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Review

Heat tolerance in plants: An overview

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Abstract

Heat stress due to increased temperature is an agricultural problem in many areas in the world. Transitory or constantly high temperatures cause an array of morpho-anatomical, physiological and biochemical changes in plants, which affect plant growth and development and may lead to a drastic reduction in economic yield. The adverse effects of heat stress can be mitigated by developing crop plants with improved thermotolerance using various genetic approaches. For this purpose, however, a thorough understanding of physiological responses of plants to high temperature, mechanisms of heat tolerance and possible strategies for improving crop thermotolerance is imperative. Heat stress affects plant growth throughout its ontogeny, though heat-threshold level varies considerably at different developmental stages. For instance, during seed germination, high temperature may slow down or totally inhibit germination, depending on plant species and the intensity of the stress. At later stages, high temperature may adversely affect photosynthesis, respiration, water relations and membrane stability, and also modulate levels of hormones and primary and secondary metabolites. Furthermore, throughout plant ontogeny, enhanced expression of a variety of heat shock proteins, other stress-related proteins, and production of reactive oxygen species (ROS) constitute major plant responses to heat stress. In order to cope with heat stress, plants implement various mechanisms, including maintenance of membrane stability, scavenging of ROS, production of antioxidants, accumulation and adjustment of compatible solutes, induction of mitogen-activated protein kinase (MAPK) and calcium-dependent protein kinase (CDPK) cascades, and, most importantly, chaperone signaling and transcriptional activation. All these mechanisms, which are regulated at the molecular level, enable plants to thrive under heat stress. Based on a complete understanding of such mechanisms, potential genetic strategies to improve plant heat-stress tolerance include traditional and contemporary molecular breeding protocols and transgenic approaches. While there are a few examples of plants with improved heat tolerance through the use of traditional breeding protocols, the success of genetic transformation approach has been thus far limited. The latter is due to limited knowledge and availability of genes with known effects on plant heat-stress tolerance, though these may not be insurmountable in future. In addition to genetic approaches, crop heat tolerance can be enhanced by preconditioning of plants under different environmental stresses or exogenous application of osmoprotectants such as glycinebetaine and proline. Acquiring thermotolerance is an active process by which considerable amounts of plant resources are diverted to structural and functional maintenance to escape damages caused by heat stress. Although biochemical and molecular aspects of thermotolerance in plants are relatively well understood, further studies focused on phenotypic flexibility and assimilate partitioning under heat stress and factors modulating crop heat tolerance are imperative. Such studies combined with genetic approaches to identify and map genes (or QTLs) conferring thermotolerance will not only facilitate marker-assisted breeding for heat tolerance but also pave the way for cloning and characterization of underlying genetic factors which could be useful for engineering plants with improved heat tolerance.

Keywords: Heat stress; Heat tolerance; High temperature; Thermotolerance; Stress response; Heat shock proteins; Tolerance mechanism; Molecular breeding; Transgenic plants

Contents

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1. Introduction

Heat stress is often defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. In general, a transient elevation in temperature, usually 10–15 °C above ambient, is considered heat shock or heat stress. However, heat stress is a complex function of intensity (temperature in degrees), duration, and rate of increase in temperature. The extent to which it occurs in specific climatic zones depends on the probability and period of high temperatures occurring during the day and/or the night. Heat tolerance is generally defined as the ability of the plant to grow and produce economic yield under high temperatures. However, while some researchers believe that night temperatures are major limiting factors others have argued that day and night temperatures do not affect the plant independently and that the diurnal mean temperature is a better predictor of plant response to high temperature with day temperature having a secondary role (Peet and Willits, 1998).

Heat stress due to high ambient temperatures is a serious threat to crop production worldwide (Hall, 2001). Gaseous emissions due to human activities are substantially adding to the existing concentrations of greenhouse gases, particularly CO_2, methane, chlorofluorocarbons and nitrous oxides. Different global circulation models predict that greenhouse gases will gradually increase world's average ambient temperature. According to a report of the Intergovernmental Panel on Climatic Change (IPCC), global mean temperature will rise 0.3 °C per decade (Jones et al., 1999) reaching to approximately 1 and 3 °C above the present value by years 2025 and 2100, respectively, and leading to global warming. Rising temperatures may lead to altered geographical distribution and growing season of agricultural crops by allowing the threshold temperature for the start of the season and crop maturity to reach earlier (Porter, 2005).

At very high temperatures, severe cellular injury and even cell death may occur within minutes, which could be attributed to a catastrophic collapse of cellular organization (Schöffl et al., 1999). At moderately high temperatures, injuries or death may occur only after long-term exposure. Direct injuries due to high temperatures include protein denaturation and aggregation, and increased fluidity of membrane lipids. Indirect or slower heat injuries include inactivation of enzymes in chloroplast and mitochondria, inhibition of protein synthesis, protein degradation and loss of membrane integrity (Howarth, 2005). Heat stress also affects the organization of microtubules by splitting and/or elongation of spindles, formation of microtubule asters in mitotic cells, and elongation of phragmoplast microtubules (Smertenko et al., 1997). These injuries eventually lead to starvation, inhibition of growth, reduced ion flux, production of toxic compounds and reactive oxygen species (ROS) (Schöffl et al., 1999; Howarth, 2005).

Immediately after exposure to high temperatures and perception of signals, changes occur at the molecular level altering the expression of genes and accumulation of transcripts, thereby leading to the synthesis of stress-related proteins as a stress-tolerance strategy (Iba, 2002). Expression of heat shock proteins (HSPs) is known to be an important adaptive strategy in this regard (Feder and Hoffman, 1999). The HSPs, ranging in molecular mass from about 10 to 200 kDa, have chaperone-like functions and are involved in signal transduction during heat stress (Schöffl et al., 1999). The tolerance conferred by HSPs results in improved physiological phenomena such as photosynthesis, assimilate partitioning, water and nutrient use

Table 1
Threshold high temperatures for some crop plants

Crop plants	Threshold temperature (°C)	Growth stage	References
Wheat	26	Post-anthesis	Stone and Nicolás (1994)
Corn	38	Grain filling	Thompson (1986)
Cotton	45	Reproductive	Rehman et al. (2004)
Pearl millet	35	Seedling	Ashraf and Hafeez (2004)
Tomato	30	Emergence	Camejo et al. (2005)
Brassica	29	Flowering	Morrison and Stewart (2002)
Cool season pulses	25	Flowering	Siddique et al. (1999)
Groundnut	34	Pollen production	Vara Prasad et al. (2000)
Cowpea	41	Flowering	Patel and Hall (1990)
Rice	34	Grain yield	Morita et al. (2004)

efficiency, and membrane stability (Camejo et al., 2005; Ahn and Zimmerman, 2006; Momcilovic and Ristic, 2007). Such improvements make plant growth and development possible under heat stress. However, not all plant species or genotypes within species have similar capabilities in coping with the heat stress. There exists tremendous variation within and between species, providing opportunities to improve crop heat-stress tolerance through genetic means. Some attempts to develop heat-tolerant genotypes via conventional plant breeding protocols have been successful (Ehlers and Hall, 1998; Camejo et al., 2005). Recently, however, advanced techniques of molecular breeding and genetic engineering have provided additional tools, which could be employed to develop crops with improved heat tolerance and to combat this universal environmental adversary. However, to assure achievement of success in this strategy, concerted efforts of plant physiologist, molecular biologists and crop breeders are imperative.

This review accentuates on plant responses and adaptations to heat stress at the whole plant, cellular and sub-cellular levels, tolerance mechanisms and strategies for genetic improvement of crops with heat-stress tolerance.

2. Heat-stress threshold

A threshold temperature refers to a value of daily mean temperature at which a detectable reduction in growth begins. Upper and lower developmental threshold temperatures have been determined for many plant species through controlled laboratory and field experiments. A lower developmental threshold or a base temperature is one below which plant growth and development stop. Similarly, an upper developmental threshold is the temperature above which growth and development cease. Knowledge of lower threshold temperatures is important in physiological research as well as for crop production. 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). Cool season and temperate crops often have lower threshold temperature values compared to tropical crops. Upper threshold temperatures also differ for different plant species and genotypes within species. However, determining a consistent upper threshold temperature is difficult because the plant behavior may differ depending on other environmental conditions (Miller et al., 2001). In tomato, for example, when the ambient temperature exceeds 35 °C, its seed germination, seedling and vegetative growth, flowering and fruit set, and fruit ripening are adversely affected. For other plant species, the higher threshold temperature may be lower or higher than 35 °C. Upper threshold temperatures for some major crop species are diplayed in Table 1. High temperature sensitivity is particularly important in tropical and subtropical climates as heat stress may become a major limiting factor for field crop production.

Brief exposure of plants to high temperatures during seed filling can accelerate senescence, diminish seed set and seed weight, and reduce yield (Siddique et al., 1999). This is because under such conditions plants tend to divert resources to cope with the heat stress and thus limited photosynthates would be available for reproductive development. Another effect of heat stress in many plant species is induced sterility when heat is imposed immediately before or during anthesis. Pulse legumes are particularly sensitive to heat stress at the bloom stage; only a few days of exposure to high temperatures (30–35 °C) can cause heavy yield losses through flower drop or pod abortion (Siddique et al., 1999). In general, base and upper threshold temperatures vary in plant species belonging to different habitats. Thus, it is highly desirable to appraise threshold temperatures of new cultivars to prevent damages by unfavorable temperatures during the plant ontogeny.

3. Plant responses to heat stress

3.1. Morpho-anatomical and phenological responses

3.1.1. Morphological symptoms

In tropical climates, excess of radiation and high temperatures are often the most limiting factors affecting plant growth and final crop yield. High temperatures can cause considerable pre- and post-harvest damages, including scorching of leaves and twigs, sunburns on leaves, branches and stems, leaf senescence and abscission, shoot and root growth inhibition, fruit discoloration and damage, and reduced yield (Guilioni et al., 1997; Ismail and Hall, 1999; Vollenweider and Gunthardt-Goerg, 2005). Similarly, in temperate regions, heat stress has been reported as one of the most important causes of reduction in yield and dry matter production in many crops, including maize (Giaveno and Ferrero, 2003).

High-temperature-induced modifications in plants may be direct as on existing physiological processes or indirect in altering the pattern of development. These responses may differ from one phenological stage to another. For example, long-term effects of heat stress on developing seeds may include delayed germination or loss of vigor, ultimately leading to reduced emergence and seedling establishment. Under diurnally varying temperatures, coleoptile growth in maize reduced at 40 °C and ceased at 45 °C (Weaich et al., 1996). High temperatures caused significant declines in shoot dry mass, relative growth rate and net assimilation rate in maize, pearl millet and sugarcane, though leaf expansion was minimally affected (Ashraf and Hafeez, 2004; Wahid, 2007). Major impact of high temperatures on shoot growth is a severe reduction in the first internode length resulting in premature death of plants (Hall, 1992). For example, sugarcane plants grown under high temperatures exhibited smaller internodes, increased tillering, early senescence, and reduced total biomass (Ebrahim et al., 1998).

Heat stress, singly or in combination with drought, is a common constraint during anthesis and grain filling stages in many cereal crops of temperate regions. For example, heat stress lengthened the duration of grain filling with reduction in kernel growth leading to losses in kernel density and weight by up to 7% in spring wheat (Guilioni et al., 2003). Similar reductions occurred in starch, protein and oil contents of the maize kernel (Wilhelm et al., 1999) and grain quality in other cereals under heat stress (Maestri et al., 2002). In wheat, both grain weight and grain number appeared to be sensitive to heat stress, as the number of grains per ear at maturity declined with increasing temperature (Ferris et al., 1998). In temperate and tropical lowlands, heat susceptibility is a cause of yield loss in common bean, *Phaseolus vulgaris* (Rainey and Griffiths, 2005) and groundnut, *Arachis hypogea* (Vara Prasad et al., 1999). In tomato, reproductive processes were adversely affected by high temperature, which included meiosis in both male and female organs, pollen germination and pollen tube growth, ovule viability, stigmatic and style positions, number of pollen grains retained by the stigma, fertilization and post-fertilization processes, growth of the endosperm, proembryo and fertilized embryo (for a review see (Foolad, 2005). Also, the most noticeable effect of high temperatures on reproductive processes in tomato is the production of an exserted style (i.e., stigma is elongated beyond the anther cone), which may prevent self-pollination. Poor fruit set at high temperature has also been associated with low levels of carbohydrates and growth regulators released in plant sink tissues (Kinet and Peet, 1997). Growth chamber and greenhouse studies suggest that high temperature is most deleterious when flowers are first visible and sensitivity continues for 10–15 days. Reproductive phases most sensitive to high temperature are gametogenesis (8–9 days before anthesis) and fertilization (1–3 days after anthesis) in various plants (Foolad, 2005). Both male and female gametophytes are sensitive to high temperature and response varies with genotype; however, ovules are generally less heat sensitive than pollen (Peet and Willits, 1998). Overall, based on the available studies, it seems that plant responses to high temperature vary with plant species and phenological stages. Reproductive processes are markedly affected by high temperatures in most plants, which ultimately affect fertilization and post-fertilization processes leading to reduced crop yield.

3.1.2. Anatomical changes

Although limited details are available, anatomical changes under high ambient temperatures are generally similar to those under drought stress. At the whole plant level, there is a general tendency of reduced cell size, closure of stomata and curtailed water loss, increased stomatal and trichomatous densities, and greater xylem vessels of both root and shoot (Añon et al., 2004). In grapes (*Vitis vinifera*), heat stress severely damaged the mesophyll cells and increased permeability of plasma membrane (Zhang et al., 2005). With the onset of high temperature regime, *Zygophyllum qatarense* produced polymorphic leaves and tended to reduce transpirational water loss by showing bimodal stomatal behavior (Sayed, 1996). At the sub-cellular level, major modifications occur in chloroplasts, leading to significant changes in photosynthesis. For example, high temperatures reduced photosynthesis by changing the structural organization of thylakoids (Karim et al., 1997). Studies have revealed that specific effects of high temperatures on photosynthetic membranes result in the loss of grana stacking or its swelling. In response to heat stress, chloroplasts in the mesophyll cells of grape plants became round in shape, the stroma lamellae became swollen, and the contents of vacuoles formed clumps, whilst the cristae were disrupted and mitochondria became empty (Zhang et al., 2005). Such changes result in the formation of antenna-depleted photosystem-II (PSII) and hence reduced photosynthetic and respiratory activities (Zhang et al., 2005). In general, it is evident that high temperature considerably affects anatomical structures not only at the tissue and cellular levels but also at the sub-cellular level. The cumulative effects of all these changes under high temperature stress may result in poor plant growth and productivity.

3.1.3. Phenological changes

Observation of changes in plant phenology in response to heat stress can reveal a better understanding of interactions between stress atmosphere and the plant. Different phenological stages differ in their sensitivity to high temperature; however, this depends on species and genotype as there are great inter- and intra-specific variations (Wollenweber et al., 2003; Howarth, 2005). Heat stress is a major factor affecting the rate of plant development, which may be increasing to a certain limit and decreasing afterwards (Hall, 1992; Marcum, 1998; Howarth, 2005).

The developmental stage at which the plant is exposed to the stress may determine the severity of possible damages experienced by the crop. It is, however, unknown whether damaging effects of heat episodes occurring at different developmental stages are cumulative (Wollenweber et al., 2003). Vulnerability of species and cultivars to high temperatures may vary with the stage of plant development, but all vegetative and reproductive stages are affected by heat stress to some extent. During vegetative stage, for example, high day temperature can damage leaf gas exchange properties. During reproduction, a short period of heat stress can cause significant increases in floral buds and

opened flowers abortion; however, there are great variations in sensitivity within and among plant species (Guilioni et al., 1997; Young et al., 2004). Impairment of pollen and anther development by elevated temperatures is another important factor contributing to decreased fruit set in many crops at moderate-to-high temperatures (Peet et al., 1998; Sato et al., 2006). The staple cereal crops can tolerate only narrow temperature ranges, which if exceeded during the flowering phase can damage fertilization and seed production, resulting in reduced yield (Porter, 2005). Under high temperature conditions, earlier heading is advantageous in the retention of more green leaves at anthesis, leading to a smaller reduction in yield (Tewolde et al., 2006). Furthermore, high temperatures during grain filling can modify flour and bread quality and other physico-chemical properties of grain crops such as wheat (Perrotta et al., 1998), including changes in protein content of the flour (Wardlaw et al., 2002). Thus, for crop production under high temperatures, it is important to know the developmental stages and plant processes that are most sensitive to heat stress, as well as whether high day or high night temperatures are more injurious. Such insights are important in determining heat-tolerance potential of crop plants.

3.2. Physiological responses

3.2.1. Waters relations

Plant water status is the most important variable under changing ambient temperatures (Mazorra et al., 2002). In general, plants tend to maintain stable tissue water status regardless of temperature when moisture is ample; however, high temperatures severely impair this tendency when water is limiting (Machado and Paulsen, 2001). Under field conditions, high temperature stress is frequently associated with reduced water availability (Simoes-Araujo et al., 2003). In *Lotus creticus*, for example, elevated night temperatures caused a greater reduction in leaf water potential of water-stressed as compared to non-stressed plants (Añon et al., 2004). In sugarcane, leaf water potential and its components were changed upon exposure to heat stress even though the soil water supply and relative humidity conditions were optimal, implying an effect of heat stress on root hydraulic conductance (Wahid and Close, 2007). Similarly, in tomato heat stress perturbed the leaf water relations and root hydraulic conductivity (Morales et al., 2003). In general, during daytime enhanced transpiration induces water deficiency in plants, causing a decrease in water potential and leading to perturbation of many physiological processes (Tsukaguchi et al., 2003). High temperatures seem to cause water loss in plants more during daytime than nighttime.

3.2.2. Accumulation of compatible osmolytes

A key adaptive mechanism in many plants grown under abiotic stresses, including salinity, water deficit and extreme temperatures, is accumulation of certain organic compounds of low molecular mass, generally referred to as compatible osmolytes (Hare et al., 1998; Sakamoto and Murata, 2002). Under stress, different plant species may accumulate a variety of osmolytes such as sugars and sugar alcohols (polyols), proline, tertiary and quaternary ammonium compounds, and tertiary sulphonium compounds (Sairam and Tyagi, 2004). Accumulation of such solutes may contribute to enhanced stress tolerance of plants, as briefly described in below.

Glycinebetaine (GB), an amphoteric quaternary amine, plays an important role as a compatible solute in plants under various stresses, such as salinity or high temperature (Sakamoto and Murata, 2002). Capacity to synthesize GB under stress conditions differs from species to species (Ashraf and Foolad, 2007). For example, high level of GB accumulation was reported in maize (Quan et al., 2004) and sugarcane (Wahid and Close, 2007) due to desiccating conditions of water deficit or high temperature. In contrast, plant species such as rice (*Oryza sativa*), mustard (*Brassica* spp.), Arabidopsis (*Arabidopsis thaliana*) and tobacco (*Nicotiana tabacum*) naturally do not produce GB under stress conditions. However, genetic engineering has allowed the introduction of GB-biosynthetic pathways into GB-deficit species (Sakamoto and Murata, 2002; Quan et al., 2004).

Like GB, proline is also known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Kavi Kishore et al., 2005). In assessing the functional significance of accumulation of compatible solutes, it is suggested that proline or GB synthesis may buffer cellular redox potential under heat and other environmental stresses (Wahid and Close, 2007). Similarly, accumulation of soluble sugars under heat stress has been reported in sugarcane, which entails great implications for heat tolerance (Wahid and Close, 2007). Under high temperatures, fruit set in tomato plants failed due to the disruption of sugar metabolism and proline transport during the narrow window of male reproductive development (Sato et al., 2006). Hexose sensing in transgenic plants engineered to produce trehalose, fructans or mannitol may be an important contributory factor to the stress-tolerant phenotypes (Hare et al., 1998).

Among other osmolytes, γ-4-aminobutyric acid (GABA), a non-protein amino acid, is widely distributed throughout the biological world to act as a compatible solute. GABA is synthesized from the glutamic acid by a single step reaction catalyzed by glutamate decarboxylase (GAD). An acidic pH activates GAD, a key enzyme in the biosynthesis of GABA. Episodes of high tempeartures increase the cytosolic level of Ca, which leads to calmodulin-mediated activation of GAD (Taiz and Zeiger, 2006). Several other studies show that various environmental stresses increase GABA accumulation through metabolic or mechanical disruptions, thus leading to cytosolic acidification. Kinetics of GABA in plants show a stress-specific pattern of accumulation, which is consistent with its physiological role in the mitigation of stress effects. Rapid accumulation of GABA in stressed tissues may provide a critical link in the chain of events stemming from perception of environmental stresses to timely physiological responses (Kinnersley and Turano, 2000).

In summary, because of significant roles of osmolytes in response to environmenal stresses in plants, crop stress (e.g., heat) tolerance might be enhanced by increased accumulation of compatible solutes through traditional plant breeding, marker-assisted selection (MAS) or genetic engineering (GE) approaches (for a review see (Ashraf and Foolad, 2007).

3.2.3. Photosynthesis

Alterations in various photosynthetic attributes under heat stress are good indicators of thermotolerance of the plant as they show correlations with growth. Any constraint in photosynthesis can limit plant growth at high temperatures. Photochemical reactions in thylakoid lamellae and carbon metabolism in the stroma of chloroplast have been suggested as the primary sites of injury at high temperatures (Wise et al., 2004). Chlorophyll fluorescence, the ratio of variable fluorescence to maximum fluorescence (F_v/F_m), and the base fluorescence (F_0) are physiological parameters that have been shown to correlate with heat tolerance (Yamada et al., 1996). Increasing leaf temperatures and photosynthetic photon flux density influence thermotolerance adjustments of PSII, indicating their potential to optimise photosynthesis under varying environmental conditions as long as the upper thermal limits do not exceed (Salvucci and Crafts-Brandner, 2004b; Marchand et al., 2005). In tomato genotypes differing in their capacity for thermotolerance as well as in sugarcane, an increased chlorophyll *a*:*b* ratio and a decreased chlorophyll:carotenoids ratio were observed in the tolerant genotypes under high temperatures, indicating that these changes were related to thermotolerance of tomato (Camejo et al., 2005; Wahid and Ghazanfar, 2006). Furthermore, under high temperatures, degradation of chlorophyll *a* and *b* was more pronounced in developed compared to developing leaves (Karim et al., 1997, 1999). Such effects on chlorophyll or photosynthetic apparatus were suggested to be associated with the production of active oxygen species (Camejo et al., 2006; Guo et al., 2006).

PSII is highly thermolabile, and its activity is greatly reduced or even partially stopped under high temperatures (Bukhov et al., 1999; Camejo et al., 2005), which may be due to the properties of thylakoid membranes where PSII is located (Mcdonald and Paulsen, 1997). Heat stress may lead to the dissociation of oxygen evolving complex (OEC), resulting in an imbalance between the electron flow from OEC toward the acceptor side of PSII in the direction of PSI reaction center (Fig. 1) (De Ronde et al., 2004). Heat stress causes dissociation of a manganese (Mn)-stabilizing 33-kDa protein at PSII reaction center complex followed by the release of Mn atoms (Yamane et al., 1998). Heat stress may also impair other parts of the reaction center, e.g., the D1 and/or the D2 proteins (De Las Rivas and Barber, 1997). In wheat, high temperatures and excessive light damaged different sites of PSII, which implied different pathways for the recovery of its functional activity (Sharkova, 2001). In barley, heat pulses abruptly damaged the PSII units and caused loss of their capacity of oxygen evolution leading to a restricted electron transport, which was totally abolished after four hours (Toth et al., 2005). This implied that the degradation of the impaired PSII units occurred in the light during this period of time. Following this, *de novo* synthesis of PSII units in the light gave a gradual rise to the observed PSII activities. These effects can result from different events, including inhibition of electron transport activity and limited generation of reducing powers for metabolic functions (Allakhverdieva et al., 2001). In field-grown Pima cotton under high temperatures, leaf photosynthesis was functionally limited by photosynthetic electron transport and ribulose-1,5-bisphosphate (RuBP) regeneration capacity, but not rubisco activity (Wise et al., 2004). On the other hand, under high temperatures, PSI stromal enzymes and chloroplast envelops are thermostable and in fact PSI driven cyclic electron pathway, capable of contributing to thylakoid proton gradient, is activated (Bukhov et al., 1999).

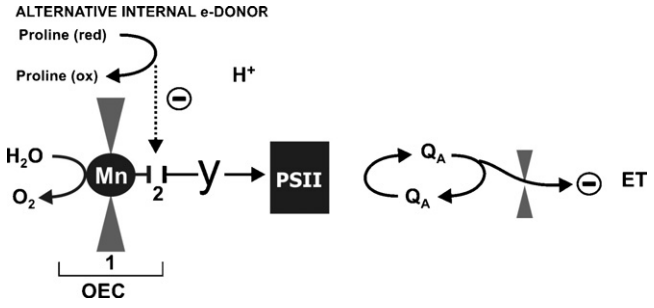

Fig. 1. Heat induced inhibition of oxygen evolution and PSII activity. Heat stress leads to either (1) dissociation or (2) inhibition of the oxygen evolving complexes (OEC). This enables an alternative internal e^--donor such as proline instead of H_2O to donate electrons to PSII. Reproduced with permission from De Ronde et al. (2004).

High temperature influences the photosynthetic capacity of C_3 plants more strongly than in C_4 plants. It alters the energy distribution and changes the activities of carbon metabolism enzymes, particularly the rubisco, thereby altering the rate of RuBP regeneration by the disruption of electron transport and inactivation of the oxygen evolving enzymes of PSII (Salvucci and Crafts-Brandner, 2004b). Heat shock reduces the amount of photosynthetic pigments (Todorov et al., 2003), soluble proteins, rubisco binding proteins (RBP) and large- (LS) and small-subunits (SS) of rubisco in darkness but increases them in light, indicating their roles as chaperones and HSPs (Kepova et al., 2005). Moreover, under heat stress, starch or sucrose synthesis is greatly influenced as observed from reduced activities of sucrose phosphate synthase (Chaitanya et al., 2001), ADP-glucose pyrophosphorylase and invertase (Vu et al., 2001).

In any plant species, the ability to sustain leaf gas exchange under heat stress has a direct relationship with heat tolerance. During the vegetative stage, high day temperature can cause damage to compensated leaf photosynthesis, reducing CO_2 assimilation rates (Hall, 1992). Increased temperatures curtail photosynthesis and increase CO_2 transfer conductance between intercellular spaces and carboxylation sites. Stomatal conductance (gs) and net photosynthesis (Pn) are inhibited by moderate heat stress in many plant species due to decreases in the activation state of rubisco (Crafts-Brander and Salvucci, 2002; Morales et al., 2003). Although with an increase in temperature rubisco catalytic activity increases, a low affinity of the enzyme for CO_2 and its dual nature as an oxygenase limits the possible increases in Pn. For example, in maize the Pn was inhibited at leaf temperatures above 38 °C and inhibition was much more severe when temperature was increased abruptly rather than gradually. However, this inhibition was independent of stomatal response to high temperature (Crafts-Brander and Salvucci, 2002). Despite observed negative effects of high temperature, the optimum temperature for leaf photosynthesis is likely to increase with elevated levels of atmospheric CO_2. Several studies have concluded that CO_2-induced increases in crop yields are much more plausi-

ble in warm- than in cool-season crops. Thus, despite its other potential negative implications, global warming may not greatly affect the overall Pn.

A well-known consequence of elevated temperature in plants is the damage caused by heat-induced imbalance in photosynthesis and respiration; in general the rate of photosynthesis decreases while dark- and photo-respiration rates increase considerably under high temperatures. Also, rate of biochemical reactions decreases and enzyme inactivation and denaturation take place as the temperature increases leading to severely reduced photosynthesis (Nakamoto and Hiyama, 1999). However, the magnitude of such alterations in response to heat stress differs with species and genotypes (Mcdonald and Paulsen, 1997). Furthermore, it has been determined that the photosynthetic CO_2 assimilation rate is less affected by heat stress in developing leaves than in completely developed leaves. Heat stress normally decreases the duration of developmental phases leading to smaller organs, reduced light perception and carbon assimilation processes including transpiration, photosynthesis and respiration (Stone, 2001). Nonetheless, photosynthesis is considered as the physiological process most sensitive to high temperatures, and that rising atmospheric CO_2 content will drive temperature increases in many already stressful environments. This CO_2-induced increase in plant high-temperature tolerance may have a substantial impact on both the productivity and distribution of many crop species in future.

3.2.4. Assimilate partitioning

Under low to moderate heat stress, a reduction in source and sink activities may occur leading to severe reductions in growth, economic yield and harvest index. Assimilate partitioning, taking place via apoplastic and symplastic pathways under high temperatures, has significant effects on transport and transfer processes in plants (Taiz and Zeiger, 2006). However, considerable genotypic variation exists in crop plants for assimilate partitioning, as for example among wheat genotypes (Yang et al., 2002). To elucidate causal agents of reduced grain filling in wheat under high temperatures, Wardlaw (1974) examined three main components of the plant system including source (flag leaf blade), sink (ear), and transport pathway (peduncle). It was determined that photosynthesis had a broad temperature optimum from 20 to 30 °C, however it declined rapidly at temperatures above 30 °C. The rate of ^{14}C assimilate movement out of the flag leaf (phloem loading), was optimum around 30 °C, however, the rate of movement through the stem was independent of temperature from 1 to 50 °C. It was concluded that, at least in wheat, temperature effects on translocation result indirectly from temperature effects on source and sink activities. From such results, increased mobilization efficiency of reserves from leaves, stem or other plant parts has been suggested as a potential strategy to improve grain filling and yield in wheat under heat stress. This suggestion, however, is based on present limited knowledge of physiological basis of assimilate partitioning under high temperature stress. Further investigation in this area may lead to improved crop production efficiency under high-temperature stress.

3.2.5. Cell membrane thermostability

Sustained function of cellular membranes under stress is pivotal for processes such as photosynthesis and respiration (Blum, 1988). Heat stress accelerates the kinetic energy and movement of molecules across membranes thereby loosening chemical bonds within molecules of biological membranes. This makes the lipid bilayer of biological membranes more fluid by either denaturation of proteins or an increase in unsaturated fatty acids (Savchenko et al., 2002). The integrity and functions of biological membranes are sensitive to high temperature, as heat stress alters the tertiary and quaternary structures of membrane proteins. Such alterations enhance the permeability of membranes, as evident from increased loss of electrolytes. The increased solute leakage, as an indication of decreased cell membrane thermostability (CMT), has long been used as an indirect measure of heat-stress tolerance in diverse plant species, including soybean (Martineau et al., 1979), potato and tomato (Chen et al., 1982), wheat (Blum et al., 2001), cotton (Ashraf et al., 1994), sorghum (Marcum, 1998), cowpea (Ismail and Hall, 1999) and barley (Wahid and Shabbir, 2005). Electrolyte leakage is influenced by plant/tissue age, sampling organ, developmental stage, growing season, degree of hardening and plant species. In maize, injuries to plasmalemma due to heat stress were much less severe in developing than in mature leaves (Karim et al., 1997, 1999). It was determined that an increase in saturated fatty acids in mature leaves elevated melting temperature of plasma membranes and thus reducing heat tolerance of the plant. In *Arabidopsis* plants grown under high temperature, total lipid content in membranes decreased to about one-half and the ratio of unsaturated to saturated fatty acids decreased to one-third of the levels at normal temperatures (Somerville and Browse, 1991). It should be noted, however, that in some species heat tolerance does not correlate with the degree of lipid saturation, suggesting that factors other than membrane stability might be limiting the growth at high temperatures.

The relationship between CMT and crop yield under high temperatures may vary from plant to plant and invokes for study of individual crops before using it as an important physiological selection criterion. For example, whereas a significant relationship between CMT and yield was observed in a few plant species such as sorghum (Sullivan and Ross, 1979), no such relationship was observed in soybean (Martineau et al., 1979) or wheat (Shanahan et al., 1990). Thus, the major cause(s) of yield suppression under heat stress remain largely elusive and deserve further experimentation.

3.2.6. Hormonal changes

Plants have the ability to monitor and adapt to adverse environmental conditions, though the degree of adaptability or tolerance to specific stresses varies among species and genotypes. Hormones play an important role in this regard. Cross-talk in hormone signaling reflects an organism's ability to integrate different inputs and respond appropriately. Hormonal homeostasis, stability, content, biosynthesis and compartmentalization are altered under heat stress (Maestri et al., 2002).

Abscisic acid (ABA) and ethylene (C_2H_4), as stress hormones, are involved in the regulation of many physiological

properties by acting as signal molecules. Different environmental stresses, including high temperature, result in increased levels of ABA. For example, recently it was determined that in creeping bentgrass (*Agrostis palustris*), ABA level did not rise during heat stress, but it accumulated upon recovery from stress suggesting a role during the latter period (Larkindale and Huang, 2005). However, the action of ABA in response to stress involves modification of gene expression. Analysis of ABA-responsive promoters revealed several potential *cis*- and *trans*-acting regulatory elements (Swamy and Smith, 1999). ABA mediates acclimation/adaptation of plants to desiccation by modulating the up- or down-regulation of numerous genes (Xiong et al., 2002). Under field conditions, where heat and drought stresses usually coincide, ABA induction is an important component of thermotolerance, suggesting its involvement in biochemical pathways essential for survival under heat-induced desiccation stress (Maestri et al., 2002). Other studies also suggest that induction of several HSPs (e.g., HSP70) by ABA may be one mechanism whereby it confers thermotolerance (Pareek et al., 1998). More so, heat shock transcription factor 3 acts synergistically with chimeric genes with a small HSP promoter, which is ABA inducible (Rojas et al., 1999).

A gaseous hormone, ethylene regulates almost all growth and developmental processes in plants, ranging from seed germination to flowering and fruiting as well as tolerance to environmental stresses. Measurement of the rate of ethylene released per unit amount of tissue provides information on the relative changes in cellular concentration of C_2H_4. Ethylene has nearly full biological activity at $1\,\mu L\,L^{-1}$, corresponding to 6.5×10^{-9} M at 25 °C. However, the levels of ethylene or the enzymes involved in ethylene biosynthesis vary at different time intervals during the day. For instance, the endogenous concentration of 1-amino-cyclopropane-1-carboxylic acid (ACC), a precursor of ethylene biosynthesis, measured at predawn and at maximum solar radiation during a summer drought in rosemary (*Rosmarinus officinalis*) showed a sharp distinction between the two times, which was positively correlated with the intensity of incident solar radiations (Munne-Bosch et al., 2002).

Heat stress changes ethylene production differently in different plant species (Arshad and Frankenberger, 2002). For example, while ethylene production in wheat leaves was inhibited slightly at 35 °C and severely at 40 °C, in soybean ethylene production in hypocotyls increased by increasing temperature up to 40 °C and it showed inhibition at 45 °C. Despite the fact that ACC accumulated in both species at 40 °C, its conversion into ethylene occurred only in soybean hypocotyls but not in wheat. Wheat leaves transferred to 18 °C followed by a short exposure to 40 °C showed an increase in ethylene production after 1 h lag period, possibly due to conversion of accumulated ACC to ethylene during that period (Tan et al., 1988). Similarly, creeping bentgrass showed ethylene production upon recovery, but not when under heat stress (Larkindale and Huang, 2005). Temperatures up to 35 °C have been shown to increase ethylene production and ripening of propylene-treated kiwifruit, but temperature above 35 °C inhibits ripening by inhibiting ethylene production, although respiration continues until the tissue disintegration (Antunes and Sfakiotakis, 2000). In pepper (*Piper nigrum*), increase in the level of ACC was positively correlated with high temperatures (Huberman et al., 1997). Exposure of imbibed sunflower seed to 45 °C for 48 h induced a state of thermodormancy, which appeared to associate with the loss of seed's ability to convert ACC to ethylene (Corbineau et al., 1989). However, treatment with 2.5 mM ethephon or $55\,\mu L\,L^{-1}$ ethylene improved germination of the seed at 25 °C. Ethylene may overcome the inhibitory effect of high temperature on thermosensitive lettuce seed due to increased α-mannanase activity, which helps weakening of the endosperm and facilitates germination (Nascimento et al., 2004). High temperature-induced abscission of reproductive organs relates to an increased ACC level; this is accompanied with both reduced levels and transport capacity of auxins to reproductive organs (Huberman et al., 1997). The effect of pre-harvest temperature on ripening characteristics of shaded and sun-exposed apple fruits indicated that the former treatment produced up to 90% less ethylene than the latter (Klein et al., 2001). In maize, ethylene production was highest at the top ear and lowest at the middle ear, suggesting that ethylene plays an important role in assimilate partitioning to grain filling (Fenglu et al., 1997).

Among other hormones, salicylic acid (SA) has been suggested to be involved in heat-stress responses elicited by plants. SA is an important component of signaling pathways in response to systemic acquired resistance (SAR) and the hypersensitive response (HR) (Kawano et al., 1998). SA stabilizes the trimers of heat shock transcription factors and aids them bind heat shock elements to the promoter of heat shock related genes. Long-term thermotolerance can be induced by SA, in which both Ca^{2+} homeostasis and antioxidant systems are thought to be involved (Wang and Li, 2006b). Sulphosalicylic acid (SSA), a derivative of SA, treatment can effectively remove H_2O_2 and increase heat tolerance. In this regard, catalase (CAT) plays a key role in removing H_2O_2 in cucumber (*Cucumus sativus*) seedlings treated with SSA under heat stress. In contrast, while glutathione peroxidase (GPX), ascorbate peroxidase (APX) and glutathione reductase (GR) showed higher activities in all SSA treatments under heat stress, they were not key enzymes in removing H_2O_2 (Shi et al., 2006). Thermotolerance of plants also can be enhanced by spraying leaves with acetyl-SA (Dat et al., 1998). Likewise, methyl salicylate (MeSA), a derivative of SA, has multiple functions. In addition to acting as signal molecule, it gives thermotolerance to holm oak (*Quercus ilex*) by enhanced xanthophylls de-epoxidation and content of ascorbate, antioxidants and α-tocopherol in leaves (Llusia et al., 2005; Wang and Li, 2006a).

The effects of gibberellins and cytokinins on high temperature tolerance are opposite to that of ABA. An inherently heat-tolerant dwarf mutant of barley impaired in the synthesis of gibberellins was repaired by application of gibberellic acid, whereas application of triazole paclobutrazol, a gibberellin antagonist, conferred heat tolerance (Vettakkorumakankav et al., 1999). In creeping bentgrass, the levels of various cytokinins, zeatin (Z), zeatin riboside (ZR), dihydrogen zeatin riboside (DHZR) and isopentinyl adenosine (iPA), showed dramatic decreases by the 5th day in root and 10th day in shoot, which were correlated with decreased dry matter production

(Liu and Huang, 2005). In a dwarf wheat variety, high-temperature-induced decrease in cytokinin content was found to be responsible for reduced kernel filling and its dry weight (Banowetz et al., 1999). Another class of hormones, brassinosteroids have recently been shown to confer thermotolerance to tomato and oilseed rape (*Brassica napus*), but not to cereals (Dhaubhadel et al., 1999). The potential roles of other phytohormones in plant thermotolerance are yet unknown.

3.2.7. Secondary metabolites

Most of the secondary metabolites are synthesized from the intermediates of primary carbon metabolism via phenylpropanoid, shikimate, mevalonate or methyl erythritol phosphate (MEP) pathways (Wahid and Ghazanfar, 2006). High-temperature stress induces production of phenolic compounds such as flavonoids and phenylpropanoids. Phenylalanine ammonia-lyase (PAL) is considered to be the principal enzyme of the phenylpropanoid pathway. Increased activity of PAL in response to thermal stress is considered as the main acclimatory response of cells to heat stress. Thermal stress induces the biosynthesis of phenolics and suppresses their oxidation, which is considered to trigger the acclimation to heat stress for example as in watermelon, *Citrulus vulgaris* (Rivero et al., 2001).

Carotenoids are widely known to protect cellular structures in various plant species irrespective of the stress type (Havaux, 1998; Wahid and Ghazanfar, 2006; Wahid, 2007). For example, the xanthophyll cycle (the reversible interconversion of two particular carotenoids, violaxanthin and zeaxanthin) has evolved to play this essential role in photoprotection. Since zeaxanthin is hydrophobic, it is found mostly at the periphery of the light-harvesting complexes, where it functions to prevent peroxidative damage to the membrane lipids triggered by ROS (Horton, 2002). Recent studies have revealed that carotenoids of the xanthophyll family and some other terpenoids, such as isoprene or α-tocopherol, stabilize and photoprotect the lipid phase of the thylakoid membranes (Havaux, 1998; Sharkey, 2005; Velikova et al., 2005). When plants are exposed to potentially harmful environmental conditions, such as strong light and/or elevated temperatures, the xanthophylls including violaxanthin, antheraxanthin and zeaxanthin partition between the light-harvesting complexes and the lipid phase of the thylakoid membranes. The resulting interaction of the xanthophyll molecules and the membrane lipids brings about a decreased fluidity (thermostability) of membrane and a lowered susceptibility to lipid peroxidation under high temperatures (Havaux, 1998).

Phenolics, including flavonoids, anthocyanins, lignins, etc., are the most important class of secondary metabolites in plants and play a variety of roles including tolerance to abiotic stresses (Chalker-Scott, 2002; Wahid and Ghazanfar, 2006; Wahid, 2007). Studies suggest that accumulation of soluble phenolics under heat stress was accompanied with increased phenyl ammonia lyase (PAL) and decreased peroxidase and polyphenol lyase activities (Rivero et al., 2001). Anthocyanins, a subclass of flavonoid compounds, are greatly modulated in plant tissues by prevailing high temperature; low temperature increases and elevated temperature decreases their concentration in buds and fruits (Sachray et al., 2002). For example, high temperature decreases synthesis of anthocyanins in reproductive parts of red apples (Tomana and Yamada, 1988), chrysanthemums (Shibata et al., 1988) and asters (Sachray et al., 2002). One of the causes of low anthocyanin concentration in plants at high temperatures is a decreased rate of its synthesis and stability (Sachray et al., 2002). On the other hand, vegetative tissues under high temperature stress show an accumulation of anthocyanins including rose and sugarcane leaves (Wahid and Ghazanfar, 2006). It has been suggested that in addition to their role as UV screen, anthocyanins serve to decrease leaf osmotic potential, which is linked to increased uptake and reduced transpirational loss of water under environmental stresses including high temperature (Chalker-Scott, 2002). These properties may enable the leaves to respond quickly to changing environmental conditions.

Isoprenoids, another class of plant secondary products, are synthesized via mevalonate pathway (Taiz and Zeiger, 2006). Being of low molecular weight and volatile in nature, their emission from leaves has been reported to confer heat-stress tolerance to photosynthesis apparati in different plants (Loreto et al., 1998). Studies have revealed that their biosynthesis is cost effective. While deriving considerable amount of photosynthates, they show compensatory benefits as to heat tolerance (Funk et al., 2004). Plants capable of emitting greater amounts of isoprene generally display better photosynthesis under heat stress, thus there is a relationship between isoprene emission and heat-stress tolerance (Velikova and Loreto, 2005). Sharkey (2005) opined that isoprene production protects the PSII from the damage caused by ROS, including H_2O_2, produced during heat-induced oxygenase action of rubisco, even though the photosynthetic rate approaches zero. It is proposed that endogenous production of isoprene protects the biological membranes from damaging effects by directly reacting with oxygen singlets ($^1O_2$) by means of isoprene-conjugate double bond (Velikova et al., 2005).

In summary, like other stresses, heat stress causes accumulation of secondary metabolites of multifarious nature in plants. However, the specific roles they play in enhancing heat-stress tolerance seem to be different and warrant further elucidation.

3.3. Molecular responses

3.3.1. Oxidative stress and antioxidants

In addition to tissue dehydration, heat stress may induce oxidative stress. For example, generation and reactions of activated oxygen species (AOS) including singlet oxygen ($^1O_2$), superoxide radical (O^{2-}), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are symptoms of cellular injury due to high temperature (Liu and Huang, 2000). AOS cause the autocatalytic peroxidation of membrane lipids and pigments thus leading to the loss of membrane semi-permeability and modifying its functions (Xu et al., 2006).

Superoxide radical is regularly synthesized in the chloroplast and mitochondrion and some quantities are also produced in microbodies (Fig. 2). The scavenging of O^{2-} by superoxide dismutase (SOD) results in the production of H_2O_2, which is removed by APX or CAT. However, both O^{2-} and H_2O_2 are not as toxic as the (OH^-), which is formed by the combination of

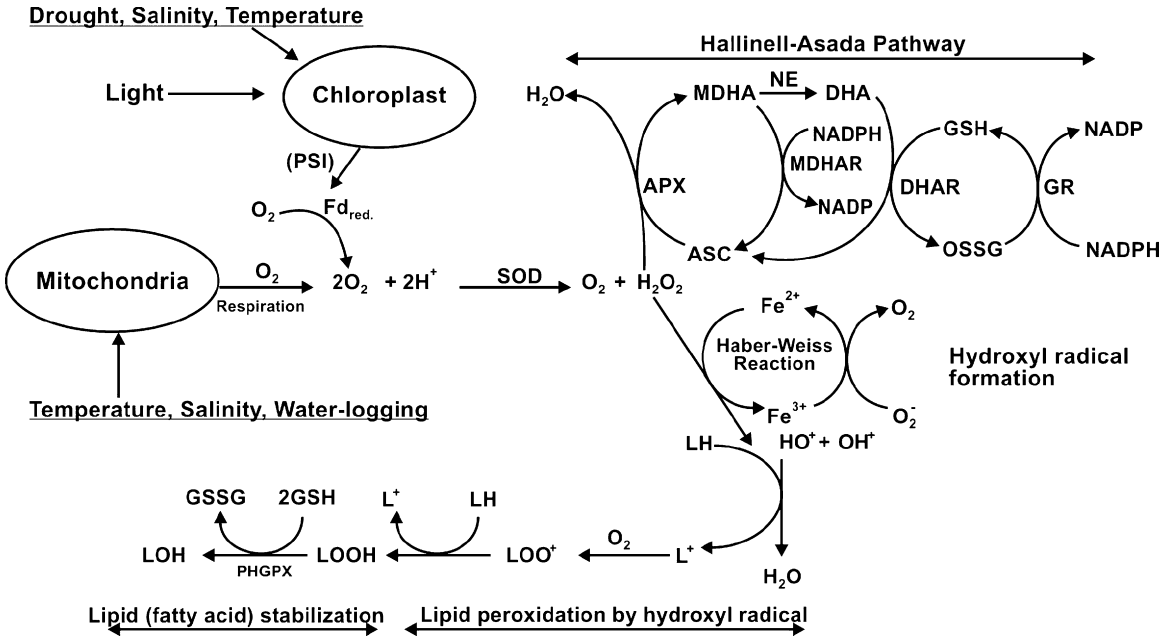

Fig. 2. Schematic presentation for generation and scavenging of superoxide radical, hydrogen peroxide, hydroxyl radical-induced lipid peroxidation and glutathione peroxidase-mediated fatty acid stabilization under environmental stresses. APX, ascorbate peroxidase; ASC, ascorbate; DHA, dehydroascorbate; DHAR, dehydroascorbate reductase; Fd, ferredoxin; GR, glutathione reductase; GSH, red glutathione; GSSG, oxi-glutathione; HO, hydroxyl radical; LH, lipid; L, LOO; LOOH, unstable lipid radicals and hydroperoxides; LOH, stable lipid (fatty acid); MDHA, monodehydro-ascorbate; MDHAR, mono dehydro-ascorbate reductase; NE, non-enzymatic reaction; PHGPX, phospholipid-hydroperoxide glutathione peroxidase; SOD, superoxide dismutase. Reproduced with permission from Sairam and Tyagi (2004).

O^{2-} and H_2O_2 in the presence of trace amounts of Fe^{2+} and Fe^{3+} by the Haber–Weiss reaction. The OH^- can damage chlorophyll, protein, DNA, lipids and other important macromolecules, thus fatally affecting plant metabolism and limiting growth and yield (Sairam and Tyagi, 2004).

As depicted in Fig. 2, plants have developed a series of both enzymatic and non-enzymatic detoxification systems to counteract AOS, thereby protecting cells from oxidative damage (Sairam and Tyagi, 2004). For example, overexpression of SOD in plants affect a number of physiological phenomena, which include the removal of H_2O_2, oxidation of toxic reductants, biosynthesis and degradation of lignin in cell walls, auxin catabolism, defensive responses to wounding, defense against pathogen or insect attack, and some respiratory processes (Scandalios, 1993). More specifically, expression and activation of APX is related to the appearance of physiological injuries caused in plants by thermal stress (Mazorra et al., 2002).

Decrease in antioxidant activity in stressed tissues results in higher levels of AOS that may contribute to injury (Fadzillah et al., 1996). Protection against oxidative stress is an important component in determining the survival of a plant under heat stress. Studies on heat-acclimated versus non-acclimated cool season turfgrass species suggested that the former had lower production of ROS as a result of enhanced synthesis of ascorbate and glutathione (Xu et al., 2006). Available data suggest that some signaling molecules may cause an increase in the antioxidant capacity of cells (Gong et al., 1997; Dat et al., 1998). Certainly further research is necessary to identify the signaling molecules, which enhanced production of antioxidants in cells exposed to heat stress.

3.3.2. Stress proteins

Expression of stress proteins is an important adaptation to cope with environmental stresses. Most of the stress proteins are soluble in water and therefore contribute to stress tolerance presumably via hydration of cellular structures (Wahid and Close, 2007). Although heat shock proteins (HSPs) are exclusively implicated in heat-stress response, certain other proteins are also involved.

3.3.2.1. Heat shock proteins. Synthesis and accumulation of specific proteins are ascertained during a rapid heat stress, and these proteins are designated as HSPs. Increased production of HSPs occurs when plants experience either abrupt or gradual increase in temperature (Nakamoto and Hiyama, 1999; Schöffl et al., 1999). Induction of HSPs seems to be a universal response to temperature stress, being observed in all organisms ranging from bacteria to human (Vierling, 1991). Plants of arid and semi-arid regions may synthesize and accumulate substantial amounts of HSPs. Certain HSPs are also expressed in some cells under cyclic or developmental control (Hopf et al., 1992). In this case, the expression of HSPs is restricted to certain stages of development, such as embryogenesis, germination, pollen development and fruit maturation (Prasinos et al., 2005). In higher plants, HSPs are usually induced under heat shock at any stage of development and major HSPs are highly homologous among distinct organisms (Vierling, 1991). HSP-triggered thermotolerance is attributed to the observations that (a) their induction coincides with the organism under stress, (b) their biosynthesis is extremely fast and intensive, and (c) they are induced in a wide variety of cells and organisms.

Three classes of proteins, as distinguished by molecular weight, account for most HSPs, *viz.*, HSP90, HSP70 and low molecular weight proteins of 15–30 kDa. The special importance of small HSPs in plants is suggested by their unusual abundance and diversity. The proportions of these three classes differ among plant species. HSP70 and HSP90 mRNAs can increase ten-fold, while low molecular weight (LMW) HSPs can increase as much as 200-fold. Other proteins, such as 110 kDa polypeptides and ubiquitin, though less important, are also considered to be HSPs (Feussner et al., 1997). All small-HSPs in plants are encoded by six nuclear gene families, each gene family corresponding to proteins found in distinct cellular compartments like cytosol, chloroplast, endoplasmic reticulum (ER), mitochondria and membranes. Some nuclear-encoded HSPs accumulate in the cytosol at low (27 °C) and high (43 °C) temperatures, but they accumulate in chloroplast at moderate (~37 °C) temperatures (Waters et al., 1996). The gene for a nuclear-encoded HSP, *Hsa32*, encoding a 32 kDa protein, has been cloned in tomato (Liu et al., 2006).

Immuno-localization studies have determined that HSPs normally associate with particular cellular structures, such as cell wall, chloroplasts, ribosomes and mitochondria (Nieto-Sotelo et al., 2002; Yang et al., 2006). When maize, wheat and rye seedlings were subjected to heat shocks (42 °C), whereas five mitochondrial LMW-HSPs (28, 23, 22, 20 and 19 kDa) were expressed in maize, only one (20 kDa) was expressed in wheat and rye, suggesting the reason for higher heat tolerance in maize than in wheat and rye (Korotaeva et al., 2001). In another study, a heat-tolerant maize line (ZPBL-304) exhibited increased amounts of chloroplast protein synthesis elongation factor under heat stress, which was related to the development of heat tolerance (Moriarty et al., 2002). In tomato plants under heat stress, HSPs aggregate into a granular structure in the cytoplasm, possibly protecting the protein biosynthesis machinery (Miroshnichenko et al., 2005). Presence of HSPs can prevent denaturation of other proteins caused by high temperature. The conformational dynamism and aggregate state of small HSPs may be crucial for their functions in thermoprotection of plant cells from detrimental effects of heat stress (Schöffl et al., 1999; Iba, 2002). The ability of small HSPs to assemble into heat shock granules (HSGs) and their disintegration is a prerequisite for survival of plant cells under continuous stress conditions at sublethal temperatures (Miroshnichenko et al., 2005).

In response to high temperatures, specific HSPs have been identified in different plant species. For example, HSP68, which is localized in mitochondria and normally constitutively expressed, was determined to have increased expression under heat stress in cells of potato, maize, tomato, soybean and barley (Neumann et al., 1993). Another HSP identified in maize is a nucleus-localized protein, HSP101, which belongs to the campylobacter invasion antigen B (CiaB) protein sub-family, whose members promote the renaturation of protein aggregates, and are essential for the induction of thermotolerance. Levels of HSP101 increased in response to heat shock, more abundantly in developing tassel, ear, silks, endosperm and embryo and less abundantly in vegetative and floral meristematic regions, mature pollen, roots and leaves (Young et al., 2001). In addition, heat treatment increases the level of other maize HSPs, which are associated with plant ability to withstand heat stress. For example, A 45-kDa HSP was found to play a major role in recovery from heat stress (Ristic and Cass, 1992). Different studies have determined that cytosolic accumulations of nuclear encoded chloroplast proteins were reversible (within 3 h) following return to normal growth temperature in many seed bearing species (Heckathorn et al., 1998).

There are considerable variations in patterns of HSP production in different species and even among genotypes within species (Wood et al., 1998). Furthermore, the ability to synthesize characteristic proteins at 40 °C and the intensity and duration of synthesis differ among various tissues examined within the same plant. Fast accumulation of HSPs in sensitive organs/tissues can play an important role in protection of metabolic apparati of the cell, thereby acting as a key factor for plant's adaptation to, and survival under, stress. In different plant species, elongating segments of primary roots exhibited a strong ability to synthesize nucleus-localized HSPs, which had roles in thermotolerance (Nieto-Sotelo et al., 2002). Under heat-stress conditions, while synthesis of a typical set of HSPs was induced in male tissues of maize flowers undergoing pollen formation, the mature pollen showed no synthesis of HSPs and thus were sensitive to heat stress and responsible for the failure of fertilization at high temperatures (Dupuis and Dumas, 1990). In another study, germinating maize pollen showed induction of 64 and 72 kDa peptides of HSPs under heat stress (Frova et al., 1989) whilst in the whole plant expression of a 45 kDa HSP was responsible for the heat tolerance (Ristic et al., 1996). Similar variation in the expression of HSPs can be found in other plant species.

The mechanism by which HSPs contribute to heat tolerance is still enigmatic though several roles have been ascribed to them. Many studies assert that HSPs are molecular chaperones insuring the native configuration and functionality of cell proteins under heat stress. There is considerable evidence that acquisition of thermotolerance is directly related to the synthesis and accumulation of HSPs (Bowen et al., 2002). For instance, HSPs provide for new or distorted proteins to fold into shapes essential for their normal functions. They also help shuttling proteins from one compartment to another and transporting old proteins to "garbage disposals" inside the cell. Among others, HSP70 has been extensively studied and is proposed to have a variety of functions such as protein translation and translocation, proteolysis, protein folding or chaperoning, suppressing aggregation, and reactivating denatured proteins (Zhang et al., 2005). Recently, dual role of LMW HSP21 in tomato has been described as protecting PSII from oxidative damage and involvement in fruit color change during storage at low temperatures (Neta-Sharir et al., 2005).

In many plant species, thermotolerance of cells and tissues after a heat stress is pretty much dependent upon induction of HSP70, though HSP101 has also been shown to be essential (Schöffl et al., 1999). One hypothesis is that HSP70 participates in ATP-dependent protein unfolding or assembly/disassembly reactions and it prevents protein denaturation during heat stress (Iba, 2002). Evidence for the general protective roles of HSPs

comes from the fact that mutants unable to synthesize them or the cells in which HSP70 synthesis is blocked or inactivated are more susceptible to heat injury (Burke, 2001). Heat sensitivity was associated with reduced capacity of bentgrass variants to accumulate chloroplastic HSPs (Wang and Luthe, 2003). The level of HSP22, a member of the plant small HSP super-family, remained high under continuous heat stress (Lund et al., 1998). LMW-HSPs may play structural roles in maintaining cell membrane integrity. Localization of LMW-HSPs in chloroplast membranes further suggested that these proteins protect the PSII from adverse effects of heat stress and play a role in photosynthetic electron transport (Barua et al., 2003).

3.3.2.2. Other heat induced proteins. Besides HSPs, there are a number of other plant proteins, including ubiquitin (Sun and Callis, 1997), cytosolic Cu/Zn-SOD (Herouart and Inzé, 1994) and Mn-POD (Brown et al., 1993), whose expressions are stimulated upon heat stress. For example, in *Prosopis chilensis* and soybean under heat stress, ubiquitin and conjugated-ubiquitin synthesis during the first 30 min of exposure emerged as an important mechanism of heat tolerance (Ortiz and Cardemil, 2001). Some studies have shown that heat shock induces Mn-peroxidase, which plays a vital role in minimizing oxidative damages (Iba, 2002). In a study on *Chenopodium murale*, when leaf proteins extracts from thylakoid and stromal fractions were subjected to heat stress it was determined that Cu/Zn-SOD from stromal fraction was more heat tolerant than Cu/Zn-SOD from thylakoid, and this was responsible for chloroplastic stability under heat stress (Khanna-Chopra and Sabarinath, 2004). In another study, a number of osmotin like proteins induced by heat and nitrogen stresses, collectively called Pir proteins, were found to be overexpressed in the yeast cells under heat stress conferring them resistance to tobacco osmotin (an antifungal) (Yun et al., 1997). Late embryogenesis abundant (LEA) proteins can prevent aggregation and protect the citrate synthase from desiccating conditions like heat- and drought-stress (Goyal et al., 2005). Using proteomics tool, Majoul et al. (2003) determined enhanced expressions of 25 LEA proteins in hexaploid wheat during grain filling. Geranium leaves exposed to drought and heat stress revealed expression of dehydrin proteins (25–60 kDa), which indicated a possible linkage between drought and heat-stress tolerance (Arora et al., 1998). Recently, three low-molecular-weight dehydrins have been identified in sugarcane leaves with increased expression in response to heat stress (Wahid and Close, 2007). Function of these proteins is apparently related to protein degradation pathway, minimizing the adverse effects of dehydration and oxidative stress during heat stress (Schöffl et al., 1999).

In essence, expression of stress proteins is an important adaptation toward heat-stress tolerance by plants. Of these, expression of low and high molecular weight HSPs, widely reported in a number of plant species, is the most important one. These proteins show organelle- and tissue-specific expression with deduced function like chaperones, folding and unfolding of cellular proteins, and protection of functional sites from the adverse effects of high temperature. Among other stress proteins, expression of ubiquitin, Pir proteins, LEA and dehydrins has also been established under heat stress. A main function of these proteins appears to be protection of cellular and sub-cellular structures against oxidative damage and dehydrative forces.

4. Mechanism of heat tolerance

Plants manifest different mechanisms for surviving under elevated temperatures, including long-term evolutionary phenological and morphological adaptations and short-term avoidance or acclimation mechanisms such as changing leaf orientation, transpirational cooling, or alteration of membrane lipid compositions. In many crop plants, early maturation is closely correlated with smaller yield losses under high temperatures, which may be attributed to the engagement of an escape mechanism (Adams et al., 2001). Plant's immobility limits the range of their behavioral responses to environmental cues and places a strong emphasis on cellular and physiological mechanisms of adaptation and protection. Also, plants may experience different types of stress at different developmental stages and their mechanisms of response to stress may vary in different tissues (Queitsch et al., 2000). The initial stress signals (e.g., osmotic or ionic effects, or changes in temperature or membrane fluidity) would trigger downstream signaling processes and transcription controls, which activate stress-responsive mechanisms to re-establish homeostasis and protect and repair damaged proteins and membranes. Inadequate responses at one or more steps in the signaling and gene activation processes might ultimately result in irreversible damages in cellular homeostasis and destruction of functional and structural proteins and membranes, leading to cell death (Vinocur and Altman, 2005; Bohnert et al., 2006). Even plants growing in their natural distribution range may experience high temperatures that would be lethal in the absence of this rapid acclimation response. Furthermore, because plants can experience major diurnal temperature fluctuations, the acquisition of thermotolerance may reflect a more general mechanism that contributes to homeostasis of metabolism on a daily basis (Hong et al., 2003). Mild stress episodes, however, should be viewed as the acceleration of a program linked to the normal termination of phytomere production during the plant cycle, rather than as an abrupt event linked to stress (Guilioni et al., 1997).

Elucidating the various mechanisms of plant response to stress and their roles in acquired stress tolerance is of great practical and basic importance. Some major tolerance mechanisms, including ion transporters, osmoprotectants, free-radical scavengers, late embryogenesis abundant proteins and factors involved in signaling cascades and transcriptional control are essentially significant to counteract the stress effects (Wang et al., 2004). Series of changes and mechanisms, beginning with the perception of heat and signaling and production of metabolites that enable plants to cope with adversaries of heat stress, have been proposed (Fig. 3). Heat stress effects are notable at various levels, including plasma membrane and biochemical pathways operative in the cytosol or cytoplasmic organelles (Sung et al., 2003). Initial effects of heat stress, however, are on plasmalemma, which shows more fluidity of lipid bilayer under stress. This leads to the induction of Ca^{2+} influx and cytoskeletal reorganization, resulting in the upregulation of mitogen acti-

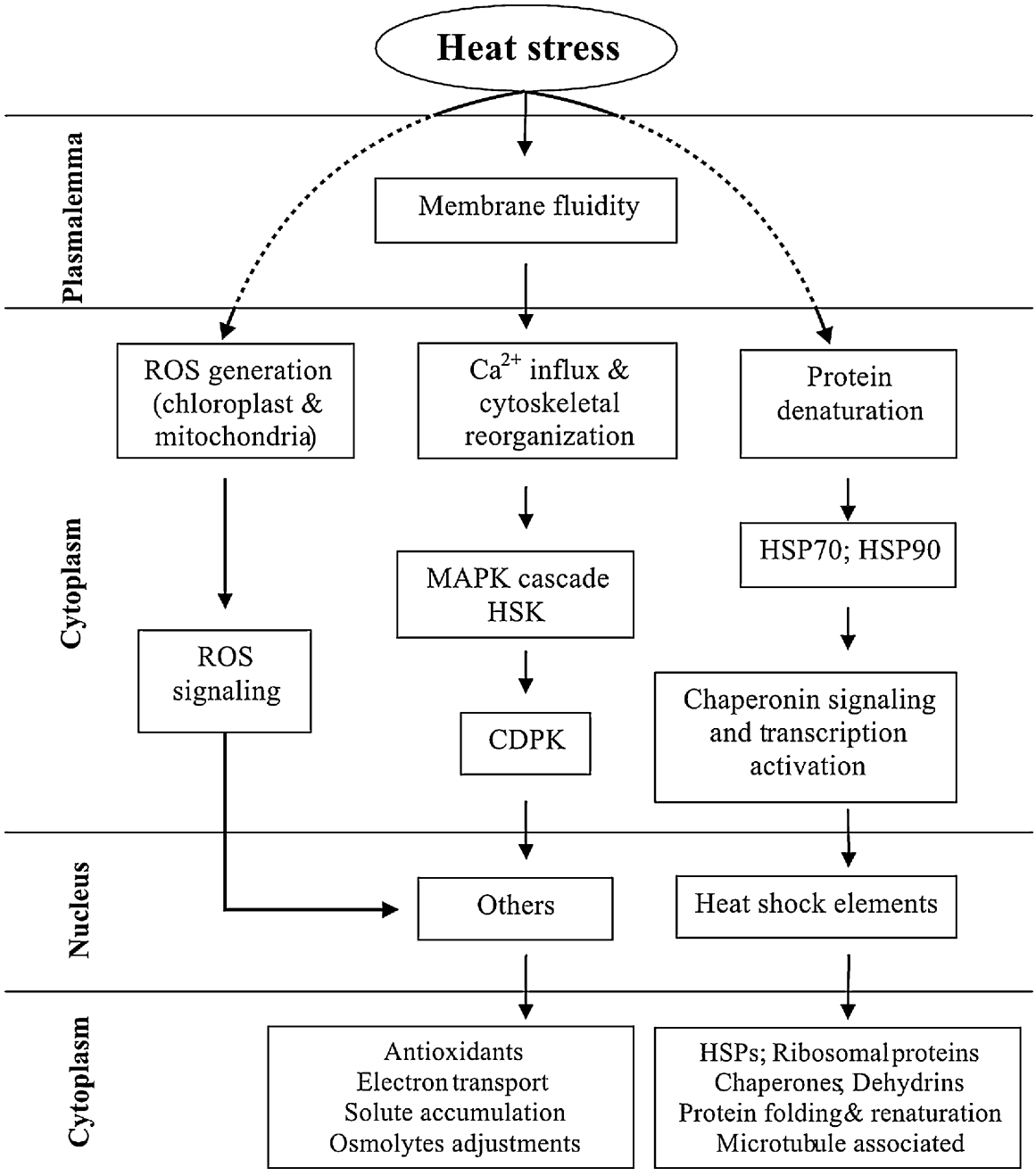

Fig. 3. Proposed heat-stress tolerance mechanisms in plants. MAPK, mitogen activated protein kinases; ROS, reactive oxygen species; HAMK, heat shock activated MAPK; HSE, heat shock element; HSPs, heat shock proteins; CDPK, calcium dependent protein kinase; HSK, histidine kinase. Partly adopted from Sung et al. (2003).

vated protein kinases (MAPK) and calcium dependent protein kinase (CDPK). Signaling of these cascades at nuclear level leads to the production of antioxidants and compatible osmolytes for cell water balance and osmotic adjustment. Production of ROS in the organelles (e.g., chloroplast and mitochondria) is of great significance for signaling as well as production of antioxidants (Bohnert et al., 2006). The antioxidant defense mechanism is a part of heat-stress adaptation, and its strength is correlated with acquisition of thermotolerance (Maestri et al., 2002). Accordingly, in a set of wheat genotypes, the capacity to acquire thermotolerance was correlated with activities of CAT and SOD, higher ascorbic acid content, and less oxidative damage (Sairam and Tyagi, 2004).

One of the most closely studied mechanisms of thermotolerance is the induction of HSPs, which, as described in above, comprise several evolutionarily conserved protein families. However, each major HSP family has a unique mechanism of action with chaperonic activity. The protective effects of HSPs can be attributed to the network of the chaperone machinery, in which many chaperones act in concert. An increasing number of studies suggest that the HSPs/chaperones interact with other stress-response mechanisms (Wang et al., 2004). The

HSPs/chaperones can play a role in stress signal transduction and gene activation (Nollen and Morimoto, 2002) as well as in regulating cellular redox state (Arrigo, 1998). They also interact with other stress-response mechanisms such as production of osmolytes (Diamant et al., 2001) and antioxidants (Panchuk et al., 2002).

Membrane lipid saturation is considered as an important element in high temperature tolerance. In a mutant wheat line with increased heat resistance, heat treatment increased relative quantities of linolenic acid (among galactolipids) and *trans*-3-hexaldecanoic acid (among phospholipids), when compared with the wild type (Behl et al., 1996). Currently, it is unknown whether a higher or a lower degree of membrane lipid saturation is beneficial for high-temperature tolerance (Klueva et al., 2001). The contribution of lipid and protein components to membrane function under heat stress needs further investigation. Localization of LMW-HSPs with chloroplastic membranes upon heat stress suggests that they play a role in protecting photosynthetic electron transport (Heckathorn et al., 1998).

An important component of thermotolerance is changes in gene expression. Heat stress is known to swiftly alter patterns of gene expression (Yang et al., 2006), inducing expression of the HSP complements and inhibiting expression of many other genes (Yost and Lindquist, 1986). The mRNAs encoding non-heat-stress-induced proteins are destabilized during heat stress. Heat stress may also inhibit splicing of some mRNAs. Earlier it was hypothesized that HSP-encoding mRNAs could not be processed properly due to the absence of introns in the corresponding genes (Yost and Lindquist, 1986). Subsequently it was shown that some HSP-encoding genes have introns and, under heat-stress conditions, their mRNAs were correctly spliced (Visioli et al., 1997). However, the mechanism of preferential post-transcription modification and translation of HSP-encoding mRNA under heat stress remains yet to be elucidated.

5. Acquired thermotolerance

Thermotolerance refers to the ability of an organism to cope with excessively high temperatures. It has long been known that plants, like other organisms, have the ability to acquire thermotolerance rather rapidly, may be within hours, so to survive under otherwise lethal high temperatures (Vierling, 1991). The acquisition of thermotolerance is an autonomous cellular phenomenon and normally results from prior exposure to a conditioning pretreatment, which can be a short but sublethal high temperature. The acquisition of high level of thermotolerance protects cells and organisms from a subsequent lethal heat stress. Thermotolerance can also be induced by a gradual increase in temperature to lethal highs, as would be experienced under natural conditions (Vierling, 1991), and induction in this way involves a number of processes. Using Arabidopsis mutants, it was shown that, apart from heat shock proteins (HSP32 and HSP101), ABA, ROS and SA pathways are involved in the development and maintenance of acquired thermotolerance (Larkindale and Huang, 2005; Charng et al., 2006). Adaptive mechanisms that protect cells from protoxic effects of heat stress are key factors in acquisition of thermotolerance. Examination of the adverse effects caused by temperature extremes can reveal useful information, in particular as heat-stress responses in plants are similar to those of other stresses, including cold and drought (Rizhsky et al., 2002).

The heat shock response (HSR), defined as a transient reprogramming of gene expression, is a conserved biological reaction of cells and organisms to elevated temperatures (Schöffl et al., 1999). HSR has been of great interest for studying molecular mechanisms of stress tolerance and regulation of gene expression in plants. The temperature for the induction of HSR coincides with optimum growth temperature for any given species, which is normally 5–10 °C above normothermic conditions. The features of this response include induction of HSPs and subsequently acquisition of a higher level of thermotolerance. The transient synthesis of HSPs suggests that the signal triggering the response is either lost, inactivated or no longer recognized under conditions of long-term heat treatment (Schöffl, 2005). The involvement of HSPs in heat-stress tolerance is a logical model, but direct support for function of HSPs in promoting thermotolerance has been difficult to obtain (Burke, 2001; Schöffl, 2005).

In summary, thermotolerance acquired by plants via autonomous synthesis of pertinent compounds or induced via gradual exposure to heat episodes, though cost intensive, is an important and potentially vital strategy. This phenomenon is principally related to display of heat shock response and accomplished by reprogramming of gene expression, allowing plants to cope with the heat stress.

6. Temperature sensing and signaling

Perception of stress and relay of the signal for turning on adaptive response mechanisms are key steps towards plant stress tolerance. There are multiple stress perceptions and signaling pathways, some of which are specific while others may be involved in cross-talk at various steps (Chinnusamy et al., 2004). General responses to stress involve signaling of the stress via the redox system. Chemical signals such as ROS, Ca^{2+} and plant hormones activate genomic re-programing via signal cascades (Joyce et al., 2003; Suzuki and Mittler, 2006). Although the presence of a plant thermometer has not been established, it is suggested that changing membrane fluidity plays a central role in sensing and influencing gene expression both under high and low temperatures. This suggests that sensors are located in microdomains of membranes, which are capable of detecting physical phase transition, eventually leading to conformational changes and/or phosphorylation/dephosphorylation cycles due to changes in temperature (Plieth, 1999). In this regard, a model for temperature sensing and regulation of heat shock response integrates observed membrane alterations. Changes in the ratio of saturated to unsaturated fatty acid on the set point of temperature for the heat shock response (HSR) alters activities of HSFs.

Rigidification of thylakoid membranes appears to invoke altered expression profiles of heat shock genes, suggesting that the temperature sensing mechanism may be located on the thylakoid membrane (Horváth et al., 1998). The prospect of the thylakoid membrane acting as a heat sensor is physiologically

crucial, because it is susceptible to temperature upshift, owing to its highly unsaturated character, and the presence of photosystems, which are fragile to temperature changes (Sung et al., 2003).

Various signaling ions and molecules are involved in temperature sensing and signaling. As a signaling response to temperature stress, cytosolic Ca^{2+} sharply rises (Larkindale and Knight, 2002), which seems to be linked to the acquisition of tolerance possibly by transducing high temperature-induced signals to MAPK. MAPK cascades are important parts of signal transduction pathways in plants and thought to function ubiquitously in many responses to external signals (Kaur and Gupta, 2005). A heat-shock activated MAPK (HAMK) has been identified, the activation of which was triggered by apparent opposite changes in membrane fluidity coupled with cytoskeletal remodeling (Sangwan and Dhindsa, 2002). Ca^{2+} influx and the action of Ca-dependent protein kinases (CDPK) have been closely correlated with the expression of HSPs (Sangwan and Dhindsa, 2002). However, another study suggested that Ca^{2+} is not required for production of HSPs in plants, despite the fact that heat stress induces uptake of Ca^{2+} and induction of some calmodulin (CaM) related genes (Gong et al., 1997). As a mediator of Ca^{2+} signal, CaM is activated by binding Ca^{2+}, inducing a cascade of regulatory events and regulation of many HSP genes (Liu et al., 2003). Several studies have shown that Ca^{2+} is involved in the regulation of plant responses to various environmental stresses, including high temperature. Increasing cytosolic Ca^{2+} content under heat stress may alleviate heat injury, such as increased activity of antioxidants (Gong et al., 1997), turgor maintenance in the guard cells (Webb et al., 1996) and enable plant cells to better survive. However, excessive Ca^{2+} released into the cytosol and sustained high cytosolic Ca^{2+} concentration might be cytotoxic (Wang and Li, 1999).

Specific groups of potential signaling molecules like SA, ABA, $CaCl_2$, H_2O_2, and ACC may induce tolerance of plants to heat stress by reducing oxidative damage (Larkindale and Huang, 2004). Being molecules of somewhat novel interest in the last few years, H_2O_2 and NO have emerged to be central players in the world of plant cell signaling under stressful situations (Dat et al., 2000). A protein phosphorylation cascade has been shown to be activated by H_2O_2 is a MAPK cascade. Methyl-SA has a major signaling role in the gene activation under heat stress up to 1.8 nmol g^{-1} dry mass of tissue, beyond which it becomes lethal to cell metabolism (Llusia et al., 2005).

In short, sensing of high temperature and induction of signaling cascades are important adaptive steps in coping with adversaries of heat stress. Although numerous molecules including ROS, hormones and ethylene have been identified for the perception of heat stress cues, role of Ca^{2+} is exclusive.

7. Genetic improvement for heat-stress tolerance

Recent studies have suggested that plants experience oxidative stresses during the initial period of adjustment to any stress. Plant responses to stress progress from general to specific. Specific responses require sustained expression of genes involved in processes specific to individual stresses (Yang et al., 2006). These responses accommodate short-term reaction or tolerance to specific stresses. However, genome plasticity in plants, including genetic (e.g., directed mutation) and epigenetic (e.g., methylation, chromatin remodeling, histone acetylation) changes, allows long-term adaptation to environmental changes/conditions (Joyce et al., 2003). Such adaptations may be necessary for long-term survival or establishment of plant genotypes/species in particular environmental niches. Under agricultural systems, plants adaptation or their tolerance to environmental stresses can be manipulated by various approaches.

In general, the negative impacts of abiotic stresses on agricultural productivity can be reduced by a combination of genetic improvement and cultural practices. Genetic improvement entails development of cultivars which can tolerate environmental stresses and produce economic yield. Adjustment/modifications in cultural practices, such as planting time, plant density, and soil and irrigation managements, however, can minimize stress effects, for example by synchronizing the stress-sensitive stage of the plant with the most favorable time period of the season. In practice, to be successful in improving agricultural productivity in stress environments, both genetic improvement and adjustment in cultural practices must be employed simultaneously. Agriculturists have long been aware of desirable cultural practices to minimize adverse effects of environmental stresses on crop production. However, genetic improvement of crops for stress tolerance is relatively a new endeavor and has been considered only during the past 2–3 decades. Traditionally, most plant breeding programs have focused on development of cultivars with high yield potential in favorable (i.e., non-stress) environments. Such efforts have been very successful in improving the efficiency of crop production per unit area and have resulted in significant increases in total agricultural production (Warren, 1998). However, genetic improvement of plants for stress tolerance can be an economically viable solution for crop production under stressful environments (Blum, 1988). The progress in breeding for stress tolerance depends upon an understanding of the physiological mechanisms and genetic bases of stress tolerance at the whole plant, cellular and molecular levels. Considerable information is presently available regarding the physiological and metabolic aspects of plant heat-stress tolerance, as discussed earlier. However, information regarding the genetic basis of heat tolerance is generally scarce, though the use of traditional plant breeding protocols and contemporary molecular biological techniques, including molecular marker technology and genetic transformation, have resulted in genetic characterization and/or development of plants with improved heat tolerance. In particular, the application of quantitative trait locus (QTL) mapping has contributed to a better understanding of the genetic relationship among tolerances to different stresses. In below, a summary of such efforts and progresses is presented and discussed.

7.1. Conventional breeding strategies

Physiological and genetic investigations indicate that most abiotic stress tolerance traits are complex, controlled by more

than one gene, and highly influenced by environmental variation (Blum, 1988). The quantification of tolerance often poses serious difficulties. Direct selection under field conditions is generally difficult because uncontrollable environmental factors adversely affect the precision and repeatability of such trials. Often, no consistent high-temperature conditions can be guaranteed in field nurseries, as heat stress may or may not occur in the field. Furthermore, stress tolerance is a developmentally regulated, stage-specific phenomenon; tolerance at one stage of plant development may not be correlated with tolerance at other developmental stages. Individual stages throughout the ontogeny of the plant should be evaluated separately for the assessment of tolerance and for the identification, characterization and genetic manipulation of tolerance components. Moreover, species may show different sensitivity to heat stress at different developmental stages. For example, in tomato, though plants are sensitive to high temperatures throughout the plant ontogeny, flowering and fruit set are the most sensitive stages; fruit set is somewhat affected at day/night temperatures above 26/20 °C and is severely affected above 35/26 °C (Berry and Rafique-Uddin, 1988). Thus, partitioning of the tolerance into its developmental and physiological/genetic components may provide a better understanding of the plant's response to heat stress and facilitate development of plants with stress tolerance throughout the plant's life cycle.

A common method of selecting plants for heat-stress tolerance has been to grow breeding materials in a hot target production environment and identify individuals/lines with greater yield potential (Ehlers and Hall, 1998). Under such conditions, however, the presence of other stresses such as insect-pests has made the selection process very difficult, particularly during reproductive stage. A suggested approach has been to identify selection criteria during early stages of plant development, which may be correlated with heat tolerance during reproductive stages. Unfortunately, thus far no reliable criteria have been identified. A rather more effective approach has been development of glasshouses for heat tolerance screening. Theoretically, such nurseries can be utilized for screening throughout the plant life cycle, from seedling to reproductive stages. An advantage of glasshouse screening is that the required temperature conditions can be maintained consistently for the duration of the experiment. Also, because a key factor in screening for heat tolerance is maintaining high night temperatures, glasshouse nursery can provide such conditions more reliably than field nurseries. However, in many places in the world where high temperatures are a concern, such growth/glasshouse facilities are nonexistent or limited in size, precluding screening of large breeding populations.

A major challenge in traditional breeding for heat tolerance is the identification of reliable screening methods and effective selection criteria to facilitate detection of heat-tolerant plants. Several screening methods and selection criteria have been developed/proposed by different researchers. For example, a heat tolerance index (HTI) for growth recovery after heat exposure was proposed for sorghum (Young et al., 2001). The index is the ratio of the increase in coleoptile length after finite exposure to heat stress (e.g., 50 °C) to the increase in coleoptile length in the no-stress treatment. This approach allows a rapid and repeated recording of coleoptile length, which may be used to screen a large number of genotypes in a rather short period of time. Although this is a very cost effective and easy-to-assay technique of screening for heat tolerance, its correlation with performance under field conditions and its effectiveness in different crop species are yet unknown (Setimela et al., 2005). In some crop species such as tomato, a strong positive correlation has been observed between fruit set and yield under high temperature. Thus, evaluation of germplasm to identify sources of heat tolerance has regularly been accomplished by screening for fruit set under high temperature (Berry and Rafique-Uddin, 1988). Furthermore, although poor fruit set at high temperature cannot be attributed to a single factor, decreases in pollen germination and/or pollen tube growth are among the most commonly reported factors. Therefore, pollen viability has been suggested as an additional indirect selection criterion for heat tolerance. Also, production of viable seed is often reduced under heat stress and thus high seed set has been arguably reported as an indication of heat tolerance (Berry and Rafique-Uddin, 1988).

Because pollen viability and fertility is adversely affected by high temperature, any type of fruit and seed production that may not need sexual hybridization and fertilization may provide heat tolerance. For example, apomixes, in particular the types that assure reproduction without the need for pollination may be very useful when developing cultivars for production under high temperatures. Through genetic engineering it may be possible to insert the cassette of genes needed to confer facultative apomixis. Currently, considerable research is underway to identify genes or enzymes that may be involved in production of apomixis (Albertini et al., 2005). Among many other traits which are affected by high temperature, the non-reproductive processes include photosynthetic efficiency, assimilate translocation, mesophyll resistance and disorganization of cellular membranes (Chen et al., 1982). Breeding to improve such traits under high temperatures may result in the development of cultivars with heat tolerance attributes.

Several other issues of concern when employing traditional breeding protocols to develop heat-tolerant crop plants are as follows:

1. Identification of genetic resources with heat tolerance attributes. In many plant species, for example soybeans and tomatoes, limited genetic variations exist within the cultivated species necessitating identification and use of wild accessions. However, often there are great difficulties in both the identification and successful use of wild accessions for stress tolerance breeding (Foolad, 2005).
2. When screening different genotypes (in particular wild accessions) for growth under high temperatures, distinction must be made between heat tolerance and growth potential. Often plants with higher growth potential perform better regardless of the growing conditions.
3. When breeding for stress tolerance, often it is necessary that the derived lines/cultivars be able to perform well under both stress and non-stress conditions. Development of such genotypes is not without inherent difficulties. In some

plant species, heat tolerance is often associated with some undesirable horticultural or agronomical characteristics. In tomato, for example, two undesirable characteristics commonly observed in heat-tolerant genotypes are small fruit and restricted foliar canopy (Scott et al., 1997). The production of small fruit is most likely due to adverse effects of high temperature on the production of auxins in the fruit, and the poor canopy is due to the highly reproductive nature of the heat-tolerant genotypes.

In summary, breeding for heat tolerance is still in its infancy stage and warrants more attention than it has been given in the past. It is unfortunate that the literature contains relatively little information on breeding for heat tolerance in different crop species. However, despite all the complexity of heat tolerance and difficulties encountered during transfer of tolerance, some heat-tolerant inbred lines and hybrid cultivars with commercial acceptability have been developed and released, at least in a few crop species such as tomato (Scott et al., 1986; Scott et al., 1995). Nevertheless, to accelerate such progresses, major areas of emphasis in the future should be: (1) designing/development of accurate screening procedures; (2) identification and characterization of genetic resources with heat tolerance; (3) discerning the genetic basis of heat tolerance at each stage of plant development; (4) development and screening of large breeding populations to facilitate transfer of genes for heat tolerance to commercial cultivars (Siddique et al., 1999). The use of advanced molecular biology techniques may facilitate development of plants with improved heat tolerance, as described in below.

7.2. Molecular and biotechnological strategies

Recent genetic studies and efforts to understand/improve high-temperature tolerance of crop plants using traditional protocols and transgenic approaches have largely determined that plant heat-stress tolerance is a multigenic trait. Different components of tolerance, controlled by different sets of genes, are critical for heat tolerance at different stages of plant development or in different tissues (Howarth, 2005; Bohnert et al., 2006). Thus, the use of genetic stocks with different degrees of heat tolerance, correlation and co-segregation analyses, molecular biology techniques and molecular markers to identify tolerance QTLs are promising approaches to dissect the genetic basis of thermotolerance (Maestri et al., 2002). Most recently, biotechnology has contributed significantly to a better understanding of the genetic basis of heat tolerance. For example, several genes responsible for inducing the synthesis of HSPs have been identified and isolated in various plant species, including tomato and maize (Liu et al., 2006; Sun et al., 2006; Momcilovic and Ristic, 2007). Also, it has been determined that induction of many heat-inducible genes is attributed to the conserved heat shock elements (HSEs), which are located in the TATA box proximal 5′ flanking regions of heat shock genes (Schöffl et al., 1999). The requirement of TATA box was earlier demonstrated by deletion analysis of soybean heat shock genes in sunflower (Czarnecka et al., 1989). In addition, a number of other sequence motifs have been identified in plants that have quantitative effects on expression of certain heat shock genes. For example, there is evidence for the involvement of CCAAT box and AT-rich sequences (Czarnecka et al., 1989). The HSEs are the binding sites for the transitive heat shock transcription factor (HSF), the activation of which in higher eukaryotes is a multi-step process. In response to heat stress, HSF is converted from a monomeric to trimeric form. The trimeric HSF is localized predominantly in the nucleus and has a high affinity of binding to HSEs. It is believed that interaction of HSF with HSP70 or other HSPs results in the activation of HSF via conformational changes involving monomer to trimer transition and nuclear targeting (Schöffl et al., 1999). This expression of heat shock genes is modulated by the temperature within permissive range, thereby conferring plant thermotolerance (Yang et al., 2006). Further research has demonstrated that thermotolerance of plants also can be modulated/effected by changes in transcriptional and translational activities. Ongoing transcription is needed during stress to support a basal level of translational activity in the subsequent recovery from the stress, but it does not appear to be required for the heat-mediated increase in mRNA stability (Gallie and Pitto, 1996). In general, such activities in plants undergo rapid changes during developmental stages such as seed formation and germination, and also during abiotic stresses such as heat shock, hypoxia and wounding (Gallie et al., 1998).

Two common biotechnological approaches to study and improve plant stress tolerance include marker-assisted selection (MAS) and genetic transformation. During the past two decades, the use of these approaches has contributed greatly to a better understanding of the genetic and biochemical bases of plant stress-tolerance and, in some cases, led to the development of plants with enhanced tolerance to abiotic stress. Because of the general complexity of abiotic stress tolerance and the difficulty in phenotypic selection for tolerance, MAS has been considered as an effective approach to improve plant stress tolerance (Foolad, 2005). The use of this approach, however, requires identification of genetic markers that are associated with genes or QTLs affecting whole plant stress tolerance or individual components contributing to it. During the past two decades, substantial amounts of research has been conducted in different crop species to identify genetic markers associated with different environmental stresses, in particular drought, salinity and low temperatures. For example, molecular marker technology has allowed the identification and genetic characterization of QTLs with significant effects on stress tolerance during different stages of plant development and facilitated determination of genetic relationships among tolerance to different stresses (Foolad, 2005). Comparatively, however, limited research has been conducted to identify genetic markers associated with heat tolerance in different plant species. In *Arabidopsis*, for example, four genomic loci (QTLs) determining its capacity to acquire thermotolerance were identified using a panel of heat-sensitive mutants (Hong and Vierling, 2000). Use of restriction fragment length polymorphism (RFLP) revealed mapping of eleven QTLs for pollen germination and pollen tube growth under heat stress in maize (Frova and Sari-Gorla, 1994).

Recent advances in genetic transformation and gene expression techniques have contributed greatly to a better

understanding of the genetic and biochemical bases of plant stress-tolerance and, in some cases, led to the development of plants with enhanced tolerance to abiotic stresses. For example, a significant progress has been made in the identification of genes, enzymes or compounds with remarkable effects on plant stress tolerance at the cellular or organismal level (Apse and Blumwald, 2002; Bohnert et al., 2006). Furthermore, manipulation of the expression or production of the identified genes, proteins, enzymes, or compounds through transgenic approaches have resulted in the development of plants with enhanced stress tolerance in different plant species (Zhang et al., 2001; Rontein et al., 2002). However, a major limitation in the use of such techniques for improving plant high-temperature tolerance is that the critical factors conferring the enhanced temperature tolerance in higher plants are still poorly understood.

Initial research on molecular manipulation to improve plant heat tolerance focused on production of enzymes that detoxify reactive oxygen species, including SOD. Reactive oxygen species are induced by most types of stresses (Havaux, 1998; Sairam and Tyagi, 2004) and their production has been envisaged in stress cross-tolerance (Allan et al., 2006). In addition to increased production of SOD, many other potential approaches can be utilized to detoxify ROS and produce plants tolerant of heat stress (Zidenga, 2005). If the critical components are determined, genetic engineering technology can be utilized to incorporate thermo-tolerance into adapted cultivars. Despite the very many limitations, some progress has been made. For example, transgenic tobacco plants with altered chloroplast membranes by silencing the gene encoding chloroplast omega-3 fatty acid desaturase have been produced which produce less trienoic fatty acids and more dienoic fatty acids in their chloroplasts than the wild type. These plants exhibited greater photosynthesis and grew better than wild type plants under high temperatures (Murakami et al., 2000). *Dnak1*, a gene responsible for high salt tolerance in the cyanobacterium *Aphanothece halophytica*, when transferred into tobacco was expressed and conferred high temperature resistance (Ono et al., 2001). Development of plants capable of higher production of glycinebetaine through transformation with the *BADH* gene has been suggested as a potentially effective method to enhance heat tolerance in plants (Yang et al., 2005). Thermal stability of rubisco activase, a molecular chaperone responsible for the activity of rubisco, is important in maintaining its activity (Salvucci and Crafts-Brandner, 2004a). By transforming tobacco plants with rubisco activase gene, thermotolerance is achieved by reversible decarboxylation of rubisco—a likely protective mechanism by which the plant protects its photosynthetic apparatus (Sharkey et al., 2001).

Genetic engineering of heat shock factors (HSF) and antisense strategies are instrumental to the understanding of both the functional roles of HSPs and the regulation of HSFs. Manipulations of the HS-response in transgenic plants have the potential to improve common abiotic stress tolerance and this may have a significant impact on the exploitation of the inherent genetic potential of agronomically important plants. Transgenic overexpression of certain HSFs and HSF-fusion proteins results in an expression of HSPs at normal temperature (Iba, 2002). The increased acquired thermotolerance of transgenic lines is attributed to the higher levels of HSP chaperones. It has also been demonstrated that tomato *MT-sHSP* has a molecular chaperone function *in vitro* (Liu and Shono, 1999) and recently it has been demonstrated that *MT-sHSP* gene exhibits thermotolerance in transformed tobacco with the tomato *MT-sHSP* gene (Sanmiya et al., 2004) at the plant level. Experimental data obtained from transgenic, reverse-genetics and mutation approaches in non-cereal species confirm causal involvement of HSPs in thermotolerance in plants (Queitsch et al., 2000). However, the cellular targets of HSPs are still unknown.

In summary, transformation technology for improving plant stress tolerance is still at its infancy, and the success to date represents only a beginning. Advancements in marker technology and genetic transformation are expected to contribute significantly to the development of plants with tolerance to high temperatures in future. With the current transformation technology, it is becoming possible to transfer multiple genes, which may act synergistically and additively to improve plant stress tolerance. Future knowledge of tolerance components and the identification and cloning of responsible genes may allow transformation of plants with multiple genes and production of highly stress-tolerant transgenic plants. In addition, there is no report to date of any studies testing the performance of transgenic plants under field stress conditions. Therefore, much more work is needed to gain a clearer understanding of the genetics, biochemical and physiological basis of plant heat tolerance.

8. Induction of heat tolerance

Although genetic approaches may be beneficial in the production of heat-tolerant plants, it is likely that the newly produced plants are low yielding compared to near-isogenic heat sensitive plants. Thus, considerable attention has been devoted to the induction of heat tolerance in existing high-yielding cultivars. Among the various methods to achieve this goal, foliar application of, or pre-sowing seed treatment with, low concentrations of inorganic salts, osmoprotectants, signaling molecules (e.g., growth hormones) and oxidants (e.g., H_2O_2) as well as preconditioning of plants are common approaches. High-temperature preconditioning has been shown to drastically reduce the heat-induced damage to black spruce seedlings at moderately high temperatures (Colclough et al., 1990). Preconditioned tomato plants exhibited good osmotic adjustment by maintaining the osmotic potential and stomatal conductance, and better growth than non-conditioned plants (Morales et al., 2003). Similarly, heat acclimated, compared to non-acclimated, turfgrass leaves manifested higher thermostability, lower lipid peroxidation product malondialdehyde (MDA) and lower damage to chloroplast upon exposure to heat stress (Xu et al., 2006).

In pearl millet, pre-sowing hardening of the seed at high temperature (42 °C) resulted in plants tolerant to overheating and dehydration and showing higher levels of water-soluble proteins and lower amounts of amide-N in leaves compared to non-hardened plants (Tikhomirova, 1985). The researchers proposed that the higher heat tolerance was due to enhanced glutathione synthase activity, promoting binding of the ammo-

nia accumulated during exposure to high temperature. In tomato, it was demonstrated that heat treatment administered to plants prior to chilling stress resulted in reduced incidence and severity of chilling injury in fruit and other organs (Whitaker, 1994). In some cool season grasses, under heat stress, Ca^{2+} is required for maintenance of antioxidant activity and not for osmotic adjustment (Jiang and Haung, 2001). Under heat stress, Ca^{2+} requirement for growth is high to mitigate adverse effects of the stress (Kleinhenz and Palta, 2002). It has been shown that exogenous application of Ca^{2+} promotes plant's heat tolerance. Application of Ca^{2+} in the form of $CaCl_2$ prior to the stress treatment elevated the content of lipid peroxidation product, MDA and stimulated the activities of guaiacol peroxidase, SOD and catalase, which could be the reasons for the induction of heat tolerance (Kolupaev et al., 2005).

Among the low molecular weight organic compounds, glycinebetaine and polyamines have been successfully applied to induce heat tolerance in various plant species. For example, barley seeds pre-treated with glycinebetaine led to plants with lower membrane damage, better photosynthetic rate, improved leaf water potential and greater shoot dry mass, compared to untreated seeds (Wahid and Shabbir, 2005). Also, exogenous application of 4 mM spermidine improved tomato heat resistance by improving chlorophyll fluorescence properties, hardening and higher resistance to thermal damage of the pigment-protein complexes structure, and the activity of PSII during linear increase in temperature (Murkowski, 2001). Thus, to improve plant heat tolerance, alternative approaches to genetic means would include pre-treatment of plants or seeds with heat stress or certain mineral or organaic compounds. The success of such approach, however, depends on plant species and gentoypes and must be studied on case basis.

9. Energy economics under heat stress

Reduction in plant growth is a major consequence of growing under stress conditions. This occurs mainly due to a reduction in net photosynthesis rate and generation of reducing powers as well as interference with mitochondrial functions. It is suggested that during light reactions increased leaf temperature induces ATP synthesis to balance ATP consumption under heat stress possibly by cyclic electron flow (Bukhov et al., 1999). During dark reactions of photosynthesis, rubisco activation in Calvin cycle has been determined as a critical step, being inhibited at 35–40 °C, which results in decreased net CO_2 assimilation and the production of carbohydrates (Crafts-Brandner and Salvucci, 2000; Dubey, 2005). In mitochondria, environmental stress normally causes NAD^+ breakdown, ATP over-consumption and higher rate of respiration. This is partially due to a breakdown in the NAD^+ pool caused by the enhanced activity of poly(ADP-ribose) polymerase (PARP), which uses NAD^+ as a substrate to synthesize polymers of ADP-ribose. This poly(ADP) ribosylation is a post-translational modification of nuclear proteins that seems to be initiated by oxidative and other types of DNA damage. Stress-induced depletion of NAD^+ results in a similar depletion of energy, since ATP molecules are required to re-synthesize the depleted NAD^+ (Zidenga, 2005). Collectively, these reactions deplete the energy of the plant and enhance the production of ROS, which eventually lead to cell death (De Block et al., 2005). A strategy of improving stress tolerance in plants by maintaining the plant's energy homeostasis under stress is the production of transgenic plants with lowered poly(ADP) ribosylation activity; such transgenics appear to be tolerant to multiple stresses by preventing energy overconsumption under stress, thereby allowing normal mitochondrial respiration (De Block et al., 2005). In short, heat tolerance in plants is a cost-intensive process and consumes considerable cellular energy to cope with adversaries of high temperature.

10. Conclusion and future prospects

Plants exhibit a variety of responses to high temperatures, which are depicted by symptomatic and quantitative changes in growth and morphology. The ability of the plant to cope with or adjust to the heat stress varies across and within species as well as at different developmental stages. Although high temperatures affect plant growth at all developmental stages, later phenological stages, in particular anthesis and grain filling, are generally more susceptible. Pollen viability, patterns of assimilate partitioning, and growth and development of seed/grain are highly adversely affected. Other notable heat stress effects include structural changes in tissues and cell organelles, disorganization of cell membranes, disturbance of leaf water relations, and impedance of photosynthesis via effects on photochemical and biochemical reactions and photosynthetic membranes. Lipid peroxidation via the production of ROS and changes in antioxidant enzymes and altered pattern of synthesis of primary and secondary metabolites are also of considerable importance.

In response to heat stress, plants manifest numerous adaptive changes. The induction of signaling cascades leading to profound changes in specific gene expression is considered an important heat-stress adaptation. Although various signaling molecules are synthesized under heat stress, the role of Ca^{2+} remains critical. A fundamental heat-stress response ubiquitous to plants is the expression of HSPs, which range from low (10 kDa) to high (100 kDa) molecular mass in different species. Evidence on synthesis and accumulation of some other stress-related proteins is also available. Such stress proteins are thought to function as molecular chaperones, helping in folding and unfolding of essential proteins under stress, and ensuring three-dimensional structure of membrane proteins for sustained cellular functions and survival under heat stress.

In addition to genetic means to developing plants with improved heat tolerance, attempts have been made to induce heat tolerance in a range of plant species using different approaches. These include preconditioning of plants to heat stress and exogenous applications of osmoprotectants or plant growth-regulating compounds on seeds or whole plants. Results from such applications are promising and further research is forthcoming. Also, while some notable progress has been reported as to the development of crop plants with improved heat tolerance via traditional breeding, the prospect for engineering plants with heat tolerance is also good considering accumulating molecular information on the mechanisms of tolerance and contributing factors.

Although physiological mechanisms of heat tolerance are relatively well understood, further studies are essential to determine physiological basis of assimilate partitioning from source to sink, plant phenotypic flexibility which leads to heat tolerance, and factors that modulate plant heat-stress response. Furthermore, applications of genomics, proteomics and trascriptomics approaches to a better understanding of the molecular basis of plant response to heat stress as well as plant heat tolerance are imperative. As in the case of most other abiotic stresses, foliar plant parts are more directly impinged upon by high temperatures than roots. However, an understanding of root responses to heat stress, most likely involving root–shoot signaling, is crucial and warrants further exploration. Molecular knowledge of response and tolerance mechanisms will pave the way for engineering plants that can tolerate heat stress and could be the basis for production of crops which can produce economic yield under heat-stress conditions.

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