



Induced Mutagenesis for High-Temperature Tolerance in Crop Plants 12

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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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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*, *OsCAOI* and *SIMAPK3*) 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				
<i>Triticum aestivum</i>				
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-hexfideenoic 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	<i>Small hsps</i>	Comastri et al. (2018)
Shi4185	Gamma rays	Increased kernel weight	Fu4185 (<i>QTKw.cau-5D</i>)	Cheng et al. (2015)
Chinese Spring	Gamma rays	Chromosome pairing mutant at 30 °C	<i>TaDmc1</i>	Draeger et al. (2020)
<i>Oryza sativa</i>				
Hwacheongbyeo	N-methyl-N-nitrosourea	Slower rate of chlorophyll degradation	Hwacheong-wx (<i>sgr(t)</i> 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				
<i>Triticum aestivum</i>				
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	<i>OsCAO1</i>	Jung et al. (2020)
KY131	Sodium azide	Increased thiolated tRNA level	SLG1	Xu et al. (2020)
<i>Hordeum vulgare</i>				
Delisa, Bowman	Sodium azide and <i>N</i> -methyl- <i>N</i> -nitrosourea (MNU)	Higher temperature tolerance in BR-deficient and BR-signalling mutants	522DK, BW084	Rudolphi-Szydło et al. (2020)
Other crops				
<i>Medicago truncatula</i>				
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)
<i>Solanum lycopersicum</i>				
Ailsa Craig	CRISPR/Cas9-mediated mutagenesis	Knockout of <i>SIMAPK3</i> showed higher heat tolerance	<i>SIMAPK3</i> (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 P_{max} (light-saturated net photosynthetic rate) for which parents showed a 23% reduction in P_{max} 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 Ca²⁺-ATPase and Mg²⁺-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 mutants have been found to accumulate 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 biphosphate 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 F₂ 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 (*CAOI*) 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 *OsCAOI* 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 *OsCAOI* 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 ($\geq 35^\circ\text{C}$) 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-23 α -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 *SIMAPK3* 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 *SIMAPK3* 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
<i>Oryza sativa</i>	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
<i>Agrostis</i> sp.	Pencross	Gamma rays	Springs	1983	Japan	Direct mutant	High-temperature tolerance
<i>Gossypium</i> 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
<i>Glycine max</i>	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
<i>Solanum lycopersicum</i>	MST 32/1	Gamma rays (Cs137)	Summer Star	2019	Mauritius	Direct mutant	High temperature tolerant, improved yield and processing quality
	Amalia	Gamma rays	Doni	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
<i>Lathyrus sativus</i>	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
<i>Lactuca sativa</i>	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 *SIHSBP1* 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 crop 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 *SIMAPK3*, a map kinase gene in tomato activated several pathways which included reduced contents of H_2O_2 and $O_2^{\cdot-}$ species, downregulation of *SIRBOH1* relative expression and upregulated expression of SOD, POD, APX and CAT genes and increased transcript levels of several HSPs namely SIHSP70, SIHSP90, SIHSP100 and SIHSFA1a, SIHSFA2 and SIHSFA3 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 daunting 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	<i>OST2</i>	Stomatal response for drought tolerance	Oladosu et al. (2016)
	<i>AREB1</i>	ABA signalling-mediated drought tolerance	Roca Paixão et al. (2019)
	<i>MIR169a</i>	Drought tolerance	Zhao et al. (2016)
	<i>SIHYPRP1</i>	Salinity stress	Tran et al. (2020)
	<i>SIMAPK3</i>	ABA-dependent kinase signalling for drought tolerance	Wang et al. (2017a, b)
	<i>SINPR1</i>	Drought tolerance	Li et al. (2019)
	<i>SICBF</i>	Chilling tolerance	Li et al. (2018)
Rice	<i>OsRR22</i>	Salinity stress	Zhang et al. (2019)
	<i>OsMPK5</i>	Various abiotic stresses	Xie and Yang (2013)
	<i>OsPDS, OsMPK2, OsBADH2</i>	Various abiotic stress tolerance	Shan et al. (2013)
	<i>OsDERF1, OsPMS3, OsEPSPS, OsMSH1, OsMYB5</i>	Drought tolerance	Zhang et al. (2014a, b)
	<i>OsAOX1a, OsAOX1b, OsAOX1c, OsBEL</i>	Various abiotic stress tolerance	Xu et al. (2015)
	<i>OsSAPK2</i>	ABA signalling-mediated drought tolerance	Lou et al. (2017)
	<i>OsSRL1, OsSRL2</i>	Leaf rolling for drought tolerance	Liao et al. (2019)
	<i>OsAnn3</i>	Cold tolerance	Shen et al. (2017)
	<i>OsRR22</i>	Salt tolerance	Zhang et al. (2019)
	<i>OsNAC006</i>	Heat and drought tolerance	Wang et al. (2020)
	<i>OsCAO1</i>	Natural and induced senescence	Jung et al. (2020)
Wheat	<i>TaDREB2 and TaERF3</i>	Drought resistant	Kim et al. (2018)
Maize	<i>ARGOS8</i>	Drought tolerance	Shi et al. (2017)
Tomato	<i>SIMAPK3</i>	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-induced 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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