Heat Stress in Wheat during Reproductive and Grain-Filling Phases

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Ambient temperatures have increased since the beginning of the century and are predicted to continue rising under climate change. Such increases in temperature can cause heat stress: a severe threat to wheat production in many countries, particularly when it occurs during reproductive and grain-filling phases. Heat stress reduces plant photosynthetic capacity through metabolic limitations and oxidative damage to chloroplasts, with concomitant reductions in dry matter accumulation and grain yield. Genotypes expressing heat shock proteins are better able to withstand heat stress as they protect proteins from heat-induced damage. Heat tolerance can be improved by selecting and developing wheat genotypes with heat resistance. Wheat pre-breeding and breeding may be based on secondary traits like membrane stability, photosynthetic rate and grain weight under heat stress. Nonetheless, improvement in grain yield under heat stress implies selecting genotypes for grain size and grain weight under heat stress. Such increases in temperature can cause heat stress: a severe threat to wheat production in many countries, particularly when it occurs during reproductive and grain-filling phases. Heat stress reduces plant photosynthetic capacity through metabolic limitations and oxidative damage to chloroplasts, with concomitant reductions in dry matter accumulation and grain yield. Genotypes expressing heat shock proteins are better able to withstand heat stress as they protect proteins from heat-induced damage. Heat tolerance can be improved by selecting and developing wheat genotypes with heat resistance. Wheat pre-breeding and breeding may be based on secondary traits like membrane stability, photosynthetic rate and grain weight under heat stress. Nonetheless, improvement in grain yield under heat stress implies selecting genotypes for grain size and grain weight under heat stress.

II. IMPACT OF TERMINAL HEAT STRESS

The optimum temperature for wheat anthesis and grain filling ranges from 12 to 22°C (Table 1). Exposure to temperatures above this can significantly reduce grain yield (McDonald et al., 1983; Macías et al., 1999, 2000; Mullarkey and Jones, 2000; Tewolde et al., 2006). Heat stress during anthesis increases floret abortion (Wardlaw and Wrigley, 1994). Heat stress during the reproductive phase can cause pollen sterility, tissue dehydration, lower CO2 assimilation and increased photorespiration. Although high temperatures accelerate growth (Fischer, 1980; Kase and Catsky, 1984), they also reduce the phenology, which is not compensated for by the increased growth rate (Wardlaw and Moncur, 1995; Zahedi and Jenner, 2003). However, temperatures □30°C, during floret formation, may cause complete sterility (Saini and Aspinal, 1982). Therefore, when temperatures are elevated between anthesis to grain maturity, grain yield is reduced because of the reduced time to capture resources (Fig. 1).

### TABLE 1

<table>
<thead>
<tr>
<th>Stage</th>
<th>Temperature</th>
<th>Mean temperature (°C)</th>
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<td>Topt</td>
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<td>Tmax&gt;</td>
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<td>Anthesis</td>
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<tr>
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<tr>
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<td>Grain filling</td>
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<tr>
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<td>Tmax</td>
<td>34.3 ± 2.66</td>
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</tbody>
</table>

Note: Data taken from Al-Khatib and Paulsen (1984); Amani et al. (1996); Anjum et al. (2008); Anwar et al. (2007); Asseng et al. (2010); Barnabás et al. (2008); Blum et al. (2001); Calderini et al. (1999); Ferris et al. (1998); Fischer (1980, 1985); Fokar et al. (1998); Gibson and Paulsen (1999); Guedira et al. (2002); Pushpalatha et al. (2008); Rahman et al. (1977); Samra and Singh (2005); Shewry (2009); Spiertz et al. (2006); Stone and Nicolas (1994, 1995a, b); Stone et al. (1995); van Herwaarden et al. (1998); Wardlaw (1994); Wollenweber et al. (2003); Yang et al. (2002); Zhao et al. (2007, 2008).
FIG. 1. Influence of mean temperatures from anthesis to harvest maturity on (a) grain yield and (b) number of grains per ear in winter wheat (data from Wheeler et al., 1996a, b).

From 1950 to 1993, the increase in global daily minimum temperatures was more than double the increase in daily maximum temperatures (Easterling et al., 1997). Increased daily minimum temperature appears to have greater impact on wheat production as grain yield is more strongly negatively correlated with increasing minimum temperatures than maximum temperatures (Lobell et al., 2005). For example, in Mexico, wheat yield decreased by 10% for every 1°C increase in nighttime temperature, but the same increase in day-time temperature had no significant effect (Lobell et al., 2005). Night temperatures >20°C can reduce spikelet fertility with a concomitant reduction in grain number and size (Prasad et al., 2008a). Increased night temperatures also linearly decrease the duration of grain filling. For example, Prasad et al. (2008a) found that night temperatures of 20 and 23°C reduced the grain-filling period by 3 and 7 d, respectively.

A. Photosynthesis

Photosynthesis is the most sensitive physiological process to elevated temperature (Wahid et al., 2007) and any reduction in photosynthesis affects growth and grain yield of wheat (Al-Khatib and Paulsen, 1990, 1999). Heat stress reduces photosynthesis through disruptions in the structure and function of chloroplasts, and reductions in chlorophyll content (Xu et al., 1995). The inactivation of chloroplast enzymes, mainly induced by oxidative stress, may also reduce the rate of leaf photosynthesis. Oxidative stress may induce lipid peroxidation leading to protein degradation, membrane rupture and enzyme inactivation (Sairam et al., 2000).

1. Rubisco and Photorespiration

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is a key enzyme that regulates carboxylation during photosynthesis (Ogren, 1984). The inhibition of photosynthesis due to high temperature is often attributed to increases in the rate of photorespiration. This is because the solubility of CO₂ and O₂, and the kinetics of Rubisco are affected under high temperatures (Fig. 2; Ogren, 1984; Long et al., 2004). However, reductions in net photosynthetic rate due to high temperature are also attributed to non-photorespiratory processes (Fig. 2; Crafts-Brandner and Salvucci, 2000; Pushpalatha et al., 2007).

Rubisco activase (RCA), a cytosol-synthesized chloroplast protein, facilitates the removal of inhibitory sugar phosphates from Rubisco-active sites and thus regulates the Rubisco activity (Salvucci and Ogren, 1996). This RCA-mediated step is vital as it frees the Rubisco active site for carboxylation (Salvucci and Ogren, 1996; Spritzer and Salvucci, 2002). Upon exposure to high temperature, Rubisco is deactivated because of faster rate of end product formation or slower reactivation by RCA, resultantly Rubisco activase looses its ability in keeping the Rubisco active and efficient (Salvucci and Crafts-Brandner, 2004a, b). Demirevska-Kepova et al. (2005) observed changes in the abundance of Rubisco large and small subunits and RCA in wheat leaves upon exposure to heat stress (40°C) in darkness or in light. Heat stress for 24 h in darkness irreversibly decreased the Rubisco subunits and RCA.

Rubisco is more sensitive to increased temperatures than the rest of the enzymes involved in carboxylation. For example, significantly higher phosphoenolpyruvate (PEP) carboxylase activities and lower Rubisco activities were observed in each of the non-leaf photosynthetic organs (e.g., awn, glume, lemma, peduncle, and sheath) compared with the flag leaf blade (Xu et al., 2004). Heat stress (34/17°C) for 12 d rapidly decreased Rubisco activity, whereas the activity of PEP carboxylase increased initially but later declined in all organs. Higher PEP carboxylase/Rubisco ratios were maintained, particularly in non-leaf organs, which had higher PEP carboxylase/Rubisco ratios than the flag leaf at all times (Xu et al., 2004).

Although Rubisco catalytic activity increases with temperature, its low affinity for CO₂ and ability to act as an oxygenase limit the chance of increasing net photosynthesis with temperature (Salvucci and Crafts-Brandner, 2004a). At high temperatures, the solubility of oxygen decreases to a lesser extent than CO₂, resulting in increased photorespiration and lower photosynthesis (Lea and Leegood, 1999).

2. Photosynthetic Apparatus

Through effects on photosystem II (PSII)- and photosystem I (PSI)-mediated electron transfer, and Calvin cycle activity,
Heat stress

Limited carboxylation

Lower CO₂ solubility

Increased photorespiration

Diminished activities of PEPcase, NADP-ME, FBPase, PPDK, Rubisco

ROS production

Attack on membranes

Limited carboxylation

Down-regulation of non-cyclic e-transport

Obstructed ATP synthesis

Declined photosynthesis

FIG. 2. Possible mechanism in which photosynthesis is reduced under heat stress. Heat stress disturbs the balance between production of reactive oxygen species (ROS) and antioxidant defence causing accumulation of ROS, which induces oxidative stress. With increases in ambient temperature, CO₂ solubility in water decreases, which not only reduces carboxylation directly but also directs more electrons to form ROS and promotes photorespiration. Severe heat stress limits photosynthesis due to a decline in activities of ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco), phosphoenolpyruvate carboxylase (PEPCase), NADP-malic enzyme (NADP-ME), fructose-1, 6-bisphosphatase (FBPase) and pyruvate orthophosphate dikinase (PPDK). Heat stress increases leaf senescence and thus limits photosynthetic area. Moreover, non-cyclic electron transport is down-regulated to match the reduced requirements of NADPH production and thus reduces ATP synthesis. (Color figure available online).

exposure to heat stress (40°C) damages the photosynthetic apparatus (Baker, 1991; Sharkey, 2005). However, under moderate heat stress (<40°C), no inhibition of PSII has been observed, even though there was a substantial reduction in carbon assimilation (Law and Crafts-Brandner, 1999; Sharkey, 2005). PSII appears to be influenced by temperatures above 45°C (Sharkey, 2005) but is not severely affected by moderately high temperatures (<40°C) (Allakhverdiev et al., 2008). Nonetheless, PSII repair is impeded due to reactive oxygen species induced damage on the protein de novo synthesis system, thus reducing carbon fixation and oxygen evolution, and disrupting linear electron flow.

Prasad et al. (2008b) reported that the most important reasons for PSII sensitivity to high temperature are heat-induced increase in thylakoid membrane fluidity and electron-transport-dependent integrity of PSII. The inhibition of PSII electron transport under heat stress is often indicated by a sharp increase in the basal level of chlorophyll fluorescence that corresponds to photosynthetic inhibition (Ristic et al., 2007). Heat-stress-induced damage and disruption of the integrity of thylakoid membranes also causes the photophosphorylation to cease (Dias and Lidon, 2009).

Heat stress and excessive light may damage PSII-active sites. Heat stress may also separate the light harvesting complex-II (LHCII) from the PSII core complex physically (Schreiber and Berry, 1977). Despite increased thylakoid membrane leakiness, cyclic electron transport around PSI, stimulated by high temperature, may help in maintenance of high ATP contents and increase in pH-related non-photochemical quenching (Bukhov et al., 1999).

B. Leaf Senescence

Leaf senescence is the progressive loss of green leaf area that occurs during reproductive development of a crop (Noodén, 1988). As plants use the resources to cope with the stress, limited assimilates remain available for reproductive development. Heat stress further triggers the senescence-related metabolic changes in wheat (Al-Khatib and Paulsen, 1984; Paulsen, 1994). In addition, chlorophyll biosynthesis is inhibited under exposure to heat stress (42°C; Tewari and Tripathy, 1998), which hastens leaf senescence. The breakdown of thylakoid components is also accelerated by heat stress, leading to leaf senescence (Harding et al., 1990).
Diurnal temperature differences are also important in this regard; for instance, larger diurnal fluctuations promoted senescence of flag leaves under high-temperature conditions (Zhao et al., 2007).

C. Water Relations

Leaf relative water contents (LRWC), leaf water potential, stomatal conductance and rate of transpiration are influenced by leaf and canopy temperature (Farooq et al., 2009a, b). In dry environments, higher temperatures lead to higher vapour pressure deficits, which drive higher evaporative transpiration. As soil water is depleted, LRWC and leaf water potential decrease. However, evaporation from the leaf surface enhances leaf and canopy cooling, so overheating may be ameliorated by higher rates of transpiration.

Although trade-offs exist between heat balance, stomatal regulation and depletion rate of limited soil water, there is a tendency for most species to conserve water over temperature regulation, particularly when ambient temperatures exceed 30°C (Martínez-Ballesta, 2009). There is limited information on the dynamics of water and heat balance for wheat during reproductive and grain-filling stages, but an example of the dynamics in seedlings occurred in the study by Machado and Paulsen (2001). LRWC and leaf water potential were not affected by heat stress when soil water content was close to field capacity, but relative water contents were slightly affected by day/night temperatures of 40/35°C. When water was withheld under the highest temperature, relative water content and leaf water potential decreased as soil water content decreased to 18%, and leaves ultimately senesced and plants died. Transpiration rates also decreased with increasing temperature (from 15/10°C to 40/35°C) and plant growth decreased (Machado and Paulsen, 2001). However, soil water content decreased more rapidly when the temperature imposed was 25°C or above, which was probably due to greater soil evaporation, which was not controlled. Root temperature, however, was not controlled in their study or, as is the case in most heat stress studies, since root functioning in water and nutrient uptake is particularly sensitive to heat (reviewed by Martínez-Ballesta, 2009), elevated root temperatures may confound results. In another study, high temperatures (35/25°C) after tillering significantly reduced water potential in two wheat genotypes at anthesis and 7 and 15 d after anthesis, with a greater reduction in the genotype more susceptible to temperature stress (Almeselman et al., 2009). Sairam et al. (2000) also reported that relative water contents were substantially reduced by increased temperature.

During reproductive and grain-filling phases, water is needed for stem and peduncle elongation to raise the ear up through the unfolding leaf to the top of the canopy; cell expansion and growth of all parts of the ear; facets of flowering, such as pollen ripening, rapid extension of stamen filaments and fertilization; grain growth and filling. Water flow for many of these processes involves crossing membranes, possibly facilitated by aquaporins. Elevated temperature tends to increase hydraulic conductivity of membranes and plant tissues due to increased aquaporin activity, membrane fluidity and permeability (Martínez-Ballesta, 2009) and, to a greater degree, reduced water viscosity with increasing temperature (Cochard et al., 2007). Increasing hydraulic conductivity may be beneficial in leaves and roots, minimizing changes in leaf water potential, so stomata can stay open for longer. Alternatively, increased permeability of membranes may cause flowers and grains to dehydrate, particularly if gradients driving water flow into flowers or grains are disrupted by heat stress.

D. Grain Growth and Development

Grain development is impacted by heat stress because assimilate translocation and grain-filling duration and rate are influenced directly by changes in ambient temperature. The extent of heat-driven damage is dependent on the level of heat stress.

1. Grain Number and Size

Both grain number and weight are sensitive to elevated temperature (Ferris et al., 1998). Influence of temperature on each of these components of grain yield depends on the developmental phase at which the elevated temperature occurs. For instance, between spike initiation and anthesis, temperatures above 20°C may substantially reduce grain number per spike (Saini and Aspinall, 1982). Several events during this phenostage that influence grain number include spikelet initiation, floral organ differentiation, male and female sporogenesis, pollination and fertilization. Heat stress speeds up development of the spike (Porter and Gawith, 1999) reducing spikelet number and thus, the number of grains per spike (Saini and Aspinall, 1982). However, the period between spike initiation and anthesis is not the most sensitive period to heat stress. The most sensitive period is between the appearance of double ridges on the shoot apex and flag leaf. An inverse relationship between duration of heat stress and grain number per spike has been observed during this time (Rawson and Bagga, 1979). The reason for this sensitivity is because spikelets begin to form in the spike from ridges of tissue between ridges of undifferentiated leaf primordia, called the double ridge stage. Each spikelet meristem then starts to produce florets (McMaster, 1997). The reduction in the duration of emergence to double ridge and double ridge to anthesis reduces spikelet number per spike and grain number per spikelet (McMaster, 1997).

Although high temperature (>30°C) during floret development may cause complete sterility (Saini and Aspinall, 1982), variation among wheat genotypes has also been observed (Table 2; Gibson and Paulsen, 1999; Anjum et al., 2008; Zhao et al., 2008). Heat stress around floral initiation has severe effects on grain number. For instance, grain number per spike decreased by 4% for every 1°C (from 15–22°C) increase in the 30 d preceding anthesis (Fischer, 1985). The availability of carbohydrates for floret development is one factor determining grain number (Abbate et al., 1995; Demotes-Mainard and Jeuffroy, 2004) because inadequate availability of assimilates may cause floret
<table>
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<th>Temperature (°C)</th>
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</tbody>
</table>

References:
- *Calderini et al.* (1999)
- **Wardlaw** (1994)
- ***Spiertz et al.* (2006)
- |Anjum et al.* (2008)
- §Gibson and Paulsen (1998)
- ‡Fokar et al.* (1998)
- †Zhao et al.* (2008)

* Temperature from anthesis for a week.
** Mean temperature during anthesis–physiological maturity period.
*** Temperature during grain filling for three days.
† Temperature from pre-anthesis to maturity.
§ Temperature at 10 days after anthesis.
‡, † Temperature during grain filling.
3. Grain Filling Rate and Duration

Heat stress accelerates the rate of grain filling whereas grain filling duration is shortened (Dias and Lidon, 2009). For instance, 5°C increases in temperature above 20°C increased the rate of grain filling and reduced the filling duration by 12 days in wheat (Yin et al., 2009). Under these conditions, the supply of photoassimilates may be limited (Calderini et al., 2006). It is estimated that for every 1°C above the optimal growing temperature of 15–20°C, the duration of grain-filling is reduced by 2.8 d (Streck, 2005).

The rate of grain growth increases as temperature increases, but this apparently depends on whether the number of grains per spike is reduced (Sofield et al., 1977). In spikes where the number of grains is less affected by elevated temperature, spikelets reduce the rate of grain growth (Sofield et al., 1977). Therefore, we would expect that an increase in the grain filling rate could compensate for the shorter grain-filling period; however, this did not occur at temperatures above 30°C (Sofield et al., 1977). Other studies have also reported that the duration of grain filling under heat stress was not compensated by greater grain-filling rates (Wardlaw et al., 1980; Stone et al., 1995). Furthermore, Viswanathan and Khanna-Chopra (2001) showed that both duration and rate of grain growth were reduced by heat stress in genotypes differing in grain weight stability (Blum et al., 1994). When the photosynthetic source of assimilates is reduced by heat stress, the alternative source for grain filling is remobilized stem reserves. The demand for stem reserves under heat stress dramatically increases, ranging from 6 to 100% depending on the heat-induced reduction in photosynthesis (Blum, 1998). However, under heat stress genotypic variation exists for the contribution of stem reserves for grain filling (Yang et al., 2002). For instance, current photosynthesis can provide 63 and 65% of assimilates to grain at 20/15°C and 30/25°C day/night temperatures, respectively (Blum et al., 1998).

4. Assimilate Translocation

During grain filling, assimilates are transferred from either current assimilation or from pre-anthesis stored stem reserves (Palta et al., 1994; Blum, 1998). Under ideal conditions, 90 to 95% of carbon required for grain filling is transferred from current carbon assimilation (Kobata et al., 1992). Under water and heat stress, the relative contribution of pre-anthesis stored reserves and current assimilation changes substantially (Evans et al., 1975; Palta et al., 1994). When the photosynthetic source of assimilates is reduced by heat stress, the alternative source for grain filling is remobilized stem reserves. The demand for stem reserves under heat stress dramatically increases, ranging from 6 to 100% depending on the heat-induced reduction in photosynthesis (Blum, 1998). However, under heat stress genotypic variation exists for the contribution of stem reserves for grain filling (Yang et al., 2002). For instance, current photosynthesis can provide 63 and 65% of assimilates to grain at 20/15°C and 30/25°C day/night temperatures, respectively (Blum et al., 1998).
Tolerance to heat stress is associated with genotypes with stable photosynthesis, but also those with high capacity to store stem reserves (Blum et al., 1994).

At high temperatures, assimilate translocation which occurs through both symplastic and apoplastic pathways, is substantially reduced. Assimilate transport from flag leaf to grain is substantially reduced by temperatures above 30°C but there is no influence of temperature (from 1 to 50°C) on translocation from the stem (Wardlaw, 1974). This indicates that, in wheat, the effect of heat stress on assimilate translocation is indirect.
even though heat stress reduces the rate of assimilate transport from vegetative organs to grain (Plaut et al., 2004).

E. Grain Quality

Grain protein content and grain size are the most important characteristics determining grain quality in wheat (Coles et al., 1997). Heat stress during grain-filling phase affects the grain protein contents (Wardlaw et al., 2002; Gooding et al., 2003) through reductions in starch deposition, which influences protein concentration by allowing more nitrogen per unit of starch (Stone and Nicolas, 1998). Although the daily flow of carbon and nitrogen into grain increases with increasing temperature, carbon flow decreases per degree-day (Wardlaw et al., 1980; Daniel and Triboi, 2000). As a result, grain size is more affected by temperature than quantity of grain nitrogen (Uhlen et al., 1998; Daniel and Triboi, 2000).

Grain protein content is inversely related to grain size (Guttieri et al., 2000; Erekul and Kohn, 2006). Whilst grain protein content increases under heat stress, the functionality of protein significantly decreases (Corbellini et al., 1997), affecting end-use quality. Total grain protein content of the crop decreases under heat stress because heat stress decreases grain yield (Stone and Nicolas, 1998; Castro et al., 2007). Heat stress also decreases the duration, but not rate, of protein deposition in the grain (Castro et al., 2007). The greatest increase in grain protein content in wheat occurs when heat stress is imposed early in grain filling (Castro et al., 2007). However, exposure to heat stress decreases synthesis of glutenin, while synthesis of gliadins remains stable or increases (Majoul et al., 2003). Heat stress also decreases the sedimentation index as an effect associated with increased protein content in grain, but with decreased levels of essential amino acids (Dias et al., 2008).

III. TOLERANCE MECHANISMS OF TERMINAL HEAT STRESS

Plants tend to reduce heat-induced damage by leaf rolling, leaf shedding, reducing leaf size, thickening leaves, reducing growth duration, transpirational cooling and other adjustments in morphology and ontogeny (Wahid et al., 2007). Plant responses to heat stress are mediated by an intrinsic capacity to endure basal thermotolerance and, after acclimation, the ability to gain thermotolerance. The capability of crop plants to survive and produce good grain yield under heat stress is generally regarded as heat tolerance (Wahid et al., 2007). Changes and mechanisms that enable the plants to cope with heat stress are given in Fig. 4.

FIG. 4. Proposed cellular events and signalling cascades in a plant cell responding to heat stress. Heat stress is perceived by perturbation to cellular equilibrium, which then activates signals possibly by hydrogen peroxide (H$_2$O$_2$), abscisic acid (ABA), calcium (Ca$^{2+}$) and nitric oxide (NO). These signals then induce synthesis of specific protein kinases, which activate more downstream responses such as changes in gene expression. The response to these signalling cascades also results in changes in plant metabolism, activation and synthesis of antioxidants, synthesis of heat shock proteins, and accumulation of osmoprotectants and solutes, and reduces senescence under heat stress. (Color figure available online).
A. Antioxidant Defense System

Reactive oxygen species (ROS)—superoxide radicals, hydroxyl radicals, and hydrogen peroxide—are produced in the cells in a natural fashion, but overproduction of these compounds can be harmful (Esfandiari et al., 2007). Heat stress triggers the production and accumulation of ROS (Sairam et al., 2000; Mittler, 2002; Almeselmani et al., 2009). Hence their detoxification by antioxidant systems is important for protecting plants against heat stress (Asada, 2006; Suzuki and Mittler, 2006).

The antioxidant defense system in plants involves both enzymatic and non-enzymatic antioxidant systems. The enzymatic antioxidant system includes ascorbate peroxidase, dehydroascorbate reductase, glutathione S-transferase, superoxide dismutase, catalase, guaiacol peroxidase, and glutathione reductase (Noctor and Foyer, 1998). Non-enzymatic antioxidants include glutathione, ascorbate and tocopherols.

Superoxide dismutase converts O$_2^-$ to hydrogen peroxide, whereas catalase and peroxidases breakdown hydrogen peroxide. Catalase eliminates hydrogen peroxide by catalyzing its decomposition to H$_2$O and O$_2$. Both guaiacol peroxidase and ascorbate peroxidase can detoxify hydrogen peroxide, but both enzymes need hydrogen peroxide to be scavenged by reducing agents. Ascorbate helps scavenge OH, O$_2^-$ and hydrogen peroxide for the ascorbate-peroxidase-mediated reactions, while guaiacol scavenges ROS for guaiacol peroxidase-mediated reactions (Goyal and Asthir, 2010). Balla et al. (2009) demonstrated that upon exposure to heat stress, during the reproductive phase, activities of enzymatic antioxidants were substantially increased in heat-tolerant genotypes of wheat. The activities of catalase and superoxide dismutase have been correlated with heat stress (34/22°C) during the reproductive phase (Zhao et al., 2007), as well as the capacity to acquire thermotolerance (Almeselmani et al., 2009). Likewise, protection of wheat plants from heat-induced oxidative damage during the reproductive phase has also been correlated with non-enzymic antioxidants, such as ascorbate (Sairam et al., 2000).

B. Molecular Basis of Heat Tolerance

Expression of heat shock proteins (HSPs) is the most studied molecular response under heat stress. HSPs save proteins from heat-induced aggregation and thus during the recovery period, facilitates their re-folding (Hendrick and Hartl, 1993; Schöffl et al., 1998; Feder and Hofmann, 1999; Maestri et al., 2002). Expression of HSP genes is a fundamental response to heat stress (Rampino et al., 2009). When exposed to high temperature (>35°C), normal protein synthesis in wheat is reduced, but HSPs are produced (Blumenthal et al., 1994).

In wheat genotypes grown at 32 to 35°C, Nguyen et al. (1994) detected messenger RNAs encoding a major class of low molecular weight HSPs—HSP 16.9. In another study, where several wheat varieties were exposed to 3.2 to 3.6°C higher than normal, HSP 18 accumulated in developing grains of heat-tolerant varieties more than susceptible types (Sharma-Natu et al., 2010). Sumesh et al. (2008) also observed higher HSP 100 content at elevated temperature in a relatively tolerant variety.

Dehydrin proteins belong to Group 2 late embryogenesis abundant proteins (LEA). Dehydrins help to stabilize macromolecules against heat-induced damage (Brini et al., 2010). In wheat, for instance, DHN-5 protein helped protect and stabilize key enzymes to start metabolism (Brini et al., 2010).

C. Stay-green

Leaf senescence starts early in response to heat stress, particularly when these stresses occur during post-flowering stages of grain filling. Therefore, maintenance of leaf chlorophyll and photosynthetic capacity, called ‘stay-green,’ is considered an indicator of heat tolerance (Fokar et al., 1998). Because the loss of chlorophyll is associated with less assimilation of current carbon into grains (see above), stay-green genotypes should be better able to maintain grain filling under elevated temperatures. Certain stay-green sorghum genotypes have been found to contain higher specific leaf nitrogen contents, indicating that this trait is correlated with shoot nitrogen content (Borrell et al., 2001). The stay-green trait has been evaluated in several crops (Harris et al., 2007; Kumari et al., 2007), but breeding for this trait has been limited in wheat. Although three components—chlorophyll content at anthesis, duration of senescence, and rate of senescence—determine the stay-green feature during heat stress, the rate of senescence, not the start of senescence, is important component of stay-green (Harris et al., 2007).

IV. STRATEGIES TO IMPROVE HEAT STRESS TOLERANCE

Development and selection of crop varieties is, most often, aimed at improving yield under existing climatic conditions. With the changing climate, in particular episodes of high temperature during the reproductive phase, ideotypes with physiological, morphological, and molecular traits unique for heat tolerance are required (Semenov and Halford, 2009). Strategies to improve heat stress tolerance in wheat include crop improvement through breeding and molecular tools, and agronomic and other management practices.

A. Selection and Breeding for Heat Tolerance

Expansion of genetic variability in the wheat gene pool is important for breeding programs aimed to improve heat tolerance during reproductive and grain-filling stages. Although several reports indicate the existence of genetic diversity for heat tolerance in conventional wheat varieties (Wardlaw, 1994; Fokar et al., 1998; Calderini et al., 1999; Gibson and Paulsen, 1999; Spiertz et al., 2006; Anjum et al., 2008; Zhao et al., 2008), new sources of genetic diversity must be explored. One option is to cross-breed wheat (Triticum aestivum) with its key ancestors, Aegilops tauschii and Triticum durum.
TABLE 3
QTLs identified for heat tolerance in wheat during the reproductive phase

<table>
<thead>
<tr>
<th>Trait</th>
<th>Chromosome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of senescence</td>
<td>2A</td>
<td>Vijayalakshmi <em>et al.</em> (2010)</td>
</tr>
<tr>
<td></td>
<td>6A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6B</td>
<td></td>
</tr>
<tr>
<td>Greenness at maximum senescence</td>
<td>4B</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3A</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6B</td>
<td></td>
</tr>
<tr>
<td>SPAD chlorophyll content</td>
<td>7B</td>
<td></td>
</tr>
<tr>
<td>Fv/Fm chlorophyll fluorescence</td>
<td>7A</td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td>4A</td>
<td>Pinto <em>et al.</em> (2010)</td>
</tr>
<tr>
<td>Canopy temperature</td>
<td>4A</td>
<td></td>
</tr>
<tr>
<td>Number of grains</td>
<td>1A, 2A, 3B, 4A, 5B</td>
<td></td>
</tr>
<tr>
<td>Grain weight</td>
<td>1B, 2B, 3B, 5A, 6D</td>
<td></td>
</tr>
<tr>
<td>Days to flowering</td>
<td>2D, 7D</td>
<td>Mason <em>et al.</em> (2010)</td>
</tr>
<tr>
<td>Stay-green</td>
<td>1A, 3B, 7D</td>
<td>Kumar <em>et al.</em> (2010)</td>
</tr>
</tbody>
</table>

Through conventional breeding, genetic variability for heat tolerance amongst breeding lines/varieties can be identified. Breeding and pre-breeding involves selection and genotype screening. Selecting genotypes for high yield under heat stress is the most important strategy; it is, however, expensive due to the high cost associated with conducting yield trials in controlled environments. Selection and screening can be based on characteristics associated with better yields under heat stress. The characters used as selection criteria for heat tolerance should be: (1) strongly correlated with grain yield under heat stress; (2) rapid, stable, and easy to measure; and (3) highly heritable (Edmeades *et al.*, 2001). Potential traits for screening heat tolerance in wheat are shown in Table 4.

Genotype screening based on electrolyte leakage, an index of membrane stability, from leaves subjected to extreme temperatures, is one of the rapid screening methods (Blum, 1988; Shanahan *et al*., 1990). Electrolytes are collected from stressed tissue soaked in deionized water and quantified by measuring electrical conductivity (Ibrahim and Quick, 2001). Correlation between membrane stability and grain yield under heat stress has been found (Blum *et al*., 2001).

Reduction of tetrazolium triphenyl chloride in mitochondria may also be used as an indicator of heat tolerance. Here, tetrazolium triphenyl chloride solution is vacuum-infiltrated into leaf tissues exposed to high temperature. Cell viability is quantified by the relative level of tetrazolium triphenyl chloride reduction to formazan, which is detected by spectrophotometer (Towill and Mazur, 1974).

Genotypes may also be screened for depression of canopy temperature, flag-leaf stomatal conductance and photosynthetic rate, which are highly correlated with field performance and grain yield under heat stress (Reynolds *et al*., 1994, 1998; Amani *et al*., 1996).

Landraces are varieties adapted to their native environment. Most of today’s wheat varieties have been derived from landraces (Trethowan and Mujeeb-Kazi, 2008). Significant variability for heat tolerance exists amongst landraces. For instance,

TABLE 4
Potential traits/characters for screening wheat for heat tolerance

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Trait/character</th>
<th>Correlated with yield</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Inhibition of meiosis</td>
<td>No</td>
<td>Saini <em>et al.</em> (1983); Zeng <em>et al.</em> (1985)</td>
</tr>
<tr>
<td>2</td>
<td>Photosynthesis rate</td>
<td>Yes</td>
<td>Reynolds <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>3</td>
<td>Leaf chlorophyll content</td>
<td>Yes</td>
<td>Reynolds <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>4</td>
<td>Canopy temperature depression</td>
<td>Yes</td>
<td>Shanahan <em>et al.</em> (1990); Reynolds <em>et al.</em> (1994, 1998); Amani <em>et al.</em> (1996); Blum <em>et al.</em> (2001);</td>
</tr>
<tr>
<td>5</td>
<td>Membrane stability</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Flag-leaf stomatal conductance</td>
<td>Yes</td>
<td>Reynolds <em>et al.</em> (1994)</td>
</tr>
<tr>
<td>7</td>
<td>Grain weight</td>
<td>No clear evidence</td>
<td>Tyagi <em>et al.</em> (2003); Singha <em>et al.</em> (2006); Dias and Lidon (2009);</td>
</tr>
<tr>
<td>8</td>
<td>Tetrazolium triphenyl chloride</td>
<td>No clear evidence</td>
<td>Towill and Mazur (1974)</td>
</tr>
<tr>
<td>9</td>
<td>Early heading</td>
<td>No clear evidence</td>
<td>Tewolde <em>et al.</em> (2006)</td>
</tr>
<tr>
<td>10</td>
<td>High temperature index</td>
<td>No clear evidence</td>
<td>Rane and Nagarajan (2004)</td>
</tr>
<tr>
<td>11</td>
<td>Stay-green</td>
<td>No</td>
<td>Reynolds <em>et al.</em> (2001); Xu <em>et al.</em> (2000)</td>
</tr>
</tbody>
</table>
heat-tolerant landraces tend to have higher leaf chlorophyll contents (Hede et al., 1999) and higher stomatal conductance. These materials may be used in breeding programs aimed to induce heat tolerance in wheat.

Maintaining grain yield under heat stress during grain filling is a measure of heat tolerance (Tyagi et al., 2003; Singha et al., 2006). In this regard, Dias and Lidon (2009) proposed that high grain-filling rate and high potential grain weight can be useful selection criteria for improving heat tolerance.

Tewolde et al. (2006) reported that early-heading varieties performed better than later-heading varieties because they (1) produced fewer leaves per tiller and retained more green leaves, (2) had longer grain-filling periods, and (3) completed grain filling earlier in the season when air temperatures were lower.

Mass screening of wheat genotypes for heat tolerance may also be done for the stay-green character (Reynolds et al., 2001). A visual rating of stay-green is quick and easy way for the plant breeders to screen on mass scale (Xu et al., 2000). Nonetheless, this trait may be a disadvantage as it is associated with the tendency to retain the stem reserves (Blum, 1998).

B. Genetic Engineering for Heat Tolerance

Genetic engineering involves the introduction of individual genes, of interest, into the candidate genotypes helpful in improving tolerance against heat stress (Barnabás et al., 2008). However, wheat’s complex genome has hampered research on genetic modification compared with other plant species.

Protein synthesis elongation factor in chloroplast (EF-Tu) has been related to heat tolerance in several crops. In wheat, cultivars that accumulated more EF-Tu at maturity tolerated heat stress better than those with less EF-Tu (Ristic et al., 2008). Fu et al. (2008) expressed a maize gene coding for plastidal EF-Tu in transgenic wheat. Transgenic wheat plants had less aggregation of leaf proteins and damage to thylakoids, and higher rate of CO₂ fixation compared with non-transgenic plants under heat stress. The study demonstrated that genes, other than HSP genes, may be used to improve heat tolerance in wheat.

C. Use of Molecular Markers

Most of the traits related with the yield and heat tolerance are controlled by several genes each with minor individual but significant effects when acting together. In wheat, the quantitative trait loci (QTLs) analysis has, partly, been hindered by large genome size (Bennett et al., 1982). The polyploid nature of the genome also makes molecular analysis complicated (Barnabás et al., 2008) due to repetitions of DNA sequences.

Natural genetic variation may be used through direct selection under heat stress during the reproductive phase or through QTL mapping and subsequent marker-assisted selection. QTL mapping allows assessment of numbers, locations, magnitude of phenotypic effects, and patterns of gene action (Vinh and Paterson, 2005).

Recently, several QTLs have been identified in wheat for heat tolerance during the reproductive phase (Table 3). For instance, Kumar et al. (2010) identified three QTLs for the stay-green character. Likewise, Vijayalakshmi et al. (2010) identified QTLs for senescence-related traits. Pinto et al. (2010) identified a QTL on chromosome 4A-a for canopy temperature under heat.

In addition to QTLs for senescence/stay-green traits, several have been mapped for wheat yield and its related traits under heat stress (Table 3). For example, under heat stress a QTL at the same location as the QTL for canopy temperature accounted for 17% of yield variation (Pinto et al., 2010). Likewise, Mason et al. (2010) identified 15 and 12 QTLs associated with yield and its associated traits in 2005 and 2006, respectively. The results suggest that for heat tolerance main spike should be used for the identification of QTLs genomic regions Mason et al., 2010).

V. MANAGEMENT STRATEGIES

Agronomic strategies for mediating future increases in ambient temperature include practices that conserve water (e.g., no tillage and stubble retention), fertilization during critical growth stages and timing of sowing. For example, continuous water supply to heat-stressed wheat helped sustain grain-filling rate, duration and size (Dupont et al., 2006). Of course, this is not possible in rainfed wheat growing regions if it doesn’t rain.

Application of nitrogen, phosphorus and potassium improve plant growth under moderate heat stress (Dupont et al., 2006). When nitrogen, phosphorous and potassium are applied post-anthesis, more protein accumulates in the grain at day/night temperatures of 24/17°C, but not at 37/28°C. Application of some micronutrients such as zinc, can also improve heat tolerance in wheat (Graham and McDonald, 2001). The timing of nutrient application to coincide with key developmental stages is already regularly practiced, such as nitrogen application when the spike is 1 cm in length.

Time of sowing is another important management strategy in some regions. Although periods of elevated temperature may occur during the growing season, grain filling usually occurs when seasonal temperatures are increasing. Early planting may avoid terminal heat stress so that grain filling occurs during cooler temperatures (Loss and Siddique, 1994).

Other management strategies for improving heat tolerance, such as application of exogenous signalling compounds, osmolytes and certain inorganic salts, are less likely to have a significant impact on broadacre agriculture, and are therefore not discussed here.

VI. CONCLUSION

High temperatures causing heat stress in wheat are expected to increase in frequency across the globe. Heat stress substantially affects grain setting, duration and rate, and ultimately grain yield. Nonetheless the timing, duration and intensity of heat stress determine its impact on grain yield. The adversities of heat stress can be minimized by developing tolerant genotypes and agronomic strategies.
HEAT STRESS IN WHEAT

Even though in wheat, mechanisms of heat tolerance on a physiological basis are relatively well-understood, research into assimilate partitioning and phenotypic flexibility are needed.

Molecular knowledge of response and tolerance mechanisms to harvest higher grain yields on a sustainable basis must be explored. Likewise, use of functional genomics approaches will be helpful in understanding the molecular basis of the response of wheat to heat tolerance.

Identifying allelic sources for heat tolerance and their introgression into elite lines through conventional breeding and modern biotechnological and molecular tools (Ortiz et al., 2008) are an important area for future research. The latest genomics resources combined with ecophysiological research may be helpful to understand the genotypes and the environment interactions. An integrated system approach should be designed to study the complex quantitative traits like yield stability under heat stress.

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