Field crops and the fear of heat stress – opportunities, challenges and future directions

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Abstract

Predicted increase in temperature variability can result in short duration of heat stress episodes coinciding with vulnerable reproductive processes leading to significant reduction in floret-fertility in crops. Recent knowledge on alternations in the pollen and stigmatic morphology, pollen biochemical and lipid composition, variable sensitivity of floral reproductive organs and differential temperature thresholds across crops advances the knowledge on heat stress induced reduction in seed-set and harvest index. Rapid increase in night-time temperature, leading to narrowing diurnal temperature amplitude is a major emerging threat to sustain crop productivity. Interestingly, wild wheat (*Aegilops* spp.) with higher heat-tolerance and wild rice (*Oryza officinalis*) escaping damage by completing flowering during early morning hours, are examples of novel opportunities to breed field crops resilient to heat stress. Information on mechanisms leading to heat stress induced sterility is biased towards rice, wheat and sorghum, while the same across other field crops is limited. Hence, increasing research efforts in this direction is critical and timely.

Key words –Field crops, Flowering, Heat stress, Seed-set, Wild species

1. Introduction

Cereals, millets, oil seeds and other field crops respond differently to short and long duration heat stress exposure during different growth and developmental stages but are most vulnerable during the key reproductive stages i.e., gametogenesis and flowering (Hedhly, 2011; Prasad et al., 2014; Prasad et al., 2015; Shi et al., 2015; Singh et al., 2015). Under field conditions, these key developmental processes extend over a period ranging between 14 to 21 days depending on crop species. Field crops have different optimum and critical temperature thresholds for

1 achieving reproductive success, beyond which a series of morph-physiological processes
2 determining seed-set are affected leading to significant yield losses. The level of damage caused
3 is based on crop sensitivity and duration of heat stress exposure. Continuous efforts in
4 quantifying the impact of heat stress during the sensitive reproductive stage in crops, primarily
5 using controlled environment facilities have identified damaging temperatures lying between 30
6 and 40 °C (Fig 1). High day-time temperatures coinciding with reproductive stage can cause
7 significant damage to reproductive processes in cereals (30 to 35 °C), millets (38 to 40 °C),
8 oilseeds (35 to 36 °C) and pulses (32 to 40 °C) (Fig 1). Information on the sensitivity at a finer
9 developmental time scale, accounting for a large proportion of the damage during these
10 vulnerable stages will allow developing precise genetic and molecular interventions to minimize
11 negative impacts of heat stress. The male reproductive organ (anther/pollen or male gametophyte
12 development) have been identified to be the major factor determining seed-set under heat stress,
13 wherein loss of pollen viability and reduced pollen germination percentage on the stigmatic
14 surface leading to sterile flowers has been quantified across crops (Djanaguiraman et al., 2014;
15 Gonzalez-Schain et al., 2015; Li et al., 2015; Polowick and Sawhney, 1988). Pollen tube growth
16 and development within female tissue following pollination have documented sensitivity to heat
17 stress in wheat (Saini et al., 1983), cotton (Snider et al., 2009, 2011), chickpea (Kumar et al.,
18 2013), rice (Jagadish et al., 2010) and other crops (Kaushal et al., 2016, references within).
19 However, in majority of the self-pollinated crops, there is limited information on heat stress
20 impact on the female reproductive organs (pistil – stigma, style and ovule), which warrants
21 further detailed investigation. In addition, other mechanistic aspects such as variation in the
22 pollen and stigmatic surface morphology, lipid composition, pollen reactive oxygen species

(ROS) production damaging their membrane are either not known or less studied in most field crops.

Season-long high-temperature stress decreases biomass production, seed number, individual seed weight and yield of all grain crops, which is reflected in the harvest index (HI = grain yield/total aboveground biomass). Knowledge on temperature thresholds that can differentiate field crops with higher HI will provide additional options for deploying resilient replacement crops in scenarios faced with heat stress challenges. Another component of the climate change phenomena is the rapid increase in night temperature resulting in narrowing diurnal temperature amplitude. Recent studies indicate significant negative impact of high night temperature on yield and grain quality among field crops (Bahuguna et al., 2016; Garcia et al., 2015, 2016; Lyman et al., 2013; Prasad et al., 2011; Narayanan et al., 2015; Shi et al., 2013; Sunoj et al., 2016; Welch et al., 2010). Warmer nights negatively affect the balance between photosynthesis and night respiration rates, reducing the overall carbohydrate pool and biomass leading to reduced yield and lower HI (Bahuguna et al., 2016; Garcia et al., 2016; Shi et al., 2013).

Breeding efforts focused on increasing yield have gradually minimized or in some cases outbred the plasticity for stress response, rendering crop production vulnerable to climatic changes. Different mechanisms have been identified to minimize heat stress damage during flowering in rice, including heat escape (early morning flowering; Ishimaru et al., 2010; Julia and Dingkuhn, 2012; Hirabayashi et al., 2014), heat avoidance through transpiration cooling (Julia and Dingkuhn, 2013) and heat tolerance through resilient reproductive processes (Jagadish et al., 2010). Such systematic quantification of mechanisms or traits in other crops are unclear. Additionally, options to sustain genetic gains and simultaneously increase resilience to heat

1 stress is possible through exploring diversity in wild species for heat tolerance (example – wheat;
2 [Pradhan et al., 2012; Pradhan and Prasad, 2015]). Hence, this focused review highlights the
3 progress achieved in quantifying the degree of sensitivity on a developmental time scale during
4 the reproductive phase, comparative assessment of floral organs vulnerability and varying
5 temperature thresholds inducing changes in harvest index (HI) in different crops important for
6 global food security. Opportunities available through exploring wild species and research
7 direction for crop improvement to sustain global food production under future hotter climates are
8 highlighted and discussed.

10 **Reproductive stage vulnerability on a developmental time scale**

11 On a broader developmental scale, reproductive stages in field crops are known to be
12 more susceptible to heat stress compared to vegetative stages. However, the finer window of
13 sensitivity during reproductive stages particularly flowering is less known across different crops.
14 Among cereals such as wheat and rice, the duration taken by a spike, head or panicle,
15 respectively to complete flowering is about 5 to 6 days and across different tillers (in rice) may
16 take up to 14 days (Bahuguna et al., 2014). In oilseed legume crops such as peanut the flowering
17 duration can last longer i.e., up to 30 days due to its indeterminate nature. Efforts to minimize
18 the damage caused, through physiological, genetic or molecular approaches would require
19 identifying precise window of sensitivity to help develop high-throughput and targeted
20 phenotyping approaches. In spite of the differences in the growth habit and the flowering
21 patterns field crops including wheat, sorghum, peanut and rice are shown to have a same narrow
22 window of extreme sensitivity ranging between 5 to 9 days before anthesis (coinciding with

gametogenesis) and more so during anthesis (Prasad et al., 2001, 2014, 2015; Yoshida et al., 1981) **Fig 2.**

A finer resolution of exposure to few hours of stress supports the hypothesis that stress affects floral organs viability primarily when the flowers are open, exposing the sensitive floral organs to the external microclimate (Jagadish et al., 2008; Prasad et al., 2000, 2001;) **Fig 2.** Quantifying developmental stage sensitivity on days or preferably on hourly timescale will allow for devising high to medium throughput phenotyping protocols and process based (such as pollination, pollen germination) physiological and molecular interventions to enhance stress resilience. In addition, such information will also allow to accurately quantify impacts of short episodes of extreme temperature events projected to increase due to climate change.

Mechanistic advances in floral organs response to heat stress

In spite of the progress achieved in identifying the narrow time scale of sensitivity in some of the major field crops, the question on the role of male and female reproductive organs and their contribution towards stress induced sterility is not entirely clear. Recent findings indicate male reproductive organ to be highly sensitive while the pollen-pistil interaction leading to fertilization could be hindered due to the damage caused by heat stress on stigma, style or ovary (Fig 3). **Seed-set in field crops is primarily depending on synchrony in the development of male and female reproductive organs and coordinated signaling between pollen and ovule for normal fertilization and embryo development. Heat stress induced morph-biochemical changes including lipid compositions, imbalance in carbohydrate partitioning between pollen grains and the stigmatic tissues at the time of pollen tube development and fertilization, can result in sterile seed**

1 or pod (Fig 3 and 4). Asynchronous maturation of male and female reproductive structures,
2 abnormal ovary development (Wheat; Saini et al., 1983), reduced ovule number and longevity
3 leading to ovule abortion (common beans; Suzuki et al., 2001), degeneration/suppression of
4 embryo sac development (mustard; Polowich and Sahney 1988) and shorter duration of stigma
5 receptivity (chickpea; Kumar et al., 2013) are identified to be key sensitive factors with heat
6 stress exposure.

7
8 Heat stress induced developmental changes in pollen morphology (flattened and collapsed
9 pollen) resulted in lower pollen viability in soybean (Salem et al., 2007), barely (Sakata et al.,
10 2010) and other crops (Sage et al., 2015 reference therein). Additionally, during the extremely
11 metabolically active young microspore stage, rice anther tapetum mitochondrial
12 number has been shown to increase by 20- to 40-fold (Mamun et al., 2005), to meet the high
13 energy demand (Dolferus et al., 2011). Higher mitochondrial numbers in the reproductive organs
14 compared to vegetative tissue can lead to higher accumulation of ROS (Reactive Oxygen
15 Species). Increased anther/pollen ROS level under a short duration heat stress (42 °C, 2 h)
16 correlated with heat-sensitivity in wheat (Kumar et al., 2014) and sorghum (Djanaguiraman et
17 al., 2014). Therefore, increased production and accumulation of ROS under heat stress exposure
18 damages membrane lipids, membrane permeability and functions (Fig 4). Accumulated ROS
19 molecules can lead to denaturation of functional proteins and misfolding of newly synthesized
20 proteins in reproductive tissues across agricultural crops (Awsthi et al., 2015). Recent
21 investigations, have also revealed disruption of microtubules and cytoskeleton during pollen tube
22 growth (growing tip of pollen tube) under heat stress (Parrotta et al., 2016), thereby altering the
23 process of vesicular transport and cell wall deposition/signaling events during fertilization.

Further, impaired sucrose hydrolysis and limited energy supply or sucrose biosynthesis in sorghum pollen grain reduced the pollen viability under heat stress (Jain et al., 2007). Supply and depletion of starch or soluble sugars due to higher respiration in the pollen (due to higher number of mitochondria) can potentially determine degree of sensitivity to heat stress. Higher rate of respiration and altered balance of ROS production and scavenging enzymes are correlated with heat sensitivity under heat stress (Fig 4). Long term heat stress reduces the expression of cell wall invertase, sugar transporters and starch synthesis genes in sorghum tapetum and anther (Jain et al., 2007, 2010), leading to complete fertilization failure and seed-set. Therefore, regulated carbohydrate metabolism with better transporters activity in pollen/stigma could be one viable route to enhance thermo-tolerance during anthesis in field crops (Fig 4).

Heat stress is shown to degrade the tapetal cell wall and modify the pollen exine wall thickness (Djanaguiraman et al., 2014; Parish et al., 2012) (Fig 4). The viability of the pollen is also determined by its exine lipid composition, as lipids are known to play a critical role in pollen stigmatic surface interactions (Allen et al., 2011). Lipidomic analysis of sorghum pollen indicate a change in lipid profiles, wherein alternations in the phosphotidic lipids resulted in an increased degree of unsaturated fatty acids, reducing the pollen membrane integrity (Prasad et al., 2011). Changes in the pollen lipid composition accompanied by altered stigma receptivity reduces pollen count and with desiccated style and damaged ovary the pollen tube growth and the fertilization are hindered (Fig 3). Recent study on pearl millet showed that female reproductive tissue was relatively more susceptible to heat stress when compared to pollen grains (Gupta et al., 2015). Similarly, Fig 4 depicts that in sorghum female reproductive organs are also

negatively affected indicating their involvement in fertilization processes under heat stress, a phenomena that needs further intensive investigation across other field crops.

Long duration high temperature stress thresholds reducing harvest index in crops

Impact of heat stress over longer duration encompassing vegetative, reproductive and the grain filling phase results in distinct differences in HI. Among different field crops wheat is the most sensitive cereal with its HI beginning to drop immediately after a mean daily temperature of 16°C while other tropical and subtropical crops not experiencing the same phenomena until 26°C (Fig 5). Temperature close to 30°C and beyond leads to complete loss of yield in wheat while other cereals have a 10°C higher point at which they reach similar response. Among cereals, pearl millet has highest ceiling temperature (41°C) followed by sorghum (38°C), rice (35°C) and wheat (31°C). For legumes, the ceiling temperature for cool-season dry bean was 32°C, while for tropical soybean and peanut it was 39°C and 40°C, respectively. Interestingly, cereals having their reproductive organs exposed directly to heat load from the sun and physically positioned above or at the canopy such as wheat and rice have low thresholds before HI starts to drop significantly. Alternatively, crops such as peanut and soybean have much higher thresholds compared to rice. This differential response seen in peanut and soybean could be an artifact due to the sensitive processes during the flowering safeguarded by the canopy foliage from direct heat load from sunlight. In addition, the canopy transpiration cooling can effectively lower floral tissue temperature facilitating normal fertilization in spite of extreme high air temperatures. This phenomena is defined as heat avoidance and has been well documented in rice (Julia and Dingkuhn 2012; Weerakoon et al., 2008), which needs to be further investigated across other crops. Both sorghum and pearl millet, similar to rice, also have their reproductive organs

1 positioned above the canopy but are categorized to be more resilient to heat stress having
2 temperature thresholds of 40 and 38 °C, respectively (Fig 1). Compared to rice, wherein the
3 degree of avoidance, escape or true tolerance has been systematically quantified (Hirabayashi et
4 al., 2014; Jagadish et al., 2010; Ye et al., 2015), mechanisms inducing higher resilience in
5 sorghum and pearl millet is yet to be investigated. Hence, studies targeted towards identifying
6 appropriate adaptive response based on plant architecture, flowering pattern and effectiveness in
7 altering their microclimate in the target environment are critical for devising effective breeding
8 strategies to enhance field crops resilience to hotter climates.

9 Unlike the short duration day maximum temperature inducing reproductive sterility, rapid
10 increase in night temperatures are closely associated with season long temperature stress, as
11 extended warmer nights are predicted (IPCC, 2013) to significantly reduce agricultural
12 productivity (Welch et al., 2010). Large number of growth chamber studies have indicated
13 increase in night temperature to increase spikelet sterility thereby inducing yield losses (Coast et
14 al., 2014; Mohammed and Tarpley, 2014; Narayanan et al., 2015; Prasad and Djanaguiraman,
15 2011). However, recent field based studies indicates that high night temperature has minimal
16 effect on spikelet fertility in rice, while the major impact was reduced biomass, loss of
17 carbohydrates in different plant parts including panicles possibly due to enhanced night
18 respiration leading to reduced yield and poor quality grain (Bahuguna et al., 2016; Shi et al.,
19 2013, 2016). Recent in wheat and barley, 6 °C higher night temperature compared to ambient
20 imposed using customized heat-chambers from 3rd detectable node till 10 days post-anthesis,
21 reduced grain yield and grain number by 7 and 6% °C⁻¹, respectively, in both crops. The
22 reduction in yield was primarily attributed to reduced spike numbers indicating lesser impact on
23 seed-set (Garcia et al., 2015). Similar, field based infrastructure to support high night

temperature studies involving other crops are needed to establish if a similar differential response to high day and high night temperature as demonstrated in rice, wheat and barley, is common across field crops.

Wild species, a wealth of opportunity

Utilizing some of the extremely useful traits/genes from wild accessions into ongoing breeding programs could be challenging, but a systematic strategy can help overcome this bottleneck and benefit from the wealth of diversity housed in wild species and accessions. An excellent example has been the exploration of the time of day of flowering in rice, wherein the early morning flowering trait has been systematically phenotyped and isolated from wild rice *Oryza officinalis* and incorporated into breeding programs. Advances in the molecular technologies has allowed for targeted incorporation of this trait into popular rice cultivars, advancing their flowering time of the day to less stressful cooler hours of the morning (Hirabayashi et al., 2014; Ishimaru et al., 2010). Water-deficit stress and high temperature co-occur in field conditions and severe water-deficit leads to increased tissue temperature affecting the floral organs negatively (Satake and Yoshida, 1978). A proportion of damage under water-deficit stress is a result of increased floral organ temperature when flowering occurs during early noon under tropical conditions (Jagadish et al., 2011). Breeding crops with early morning flowering trait can be hypothesized to provide additional benefit to minimize combined abiotic stress (both heat and water-deficit stress) induced reduction in sterility. Similarly, large genetic variability for heat stress tolerance during flowering was observed among wild wheat species and accessions within species. Among them *Aegilops speltoides* and *A. geniculata* were relatively more tolerant than other species (Pradhan et al., 2012). Wheat chromosomal substitution lines having small segments of chromosome from

Haynaldia villosa or *Aegilops speltoides* in Chinese Spring background exhibited greater tolerance to heat stress during grain filling (Pradhan and Prasad, 2015). Systematic evaluation of wild species and their progenies, possessing greater variability for traits discussed above can help in breeding for increased heat stress resilience in field crops.

Future directions

Evidently a narrow genetic pool is exploited by current heat tolerance breeding programs. Novel donors with higher heat tolerance or escape as illustrated above provides ample evidence for systematic exploration of wild species and accessions. Crop responses to heat stress are generally clubbed under heat tolerance category without having investigated for heat avoidance or escape phenomena, which are equally effective under field conditions. Phenotyping approaches classifying heat stress response into the appropriate tolerance, escape or avoidance category is an essential first step towards developing stress resilient crops for the future hotter climate. Establishing field based temperature threshold or ceiling temperatures that lead to reproductive failure will significantly improve the prediction power of crop models. Advances in the phenotyping approaches using (i) biochemical means such as lipidomics or metabolomics appear to be promising to help identify robust biochemical markers to complement breeding efforts and (ii) advances in ground based or aerial (unmanned aerial vehicles) sensor technology allows for field based high-throughput phenotyping, facilitating marked expansion of the genetic base incorporated into abiotic stress breeding programs. On the field scale, multiple stresses interact and dealing with the entire complexity could be challenging and hence addressing subcomponents (such as heat stress or drought stress) independently and using advanced techniques for need based trait/gene stacking based on the target environment would be an appropriate research strategy.

1

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Figure legends

Fig 1. Day optimum and damaging temperature thresholds at reproductive stage in field crops. Optimum (OT) and damaging temperatures (DT) thresholds for cereals [barely (OT/DT:20°/30°C, Sakata et al., 2010), wheat (24°/32°C; Pradhan and Prasad, 2015), rice (30°/35°C; Satake and Yoshida, 1978), corn (30°/35°C; Ordóñez et al., 2015)], millets [pearl millet (26°/40°C; Gupta et al., 2015), finger millet (27°/40°C; Opole et al., 2010), sorghum (30°/38°C; Nguyen et al., 2013)], oilseeds [Mustard (20°/35°C; Angadi et al., 2000), rape seed (23°/35°C; Young et al., 2004), sunflower (25°/35°C; Hewezi et al., 2008), groundnut (28°/36°C; Prasad et al., 2000)] and pulses [Common bean (24°/32°C; Porch and Jahn, 2001), pea (24°/36°C; Lahlali et al., 2014), chickpea (25°/35°C; Devasirvatham et al., 2012), fababean (26°/34°C; Bishop et al., 2016), lentil (27°/35°C; Singh et al., 2016), drybean (28°/32°C; Prasad et al., 2002), mungbean (28°/40°C; Kumari and Varma, 1983), soybean (28°/40°C; Djanaguiraman et al., 2013) and cowpea (30°/36°C; Craufurd et al., 1998)], mostly synthesized from controlled environmental studies.

Fig 2. Comparative assessment of the magnitude of sensitivity on a developmental time-scale in wheat, sorghum, peanut, and rice to heat stress coinciding with reproductive stages (gametogenesis and flowering). Daily optimum (control) and heat stress treatments for wheat were 20°C (day/night: 25°/15°C) and 31°C (36°/26°C) for 5 d (A) and 2 d (B; Prasad et al., 2014). The corresponding values for sorghum were 25°C (30°/20°C) and 31°C (36°/26°C), respectively, for 5 d (C; Prasad et al., 2015); for peanut were 25°C (28°/22°C) and 31°C (40°/22°C) (D; Prasad et al., 2001) for 1 d; for rice were 30°C for 6 h (30°/24°C) and 35°C (35°/24°C) (E; Satake and Yoshida, 1978); and for genotypic responses for rice were 30°C (30°/24°C) and 38°C (38°/24°C) for 6 h during anthesis (F; Jagadish et al., 2008).

Fig. 3. Alternations in floral organs morphological and key physiological processes during anthesis in crops exposed to heat stress and control conditions. Sorghum pollen surface morphological modifications captured through scanning electron microscope (A to F), exposed to control and heat stress (A, B at 6000x magnification), with higher resolution of 12,000x (C, D) and 24,000x (E, F) showing criss-cross orientation of the exine wall (heat stress) compared to a smooth and uniform surface (control) (Prasad et al., UnPub). Complete anther dehiscence under

control conditions (G) and either partial or poor dehiscence in rice spikelets exposed to heat stress (H), with insets showing successful pollen dispersal under control conditions and pollen retained within anthers on stress exposure (Muthurajan et al., 2011). Pollen-pistil interaction is depended on the lipid signaling (see Fig 4), with heat stress reducing the pollen germinating on the stigma (in Moroberekan by 83% and IR64 by 60% with no significant change in the tolerant N22 cultivars; see Fig 2 (Jagadish et al., 2010), while in the tolerant N22 the viability is maintained similar to control (I, J). Functional pollen stigma, style and ovary in sorghum under control conditions (K, M, O respectively) while heat stress leads to dysfunctional stigma, desiccated style and damaged ovary (L, N, P respectively) (Prasad et al., UnPub).

Fig. 4. Impact of heat stress on the biochemical and nutritional aspects of the reproductive organs. Increased reactive oxygen species and altered phosphotidic lipids leading to increased unsaturated fatty acids resulting in membrane damage, tapetal dysfunction leading to pollen exine wall thickness and shortage in sugar supply to the high energy demanding reproductive organs are key mechanistic damages resulting in poor reproductive organs viability, lowering seed-set and final yield.

Fig. 5 Season long heat stress and the variation in threshold temperature induce sharp decline in the harvest index among field crops. Diurnal difference between day and night temperatures was 10°C for all treatments for all crops. Dry bean (Prasad et al., 2002); peanut (Prasad et al., 2003); rice and soybean (Boote et al., 2005); wheat, sorghum and millet (Prasad et al, UnPub).

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