The effect of high temperature stress on male and female reproduction in plants

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ARTICLE INFO

Article history:
Received 28 November 2014
Received in revised form 28 May 2015
Accepted 16 June 2015
Available online 30 July 2015

Keywords:
Climate warming
Plant male reproduction
Pollen development
Reactive oxygen species

ABSTRACT

Humanity confronts a major transition in the global environment. With the continued rise in atmospheric greenhouse gases, and wide-spread land transformation, the climate of the planet could warm to levels that have not existed for tens of millions of years (Masson-Delmotte et al., 2013). Assuming business-as-usual emissions scenarios for greenhouse gases, atmospheric CO₂ levels will double-to-triple above current values of 400 ppm within two centuries which in turn will warm the global climate by a mean of 2.5 to 8 °C by the year 2200 (Stocker et al., 2013). Such warming is not unprecedented in Earth’s history; however, it is occurring more rapidly than exists in the geological record, and in combination with multiple other changes of major global significance (Sala et al., 2000; Masson-Delmotte et al., 2013). Such changes include increases in ground-level (tropospheric) ozone concentrations, deposition of nutrients that alter soil biogeochemistry, invasions by exotic species, widespread land conversion, and the direct effects of atmospheric CO₂ enrichment on plants and insects (Vitousek, 1994; Long et al., 2004; Ashmore, 2005; Foley et al., 2005; Long and Ort, 2010). Together, these global change drivers threaten to overwhelm the resilience of natural systems and human society, with consequences that will challenge human welfare (Parry et al., 2007; Rockström et al., 2009). Agriculture, in particular, is highly vulnerable to global change as the crops we depend upon are negatively impacted by a number of the drivers, most notably climate change (Easterling et al., 2007; Schlenker and Roberts, 2009; Lobell et al., 2014). Unlike natural systems, however, agriculture is under direct human control, and thus each element of a crop production system can be manipulated to offset deleterious impacts of global change, and in some cases, exploit opportunities that may arise in a globally-altered world (for example, higher atmospheric CO₂ can be exploited to boost photosynthesis and productivity) (Ainsworth et al., 2008; Lobell, 2014; Zhu et al., 2010).

The likelihood that agriculture can adapt to global change will depend upon an understanding of how predicted changes will impact agricultural crops and management systems (Deryng et al., 2014; Lobell and Tebaldi, 2014; Siebert and Ewert, 2014). Such understanding will enable people to target the most vulnerable aspects of the cropping system and to focus on developing appropriate solutions (Ainsworth et al., 2008; Lobell, 2014). At the crop level, this will require a detailed understanding of the mechanisms of plant responses to global climate change, including the genetic controls over responses to combinations of heat, drought and
elevated atmospheric CO₂ (Long and Ort, 2010; Lobell and Gouldij, 2012). Among the global change responses particularly well studied are the effects of climate warming and atmospheric CO₂ enrichment on photosynthesis, respiration, and growth (Long et al., 2004; Ainsworth et al., 2008). While these responses are noteworthy, they are not likely the most critical threat to food security. Instead, plant reproduction, appears to be among the most sensitive of the physiological components contributing to yield (Prasad et al., 2002, 2006a,b; Barnabas et al., 2008; Hedhly et al., 2009; Reguera et al., 2012; Lobell and Gouldij, 2012; Harsant et al., 2013; Sanchez et al., 2014). In both warm and cool season crops, reproductive processes are widely noted to fail completely between 30 and 40 °C; by contrast, photosynthesis and vegetative growth of warm season crops (particularly C₄ crops) exhibit a thermal optimum of 30 to 36 °C, and it is not uncommon to observe crops with good vegetative growth and photosynthesis being completely sterile (Peng et al., 2004, 2002, 2006a,b; Cowling and Sage, 1998; Sage and Kubien, 2007; Sage et al., 2011; Harsant et al., 2013; Oort et al., 2014; Sanchez et al., 2014). Indeed, current models of crop responses to climate warming predict that for every 1 °C rise in mean local temperature during the growing season, average grain yields of our major crops such as maize and rice will decline from 5% to 17%, in part due to heat induced sterility (Peng et al., 2004; Sheehy et al., 2006; Hedhly et al., 2009; Challinor et al., 2014; Lobell and Tebaldi, 2014).

It has been argued that plant reproductive sterility is a major threat to global food security in a warming world (Reguera et al., 2012; Bita and Gerats, 2013). If this is the case, substantial effort would be warranted to identify mechanisms controlling the sterility response and develop ways to overcome the problem, be it through selective breeding or genetic engineering of new, heat tolerant varieties, or via adoption of management techniques that avoid exposure to sterilizing events. Here, we survey the literature on the effect of high temperature (HT) on plant reproduction in general. We then review the response of heat stress on male and female reproductive development in crop and model plant species to highlight our current understanding of cellular and molecular mechanisms controlling the heat sterility response. Our main objective is to provide breeders and molecular engineers with an understanding of the bottlenecks for successful reproductive development at HT.

2. High temperature and plant reproduction

2.1. General patterns

The unfavourable influence of HT on crop yields results from injury during development to reproductive units that contribute to harvest index and the responses vary with the duration and severity of the heat stress (Barnabas et al., 2008; Hedhly et al., 2009; Harsant et al., 2013). High temperatures reduce the number of flowering branches that are initiated, and thus the number of flowers per plant (Dawson and Wardlaw, 1989; Allen et al., 1995; Reddy et al., 1992; Young et al., 2004; Prasad et al., 2001, 2002; Harsant et al., 2013). Day time temperatures as low as 30 °C can induce abortion and subsequently abscission of developing floral buds as well as fruits in numerous legumes, (Konsens et al., 1991; Ahmed et al., 1992; Gross and Kigel, 1994; Guilioni et al., 1997; Djanaguiraman et al., 2013), tomato (Levy et al., 1978; Charles and Harris, 1972; Warner and Erwin, 2001), cotton (Reddy et al., 1992), pepper (Aloni et al., 2001), and canola-related species (Angadi et al., 2000; Young et al., 2004; Faraji et al., 2008). Fruit and seed dry weight are also reduced at temperatures ranging from 30 to 38 °C (Wardlaw, 1994; Prasad et al., 2002, 2008; Harsant et al., 2013; Prasad and Djanaguiraman, 2014).

Within developing flowers, male and female organs are sensitive to temperatures ≥30 °C. The impact of HT on the male reproductive development has been the primary focus of crop research for many years as it has been viewed as the most vulnerable (Sharkey and Schrader, 2006; Dolferus et al., 2011). The enhanced sensitivity of the male reproductive process is observed when reciprocal crosses using pollen from plants treated with HT reduces yields significantly more than when female plants treated with the same temperature are the receptor plants (Dupuis and Dumas, 1990; Peet et al., 1998; Young et al., 2004; Endo et al., 2009; Devarsivatham et al., 2012). Moreover, many studies on warm-season crop plants such as rice, sorghum, and soybean demonstrate temperatures in the mid-to-upper 30s °C result in reduced yields that are highly correlated with declines in pollen production as well as viability as indicated by viability stains and assessment of in vitro and in vivo pollen tube growth (Matsui et al., 1997; Prasad et al., 1999, 2001, 2002, 2003, 2006b; Djanaguiraman et al., 2013; Maruyama et al., 2013; Prasad and Djanaguiraman, 2014; Singh et al., 2015). This severity of the impact of HT on male reproduction and crop yields is best illustrated in rice where high day (≥32 °C) and night (≥29 °C) temperatures drastically reduce or eliminate yields solely through their negative effect on one or more of the sensitive stages of male reproduction (Satake and Yoshida, 1978; Peng et al., 2004; Shah et al., 2011; Laborte et al., 2012; Teixeira et al., 2012; Bagha, 2014; Nguyen et al., 2014). Notably, the major rice growing regions currently experience both high day and night temperatures during flowering (Fig. 1; Peng et al., 2004; Sheehy et al., 2006; Wassmann et al., 2009; Welch et al., 2010; Shah et al., 2011; Laborte et al., 2012; Teixeira et al., 2012; Bagha, 2014).

Although male reproduction has generally been viewed as more sensitive than female reproduction at HT, data indicates that female tissues as well as processes important for development of the male (pollen tube) within female tissues after pollination are also sensitive to HT (Snider and Oosterhuis, 2011; Gupta et al., 2015). The mechanisms regulating failure of male and female reproductive development and post-pollination processes up to the time of fertilization during exposure to HT are not clearly understood. This observation is alarming given the recognized importance of these processes to both current agriculture and sustainability of future agriculture in a warmed-world (Barnabas et al., 2008; Hedhly et al., 2009; Snider and Oosterhuis, 2011; Giorno et al., 2013; Gourdji et al., 2013).

2.2. Anther and pollen development in elevated temperatures

The anther is the region of the male reproductive structure that functions to produce pollen (reviewed in Ma, 2005; Wilson et al., 2011). It is composed of four fused lobes, each made of five cell layers that develop shortly after stamen initiation. Anther and pollen development from meiosis onward is illustrated in Fig. 2. The innermost layer of the anther is where the microspore mother cells develop and each mother cell will undergo meiosis to form uninucleate microspores that then undergo two successive mitotic divisions to give rise to a mature pollen grain. Two cell layers surrounding the microspore mother cells in anthers are the tapetum and the middle layer. Both of these layers are ephemeral and will undergo a programmed cell death after pollen wall development has ensued. The fourth cell layer of the anther, the endothecium, is positioned between the epidermis and middle layer. Endothecial cells as well as the stomium, which is a region of specialized epidermal cells, play crucial roles in anther dehiscence and thus pollen release. Dehydration of anthers facilitates anther opening, a process that is enabled by separation of stomium cells and retraction of the endothecium cells. Endothecial retraction is
facilitated by secondary cell wall thickenings (Wilson et al., 2011).

Knowledge of anther/pollen structure as well as the timing of development is essential to assess factors that lead to male sterility during HT stress or enable thermotolerance. The timing of anther maturation and duration of each particular stage of pollen development is species dependent and varies with environmental conditions (Raghavan, 1988; Hill, 1996; Harsant et al., 2013). Studies assessing the cellular and developmental aspects of anther/pollen development and the influence of HT on these parameters do so in the context of anther length (Raghavan, 1988; Hill, 1996; Harsant et al., 2013), and Alexander’s triple stain has been used as a rapid and effective way to stage pollen development as a function of anther length (Harsant et al., 2013). Other investigators use floral bud length as a proxy for anther/pollen development (Giorno et al., 2013; Smyth et al., 1990), the ‘days-before-anthesis’ as a guide for predicting the stages of anther/pollen development (Satak and Yoshida, 1978; Sakata et al., 2000; Jain et al., 2007; Giorno et al., 2013) or other phenotypic markers (Jagadish et al., 2014). The most important information from these types of studies is that anther development within a flower can span up to two weeks in wheat and tomato (Saini and Aspinall, 1982; Giorno et al., 2013), nine days in rice (Satak and Yoshida, 1978), and a week in barley and sorghum (Sakata et al., 2000; Jain et al., 2007). Meiosis or uninucleate microspore biogenesis can each take one or more days to complete (Finch and Bennett, 1972; Bennett and Kaltsikes, 1973). The length of time it takes an anther to develop in a flower and the duration for all flowers to complete their maturation on a plant has important implications for managing the impact of elevated day and night temperatures on male reproduction.

2.2.1. High temperature and uninucleate microspore development

Amongst the various stages of pollen development, the uninucleate stage is viewed as most sensitive to HT (Dolferus et al., 2011). Microscopic confirmation of abortion at this stage following exposure to temperatures ≥30 °C has been reported for cereal and eudicot crops to include rice (Fig. 3D; Bagha, 2014), wheat (Saini and Aspinall, 1982; Saini et al., 1984), sorghum (Jain et al., 2007), barley (Sakata et al., 2000; Oshino et al., 2007), snap beans (Suzuki et al., 2001), cowpea (Ahmed et al., 1992), tomato (Iwahori, 1965), Brachypodium distachyon (Harsant et al., 2013), cotton (Min et al., 2014), and garlic (Mayer et al., 2015). The uninucleate stage is more sensitive in the low 30 °C range in cool-season crops such as barley, wheat, and garlic whereas temperatures in the mid-to-upper 30 °C range effect development in warm-season crops. Uninucleate microspores also abort during cold and drought stress and this has lead to the designation of this stage as ‘an Achilles tendon’ of plant reproduction (Dolferus et al., 2011).

To further investigate the sensitivity of the uninucleate stage of pollen development, we conducted a preliminary screen that examined pollen viability at day/night growth chamber and tissue temperatures of 36/24 °C in a range of crop and wild species from hot climates. Substantial (>70%) pollen abortion at 36 °C occurred during the uninucleate stage (Figs. 3 and 4) in all species to include a maize (C₄) variety from the Mojave desert regions of the American southwest (Yuman Yellow) and a second C₄ species, Amaranthus cruentus; two chilli pepper species from warm, lowland climates of the sub-tropics; Teff, from lowland Ethiopia; and tepary bean (Phaseolus acutifolius), the most heat tolerant Phaseolus species. In tepary bean, there was evidence for reduced levels of pollen...
abortion (70% at 36 °C; Fig. 4) compared to the more temperate scarlet runner bean (*Phaseolus coccineus*), which had close to 100% abortion at 36 °C. We also examined a wild gourd species (*Cucurbita palmata*, coyote gourd) that produces flowers on vines that creep over the desert floor during the summer in the American Southwest. Day-time surface and air temperatures routinely exceed 45 °C near the *C. palmata* flowers (R Sage, unpublished), indicating a high potential for heat sterility, unless the plants have well-established tolerance mechanisms. Surprisingly, *C. palmata* exhibited 90% pollen abortion at 36 °C, indicating its reproduction is no more tolerant of elevated temperatures than many of the crop species from moderate to warm climates. Given the wide taxonomic diversity of the species examined in this screen, it is apparent that the uninucleate stage of pollen development is indeed highly sensitive to HT.

Developing anthers are a very strong resource sink and the uninucleate microspore stage is particularly susceptible to HT because tapetum cells and microspores are actively engaged in processes well known to be negatively impacted by HT—DNA, carbohydrate, protein, and lipid synthesis, and metabolite transport (Ma, 2005). Tapetal cells and microspores are symplastically isolated from other anther tissues and tapetal cells are highly metabolic in order to provision metabolites to developing microspores (Ma, 2005). The high metabolic and transport activity of the tapetum is evidenced by the large numbers of plastids, mitochondria, peroxisomes, and extensive endomembrane and cytoskeletal system involved in processing and transporting metabolites (Ma, 2005; Ariizumi and Toriyama, 2011; Bagha, 2014). Metabolism during the uninucleate stage generates substantial reactive oxygen species (ROS) in anther tissues, with the highest levels occurring in the tapetum and microspores. ROS scavenging antioxidants are essential to maintain an environment where ROS contribute to integration of development under ambient conditions (Cheeseman, 2007; Nguyen et al., 2009; Frank et al., 2009; Nguyen et al., 2010; Ariizumi and Toriyama, 2011; Mittler et al., 2011; Hu et al., 2011; Zhang et al., 2012; Bagha, 2014). Mutations in genes encoding proteins involved in carbohydrate assimilation, synthesis and secretion of proteins and lipids, ROS homeostasis, and pollen wall synthesis result in uninucleate microspore abortion (Ma, 2005; Ariizumi and Toriyama, 2011; Wilson et al., 2011; Hu et al., 2011). A finely tuned cell death program of tapetal cells terminates their role in pollen development and mutations in genes affecting the timing of tapetal cell death also result in abortion of uninucleate microspores (Ma, 2005).

Alterations in carbohydrate metabolism, reductions in starch levels of pollen grains and mistimed programmed cell death of the tapetum are traits associated with HT-induced microspore death (Dolferus et al., 2011; Bita and Gerats, 2013; De Storme and Geelen, 2014). Given the critical role of the tapetum in provisioning of carbohydrates to uninucleate microspores, researchers have posited that microspore death is caused by reduced transfer of carbohydrates to microspores from the tapetum (Dolferus et al., 2011; Parish et al., 2012; De Storme and Geelen, 2014). To test this hypothesis, investigators have assessed factors
influencing carbohydrate partitioning to microspores under HT and other abiotic stresses. High temperature stress results in changes in carbohydrate metabolism in Capsicum (Aloni et al., 2001) and Sorghum anthers that includes reduced expression of cell wall invertase genes in Sorghum tapetum and pollen (Jain et al., 2007, 2010) and reduced cell wall invertase activity in Capsicum pollen (Aloni et al., 2001). Cold and drought stress imposed at the microspore stage of pollen development also results in down-regulation of cell wall invertases and monosaccharide transporter genes in the tapetum of wheat and rice (Dorion et al., 1996; Sheoran and Saini, 1996; Ji et al., 2011; Nguyen et al., 2010). The negative impact of HT and other stresses on the timing of tapetal cell death and tapetal invertase activity is proposed to limit provision of compounds, to include hexose, to developing pollen and it has been hypothesized that this results in microspore starvation and subsequently, abortion (Parish et al., 2012; De Storme and Geelen, 2014). Mistimed tapetum programmed cell death or down-regulation of invertase genes are not universally associated with abortion of uninucleate microspores at HT (Endo et al., 2009; Bagha, 2014; Min et al., 2014). Alterations in tapetal death and carbohydrate metabolism in anthers following exposure to HT, to include invertase activity in some species, may be only part of a complex response wherein a network that normally senses elevated temperatures and re-establishes cellular homeostasis subsequently fails.

Heat sensing and response processes that generate a new cellular equilibrium to enable survival following heat stress in vegetative tissues involve a very well coordinated transcriptional reprogramming of gene expression (Saidi et al., 2011; Mittler et al., 2012; Fragkostefanakis et al., 2014). High temperatures enhance membrane fluidity and calcium influx that activates the plasmamembrane ROS producing NADPH oxidase (Konigshofer et al., 2008). Physical changes in membranes, cellular calcium and ROS, to include H$_2$O$_2$, stimulate a network of signal transduction pathways, heat shock proteins, and transcriptional regulators to re-establish cellular homeostasis (Saidi et al., 2011; Mittler et al., 2012; Fragkostefanakis et al., 2014). Uncoupling of metabolic pathways induced at HT, to include those involved in carbohydrate metabolism, result in reduced cellular energy levels and increased ROS production (Saidi et al., 2011; Mittler et al., 2012). Excessive ROS levels that could lead to cell death are prevented by ROS-scavenging pathways and molecules that can be stimulated by ROS production (Karuppanapandian et al., 2011).

Analyses of genome-wide expression profiles of anthers exposed to HT during the uninucleate stage of development have
been reported for tomato, rice, and cotton (Endo et al., 2009; Frank et al., 2009; Min et al., 2013, 2014). In all three species, elevated temperatures modulated expression of genes associated with the heat sensing and response network (Endo et al., 2009; Frank et al., 2009; Min et al., 2013, 2014). Researchers identified candidate genes that might induce male sterility or confer thermotolerance in anthers/pollen. Notably, multiple genes were identified in each study; however, there is no consensus between studies in these gene candidates reflecting either species differences or the wide variation in HT treatments. Global transcriptomes for tomato and cotton were generated for HT treatments that resulted in partial abortion of uninucleate pollen grains whereas the HT treatments resulted in complete pollen failure in rice.

Gene expression profiles were assessed in tomato and cotton anthers containing developing uninucleate pollen following short-term and long-term exposure to HT, respectively. Comparative analyses between anthers from heat-tolerant and sensitive tomato cultivars after exposure to 43–45°C for 2 h, did not reveal any significant differences from ambient temperature even though the HT treatment resulted in a higher rate of pollen abortion in the sensitive line (Frank et al., 2009). The temperature tolerant cultivar exhibited higher basal levels of some heat shock factors and heat shock proteins at ambient temperatures relative to the basal levels of the sensitive line leading the authors to conclude that these genes may be prospects to enhance pollen thermotolerance (Frank et al., 2009). In contrast to the short-term heat treatment applied to tomatoes (Frank et al., 2009), gene expression of cotton anthers from heat-tolerant and sensitive cotton lines was assessed following seven days exposure of flowers to day/night temperatures ranging from 31 to 39°C/29 to 31°C, approximately 0–4°C/1–11°C above control conditions (Min et al., 2013, 2014). These treatments induced a down-regulation of genes involved in DNA methylation and histone demethylation more so in the sensitive line leading to the conclusion that differential epigenetic modifications resulted in enhanced DNA stability and pollen viability in the temperature tolerant line (Min et al., 2014). Anther glucose and starch metabolism transcripts were differentially modulated between the two lines at HT, which, combined with lower glucose and starch levels in anthers of the sensitive line, led Min et al. (2014) to posit that pollen abortion in the sensitive line also resulted from a lack of glucose (Min et al., 2013, 2014). Overexpression of a casein kinase gene that exhibits starch synthase kinase activity in cotton was correlated with reduced glucose levels and implicated as a key player in pollen abortion (Min et al., 2013, 2014). The HT-induced casein kinase transcription was posited to act in concert with phytochrome-interacting transcription factors as master switches to disrupt auxin homeostasis in the heat sensitive line (Min et al., 2014). Auxin homeostasis is essential for successful pollen development (Sundberg and Ostergaard, 2009). High levels of the ROS species H2O2 were also associated with partial pollen abortion in the sensitive cotton line following HT treatment (Min et al., 2013). ROS and hormone signalling networks are highly integrated to produce a morphogenetic response during environmental stress (Mittler et al., 2011).

Chronic exposure of rice anthers to high day/night temperatures ≥36/29°C during development of uninucleate microspores results in complete pollen abortion in the absence of differences in tapetum and microspore starch accumulation and invertase activity between ambient and HT treatments (Endo et al., 2009; Bagha, 2014). Ultrastructural observations demonstrated that these HT treatments resulted in accumulation of H2O2 initially in the developing wall and nucleus of uninucleate microspores as well as the anther locule prior to abortion of rice pollen (Bagha, 2014). This ROS species was secondarily detected at the site of secretion of pollen wall precursors into the locule as well as the tapetum nucleus in the absence of changes in the timing of tapetal cell death (Bagha, 2014). Subsequently, uninucleate microspores became necrotic and collapsed (Fig. 5A). In the absence of acclimation to HT, high levels of apoplastic and nuclear localized H2O2 in rice microspores were proposed to induce a signal cascade leading to pollen death by necrosis (Bagha, 2014) as occurs in vegetative tissue (Gechev et al., 2006; Ashtamker et al., 2007; Suzuki and Mittler, 2006). Excessive H2O2 in the nucleus of uninucleate rice microspores may have also resulted in cell death by altering the redox balance needed in part to suppression of genes essential for metabolism of lipids needed for pollen wall synthesis and high H2O2 in the tapetum nucleus could modulate expression of these genes (Endo et al., 2009; Bagha, 2014). Finally, elevated levels of H2O2 at the tapetum/anther locule interface and the developing microspore cell wall may have challenged pollen wall integrity by directly oxidizing proteins and lipids essential for wall synthesis. The appearance of H2O2 in microspores during chronic exposure to high day/night temperatures, prior to their appearance in the tapetum indicates that developing microspores also perceive and respond to HT. Therefore, tapetal defects may not be the sole basis for HT induced male-sterility in all species, as has been proposed (Parish et al., 2012; De Storme and Geelen, 2014). This conclusion has important implications for research efforts aimed at engineering of thermotolerance of pollen development during the uninucelate stage.

### 2.2.2. High temperature and meiosis

In comparison to studies on the effect of HT on anthers and pollen during the uninucleate stage of development, much less is known about the impact of HT on anthers during meiosis. Analysis of genome expression profiles following short-term exposure of tomato and rice anthers to day temperatures that have minimal effect on pollen quality (viability) during meiosis (32 and 40°C, respectively) demonstrated that HT resulted in the modulation of heat sensing and response genes (Bita et al., 2011; Zhang et al., 2012) as noted for anthers containing uninucleate microspores that were subjected to HT (Frank et al., 2009; Endo et al., 2009; Min et al., 2013, 2014). The quantity of pollen grains that can develop in an
anther is first determined by the number of microspore mother cells initiated that undergo meiosis. Abiotic stress, including HT, is well known to have a negative impact on cell division and elongation (Poiters et al., 2007; Livanos et al., 2012; De Storme and Geelen, 2014; Bagha, 2014). The negative response of HT on mitosis gives rise to smaller anthers with fewer cells to undergo meiosis and this likely translates into fewer pollen grain numbers in many crop species and their relatives (Prasad et al., 1999, 2001, 2002, 2006b; Oshino et al., 2007; Devasirvatham et al., 2012; Harsant et al., 2013). When anthers develop at the upper limit of the HT threshold for a given species, meiosis is negatively impacted as indicated by microscopic studies on crops such as tomato (Iwahori, 1965), wheat (Saini et al., 1984), barley (Sakata et al., 2000), and rice (Bagha, 2014), and the C3 model grass Brachypodium distachyon (Harsant et al., 2013). Notably, failure during meiosis occurs when temperatures are elevated above those that induce abortion of unineucleate microspores in the same species (T Sage, unpublished observations). In barley, the arrest in anther cell division during meiosis occurs after increasing both day and night temperatures 10°C at the time of panicle initiation to 30/25°C for 10 days (Oshino et al., 2007, 2011). Cell division arrest in barley anthers was associated with alterations in auxin biosynthesis and a down-regulation of genes encoding DNA replication licensing factors, DNA polymerase, histones, 60S ribosomal proteins, and DEAD/DEAH box RNA helicases (Oshino et al., 2007, 2011). The same HT regime was posited to also result in premature progression to meiotic prophase of microspore mother cells and an early tapetal death (Oshino et al., 2007). Temperatures of 36°C for 48 h affected meiosis by inducing the formation of dyads and triads containing diploid gametes in a diploid interspecific hybrid rose (Pecrix et al., 2011) leading to the hypothesis that elevated temperatures disrupt spindle orientation (De Storme and Geelen, 2014). The formation of dyads and triads does not occur in all species at HT, however. Elevated temperatures did not induce the formation of dyads and triads in rice following exposure of anthers to temperatures that induce male sterility during meiosis (36/29°C); this temperature treatment did have a negative impact on cell wall formation during meiosis potentially through an effect on the cytoskeleton (Bagha, 2014).

A prominent phenotype of barley, rice, and other monocot anthers exposed to HT during meiosis is an empty anther locale (Fig. 5B; Sakata et al., 2000; Harsant et al., 2013; Bagha, 2014). In rice, the empty locale results from ROS-induced autophagic programmed cell death that removes all cellular components of tetrads (Bagha, 2014). Autophagy plays a central role in reproductive development under normal conditions (Bassham et al., 2006) and ROS-induced autophagy during abiotic stress, to include HT, leads to cell degradation and recycling of cellular components that can re-establish cellular homeostasis (Hauser et al., 2006; Pérez-Pérez et al., 2012; Zhou et al., 2014). Silencing of a transcription factor that is important for heat tolerance, WRKY33, inhibits HT-induced autophagy in vegetative tissue and it has been hypothesized that stress-initiated pathways may converge to activate and induce WRKY33 for induction of autophagy (Zhou et al., 2014).

2.2.3. High temperature and mature pollen

When anthers develop to maturity during exposure to day temperatures >30°C and night temperatures >29°C, it is not uncommon for them to contain mature but nonviable pollen (Schoper et al., 1986; Jagadish et al., 2010; Prasad and Djanaguiraman, 2011; Djanaguiraman et al., 2013, 2014; Harsant et al., 2013; Song et al., 2015). Even under the best of circumstances, pollen viability rapidly declines (minutes and hours) after dispersal and HT exacerbates the rate of this decline (Song et al., 2001; Stone et al., 1995; Pacini et al., 1997; Rodriguez-Riano and Dafni, 2000; Dafni and Firmage, 2000). While the mechanisms that lead to a decline in viability of mature pollen are not known, there is evidence that ROS production is involved. In Sorghum and soybean, exposure of high day and night temperatures ≥38/28°C for 7–10 days increases ROS in pollen, alters phospholipids and decreases pollen membrane integrity (Prasad and Djanaguiraman, 2011; Djanaguiraman et al., 2013, 2014). The loss of pollen viability and membrane integrity following chronic exposure to HT in these two crop species is putatively due to ROS accumulation (Prasad and Djanaguiraman, 2011; Djanaguiraman et al., 2013, 2014). Djanaguiraman et al. (2013) and Prasad and Djanaguiraman (2011) note changes in pollen membrane stability may result from alterations in phospholipid saturation levels and phospholipid species that make membranes more leaky, prone to ROS attack and disrupt tip-growth of pollen tubes during germination. Pollen plastids are critical because of their role in fatty acid synthesis, carbon balance, and energy supply (Pacini, 1996; Pacini et al., 2006; McConn and Browse, 1996; Prabhakar et al., 2010) and changes in pollen phospholipid content in Sorghum and soybean following chronic exposure to HT indicate that plastid function, to include lipid biosynthesis, was altered. This hypothesis is supported by recent reports that a plastid-targeted gene involved in ameliorating the negative effect of ROS in pollen plastids is essential for normal lipid biosynthesis and pollen viability following chronic exposure of developing anthers to 32°C in Arabidopsis (Lundsgaard-Nielsen et al., 2014).

2.2.4. High temperature, anther dehiscence, and pollen dispersal

If temperatures are conducive to mature anther and pollen development, HT at anther dehiscence can cause problems by

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**Fig. 5.** (A) Rice anther with aborted uninucleate microspores (day-time temp, 36°C; black arrowheads; Oryza glaberrima). (B) Rice anther with no pollen due to autophagic removal of tetrads during final stages of meiosis (day-time tissue temp, 36°C, Oryza sativa c.v. Notohikari). Asterisk marks empty anther locale. Bar, 100 μm.
Chloride (CeCl3). CeCl3 reacts with H2O2 to form black deposits of CeH4O4 which is visible with the transmission electron microscope. The presence of black deposits of CeH4O4 (white arrows) confirms the presence of H2O2 in the cell wall of septum cell walls that fail to degrade in a high temperature sensitive cultivar. Bars, (A) 50 μm; (B, C) 5 μm; (D, E) 50 nm. E, epidermis; En, Endothecium; L, anther locale; P, pollen grain; S, stomium.

Fig. 6. The impact of high temperatures on rice anther dehiscence. (A) Anther with pollen. The region of the anther where cell wall lysis occurs to enable pollen release from each of the anther locules is enclosed in a box. (B) High magnification of the boxed region in image A illustrating lysis of cells walls to enable pollen release from anther locules at permissive temperatures (black arrows). Cell wall degradation occurs during filament elongation 10–20 min prior to anther dehiscence. (C) Magnified view of boxed region in image A illustrating absence of cell wall lysis in a high temperature sensitive cultivar. Cell walls not degraded at high temperature are marked with black arrowheads. (D) Transmission electron micrograph illustrating degraded septum cell walls (black arrows) prior to anther dehiscence under permissive temperatures. (E) Transmission electron micrograph demonstrating intact cell walls in a high temperature sensitive cultivar. Anthers illustrated in image D and E were incubated with Cerium Chloride (CeCl3). CeCl3 reacts with H2O2 to form black deposits of CeH4O4 which is visible with the transmission electron microscope. The presence of black deposits of CeH4O4 (white arrows) confirms the presence of H2O2 in the cell wall of septum cell walls that fail to degrade in a high temperature sensitive cultivar. Bars, (A) 50 μm; (B, C) 5 μm; (D, E) 50 nm. E, epidermis; En, Endothecium; L, anther locale; P, pollen grain; S, stomium.

Impairing pollen release. Air and tissue temperatures >30 °C prior to anther opening can severely delay or prevent anther dehiscence in tomato, rice, and Brachypodium (Rudich et al., 1977; Matsu et al., 2001; Porch and Jahn, 2001; Saini and Aspinall, 1982; Jagadish et al., 2007, 2010, 2014; Harsant et al., 2013; Maruyama et al., 2013; Min et al., 2013; Song et al., 2015). A minimum number of pollen grains are required for fertilization because of pollen tube attrition during growth en route to ovules (Erbar, 2003). Thus, inhibited anther dehiscence translates into reductions in yield because it decreases pollen deposition below the minimum threshold. High temperature-induced reductions in tomato fruit set result primarily from alterations in endothecium cell wall development that prevent anther dehiscence and this effect is more significant than the negative impact on pollen development (Rudich et al., 1977; Min et al., 2013, 2014). In rice, the minimum pollen grains needed to be deposited on the stigma to effect fertilization is 10 (Satake and Yoshida, 1978). Indehiscence of rice anthers at HT is particularly problematic and considerable attention has been given to dissect factors that influence anther dehiscence and the HT sensitivity of the process (Matsu et al., 1999, 2001; Matsu and Omasa, 2002). The severity of this problem is underscored by efforts to identify and introduce an early-morning flowering QTL from wild rice into cultivated rice lines that enables plants to escape mid-day heat during flowering (Sheehy et al., 2007; Ishimaru et al., 2010; Hirabayashi et al., 2014). Early studies examining the impact of HT on anther dehiscence in rice led to a long-standing hypothesis that HT impairs the process by preventing pollen swelling, which is proposed to cause the splitting open of adjacent anther locules where pollen resides (Matsu et al., 1999). Reassessmet of this original study indicates that pollen measurements prior to dehiscence were made on pollen from excised florets and results noted “repeated pollen swelling and contraction” (Matsu et al., 1999). Reiterative changes in pollen size have not been documented in other studies which examined dehiscence of anthers still attached to the plant (Keijzer, 1987; Pacini et al., 2006; Bagha, 2014) indicating that excised anthers are an unreliable study system for examining changes in pollen size. A study using cryofixation techniques to evaluate changes in pollen size during rice anther development indicated that tissue temperatures did not influence pollen size or its relationship to surrounding anther tissues (Bagha, 2014). Moreover, pollen size did not vary between HT sensitive and HT tolerant cultivars of Oryza sativa or the African rice Oryza glaberrima at tissue temperatures of 32 °C (Bagha, 2014). Instead, HT impaired anther dehiscence in the sensitive O. sativa cultivar by influencing other developmental processes. Notably, the final stages of anther opening in rice and other species, to include maize and wheat, is dependent upon the dissolution of the walls in the layers of cells that separate adjacent anther locules (Figs. 2, 6A, B, D; Saini et al., 1984; Sanders et al., 2000; Wilson et al., 2011; Harsant et al., 2013; Bagha, 2014). HT prevents or delays cell wall dissolution such that locules remain closed and pollen is trapped within the anther (Fig. 6C, E; Bagha, 2014). In rice, the cell wall dissolution occurs during filament elongation which is approximately 10–20 min prior to anther and floret opening (Bagha, 2014). The timing of this process relative to floret opening and sensitivity of this process to HT explains why florets experiencing HTs 1 h prior to flower opening are detrimental to seed set in rice (Satake and Yoshida, 1978; Jagadish et al., 2007).

Reactive oxygen species, in particular, H2O2, assist in either cell wall loosening or cell wall stiffening (Cheeseman, 2007; Bhattacharjee, 2012; Karuppanapandian et al., 2011). Thus, a basic question is: does HT influence H2O2 production in rice anther cells at the site of cell wall lysis? In rice, a high concentration of H2O2 is present in the apoplast when cell wall lysis has not occurred during
 filament elongation in a HT sensitive cultivar at a day-time temperature of 34°C (Fig. 6D vs E). The high H$_2$O$_2$ at the cellular site of cell wall lysis may operate to make the wall more rigid. Along these lines, Jagadish et al. (2010) notes that anther indehiscence in a rice cultivar that is moderately sensitive to HT was associated with a higher expression of an enzyme that functions in cell wall rigidity. Although high apoplastic H$_2$O$_2$ may have impacted cell wall rigidity, a variety of other processes important for anther dehiscence may have also been negatively impacted to include the activity of cell wall degrading enzymes and signalling cascades, possibly due to protein oxidation (Gechev et al., 2006; Moller et al., 2007). It is also of note that this same region of the anther fails to dehisce during drought or cold stress (Porch and Jahn, 2001; Liu et al., 2006). Cold and drought also produce an abundance of ROS in vegetative tissues (Gill and Tuteja, 2010; Goldack et al., 2014).

2.2.5. High temperature, pollen germination, tube growth and ovule development, and ovule penetration by pollen tubes

Successful pollen germination on the stigma and growth of the pollen tube to the female gametophyte to deliver sperm is dependent on cell-to-cell interactions between the pollen grain/tube and transmitting tissues (stigma, style, and ovary; Sage et al., 2009; Chae and Lord, 2011). In comparison to anther and pollen development, few studies have examined the effect of HT on transmitting tissue and ovule development and in vivo post-pollination events. The current lack of studies on female reproduction is alarming given that Barnabas et al. noted in 2008 ‘there is a dearth of information on the effect of HT on female sexual generation’. Although limited in number, studies do provide evidence that HTs have a negative affect on female reproductive tissues and the growth of pollen tubes therein. Temperatures >30°C reduce stigmatic receptivity and stigmatic pollen germination (Gross and Kigel, 1994; Harsant et al., 2013), and subsequent growth within the stigma and style (Saini et al., 1983; Snider et al., 2011; Song et al., 2015) and ovule penetration (Saini et al., 1983). A lack of pollen germination on the stigma may also be attributed to HT-induced modifications in the pollen wall. Pollen wall structure and composition regulates pollen recognition, hydration and adhesion on the stigma and HT-induced modifications in pollen wall morphology have been observed in bean (Porch and Jahn, 2001), cowpea (Ahmed et al., 1992), and wheat (Prasad and Djanaguiraman, 2014). The negative effect of HT on pollen tube growth in cotton styles is linked to a decline in carbohydrates and disruption in the appropriate balance of ROS necessary to regulate pollen tube growth (Snider et al., 2011).

The most important cells produced in the female gametophyte housed within the ovule are the egg, central cell and synergids. These cells develop by mitotic divisions within the female gametophyte. The synergids secrete attractants into the micropyle that direct pollen tube growth to the ovule and synergid (Chae and Lord, 2011). ROS production within the synergid where a pollen tube delivers sperm is essential for successful fertilization (Duan et al., 2014). Exposure of ovules to extremely high temperatures (90°C for 5 min) eliminates the secretion of pollen tube attractants (Higashiyama et al., 1998). Whether or not more moderate HTs have a negative impact on the secretion of pollen tube attractants and the ROS environment in the synergid that translate into decreased penetration of ovules by pollen tubes in vivo (Saini et al., 1983) is not clear. A more direct effect of HT on the female gametophyte development is apparent at HTs, however. Exposure of wheat to 30°C for three days resulted in reduced female gametophyte expansion and division and differentiation of the egg and synergids (Saini et al., 1983). Ovules of tomato exposed to 40°C for 3 h on two consecutive days four days prior to flower opening contained a degenerated egg and synergids (Iwahori, 1965). Increasing day temperatures from a constant 24 to 35°C resulted in an increase in degenerated contents of the female gametophyte in bean (Ormrod et al., 1967) although this may have been due to an absence of viable pollen that prevented successful fertilization.

Male and female reproductive development are coordinated to insure pollen deposition at the time of stigma receptivity and this includes proper positioning of the anthers adjacent the stigma for pollen capture after anther dehiscence (Fig. 7). High temperature has been demonstrated to disrupt the coordinated development of male and female organs that alters spatial positioning of anthers relative to the stigmas or the timing of anther dehiscence and stigma/style maturation and receptivity most likely through the
negative effect of high temperature on cell division and elongation (Mitchell and Petolino, 1988; Polowick and Sawhney, 1988; Basra, 2000; Harrison et al., 2011; Giorno et al., 2013). These morphological changes can reduce or prevent pollen deposition on the stigma.

3. Conclusion

Enhancing grain yields by improving thermotolerance of the male and female reproductive processes requires a detailed understanding of the biology and physiology of the heat sensing and response mechanisms at the cellular and organismal level (Fragkostefanakis et al., 2014). The present review provides breeders and engineers with a current understanding of male and female processes that are influenced by HT stress to enable breeding programs to focus on important stages that are bottlenecked to successful development. Although numerous stages of male reproduction are impacted, the collective body of research supports the view that the uninucleate stage of male reproductive development is highly sensitive to levels of heat stress currently experienced by many crop species. Thus, high priority should be given to identification of mechanisms leading to HT sensitivity of this stage of development. A number of studies using recombinant and isogenic lines have identified QTLs for heat tolerance at the flowering stage of different species (Cao et al., 2003; Frova and Sari-Gorla, 1994; Xiao et al., 2011; Ye et al., 2012, 2015; Jha et al., 2014; Jagadish et al., 2012). Fine-mapping of one of the QTLs has lead to the identification of a conserved region on chromosome 4 that results in a 15% increase in fertility in rice at HT (Ye et al., 2015). Whether or not this QTL enhances male or female thermotolerance remains to be clarified.

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