: Considering the current scenario of global climate change, high-temperature stress is becoming a major threat limiting crop yield and productivity of crops including mungbean (*Vigna radiata* L. Wilczeck), globally. Significant yield reduction in mungbean due to high-temperature stress, especially during the reproductive stage, has been observed by various researchers. Therefore, identification of heat-tolerant mungbean lines by using different selection criteria, based on field trials evaluating various yield traits, is urgently needed. An overview of different morpho-physiological responses of mungbean under heat stress may help in formulating appropriate strategies for improving its yield potential. In addition, identification and incorporation of appropriate management strategies may enhance the productivity and sustainability of mungbean worldwide. The key findings of this chapter include the effects of heat stress on growth, reproduction and physiology of mungbean growing at different agro-climatic zones. Further, effective approaches for managing heat stress such as selection and screening of available germplasm under field trials, application of exogenous thermo-protectants and well-integrated genetic and agronomic management methods, are also discussed to improve mungbean performance under heat stress. However, the implications of the above-mentioned techniques for heat stress management require deep insight into heat tolerance mechanisms, molecular breeding, and gene characterization methods.

Keywords: Breeding, Mungbean, Heat tolerance, High temperature, Legumes.

INTRODUCTION

Rising temperatures and associated climatic disturbances are considered a serious

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threat to future agricultural output and food supply (Hanumantha Rao *et al.,* 2016). High temperature reduces agricultural yield and productivity of different crop species including grain legumes, either directly or indirectly (Basu *et al.,* 2009; Farooq *et al.,* 2018; Sita *et al.,* 2017). Currently, increased adversities of high-temperature stress on crop productivity are receiving considerable attention worldwide (Teixeira *et al.,* 2013). Previous studies have speculated that global food productivity needs to be doubled by the end of 2050 to meet the requirements of rising population and dietary shifts(Ohama *et al.,* 2017). Enhancing agricultural yields to keep pace with these increasing demands have been suggested as a favored solution to achieve this goal (Fedoroff *et al.,* 2010; Godfray *et al.,* 2010). During the current era of global warming, hot spells and warm days are likely to enhance both in intensity as well as frequency in many temperate, sub-tropical and tropical areas of the world in the coming future (Team *et al.,* 2014), which is expected to diminish the crop production. Further, in view of the complete reliance of humans on agricultural crops for meeting food demands, a deep insight regarding the sensitivity of food crops towards heat stress at different developmental stages is of principal importance (Kumar *et al.,* 2013). According to current climate model predictions, generally, the arid and semi-arid regions of the world represent the badly affected areas due to rising temperature (Vadez *et al.,* 2012; Teixeira *et al.,* 2013). Consequently, food crops, especially summer- grown crops including food legumes such as Mungbean, especially being cultivated in tropical areas, encounter frequent spells of heatwaves, coupled with soil moisture stress (Vadez *et al.,* 2012; Sita *et al.,* 2017; Farooq *et al.,* 2018). Concerning these issues, efforts are required to examine the heat sensitivity of summer crops and to develop strategies to overcome the devastating impacts of elevating temperatures. In combination with drought or other stresses, high temperature leads to world-wide extensive loss to agriculture (Mittler, 2006). Carbohydrate metabolism is also impaired due to the incongruity between photosynthesis and respiration (Ruan *et al.,* 2010). Further, intensification in heat-stress also inhibits membrane functionality and essential physiological processes, ultimately leading to cell death (Hatfield and Prueger, 2015). Aberrant metabolism due to the whole sequence of events finally ends up in the generation of reactive oxygen species (ROS) and toxic metabolites in the injured cells, which causes oxidative damage, protein denaturation, and DNA mutation(Van Breusegem *et al.,* 2001). Seed germination may also be completely inhibited depending upon plant species, intensity, and duration of the stress (Rasheed *et al.,* 2016). Heat stress also alters the stability, compartmentalization, content, and homeostasis of many molecules, especially plant growth regulators (Maestri *et al.,* 2002) Some other consequences include premature shedding of leaves, flowers, and fruits which produce unproductive tillers due to loss of entire crop cycles (Guo *et al.,* 2016).

In legumes, high temperature drastically affects various plant growth parameters (Hamada, 2001;Kumar *et al.,* 2013) such as photosynthetic efficiency (PSI and PSII), stability of thylakoid membranes (Gounaris *et al.,* 1984), electron transport channels (Srinivasan *et al.,* 1996; Sharkey, 2005), respiration (Kurets and Popov, 1998) and nitrogen fixing ability (Zahran, 1999). The adversity of constantly rising temperatures in plants is reported to be higher during the reproductive phase in comparison to the vegetative phase (Hall, 1992). Further, male reproductive structures are more vulnerable to heat than the female reproductive structures, which is evident from various heat stress studies (Dickson and Boettger 1984; Monterroso and Wien,1990; Young *et al.,* 2004). Recently, some efforts are being made in developing stress-tolerant varieties of legumes either by traditional breeding strategies or by molecular-assisted breeding methods (Varshney *et al.,* 2014; Pratap *et al.,* 2017; Mannur *et al.,* 2019). The global climatic changes are a great challenge for agriculture, especially; a progressive increase in temperature is a major concern for the crops. In temperate areas too, short and occasional increase in temperature of several degrees above the mean values reported for a certain season occur more and more often (Sgobba *et al.,* 2015). Among grain legumes, considerable yield losses have been reported in mungbean (a summer-grown legume) due to heat stress during flowering, which also affects both root and shoot growth and results in the poor crop quality (Kaur *et al.,* 2015; Sharma *et al.,* 2016). Heat stress negatively affects the pollen maturation, pollen viability, pollen germination and pollen tube growth (Sita *et al.,* 2017;Basu *et al.,* 2019). Exposure of plants to heat stress during the seed filling stage enhances senescence, decreased seed set, seed weight, and yield as reported in mungbean (Kaur *et al.,* 2015). High temperature also induces chlorosis, senescence and abscission in leaves, inhibits proliferation of roots and shoots and inhibits the yield potential (Hossain *et al.,* 2012).

GROWING CONDITIONS AND STATUS OF MUNGBEAN CULTIVATION

Mungbean (*Vigna radiata* L. Wilczek) is a valuable pulse crop in several Asian countries including India (Dahiya *et al.,* 2015; Thirumaran and Seralathan). India is one of the largest producers and consumer of mungbean contributing up to 54% of the world's production (Sehrawat *et al.,* 2013). The crop is generally grown in summer and autumn in an optimum temperature range between 27-30°C and mostly cultivated in arid and semiarid tropics at altitudes below 2000m (Singh *et al.,* 2017). The plant is an annual food legume belonging to family Fabaceae and has an indispensable role in nutrition all over the world (Pratap *et al.,* 2017). Mungbean is an economical source of plant protein ranging from 22-27% and is the main component of a balanced diet (Biswash *et al.,* 2014). Mungbean is an

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