Physiological and Agronomic Characterization of Post-Flowering Heat Stress in Winter Wheat

by

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Abstract

Post-flowering heat stress is one of the major environmental constraints for wheat (*Triticum aestivum* L.) production in the state of Kansas, where wheat is the most widely grown grain crop. Studies have shown that the optimal temperature for wheat grain development is approximately 21°C. During the grain filling stage for wheat in Kansas, it is fairly common for temperatures to reach more than 30°C and above. These scenarios have resulted in lower productivity and yield in Kansas compared to other regions of the United States. Therefore the objectives of this research project included: phenotyping seven Kansas varieties for post-flowering heat tolerance in a controlled environment growth chamber study as well as in two field experiments, estimation of spike and flag leaf senescence in wheat exposed to post-flowering heat stress, and identifying potential genetic donors for heat tolerance from winter wheat breeding lines and Near Isogenic Lines developed from Kansas State University's Wheat Breeding Program. To impose heat stress in the controlled growth chambers, plants grown at 25^{o}C were transferred to high day temperature (35^{o}C) chambers ten days after the first sign of anthesis. Under field conditions, custom built "heat tents" were placed over the wheat plots ten days after first flowering and remained until maturity. Plants grown under heat stress exhibited early senescence, indicating a shorter grain filling period compared to the controls. Early-maturing varieties recorded greater percent reductions in grain yield under heat stress. Post-flowering heat stress induced significant reductions in thousand kernel weight, grain number, harvest index, and grain yield. Spike and flag leaves effective quantum yield of PSII was reduced more drastically under growth chamber stress exposure compared to field grown plants. Significant genetic variation in the spike and flag leaf senescence initiation and the differential rate of senescence among the seven tested varieties suggested the potential for considering this

trait in breeding programs. Compared to the commercially relevant varieties, breeding lines varied less under heat stress with a few lines recording a greater degree of heat resilience and experienced little to no drop off in heat stress conditions compared to control. The reduced performance under heat stress for the seven varieties highlights the genuine need to explore wider genetic diversity, including wild wheat, to infuse greater resilience into ongoing wheat breeding programs. However, the results observed in the breeding lines indicate that introducing larger genetic diversity may aid in developing greater heat stress resilient wheat varieties for current and future changing climate.

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Chapter 1 - Literature Review

Introduction

Wheat Production – An Overview

Wheat (*Triticum aestivum* L.) is among the most widely grown food crops in the world. It is a staple in the diets of nearly 35% of the world population (Braun et al., 1998) and is the largest source of vegetable protein in low-income countries (Tack et al., 2015). Consumer demand for wheat is predicted to increase at a greater rate than for any other major crop (Braun et al., 1998) with its demand forecasted to grow by 2% annually (Bahar et al., 2011). Over the twenty-five year period, from 1989 to 2014, global wheat yield increased at an average annual rate of 1.56% (Food and Agriculture Organization of the United Nations Statistics Division [FAOSTAT], 2014). In 2014, wheat was the third largest grain crop produced globally at 729,012,175 tonnes, behind only maize and rice. The United States is typically one of the four leading countries in wheat production. In 2014, the top wheat producing nations were: China (126 million tonnes), India (96 million tonnes), Russian Federation (59.5 million tonnes), and the United States (55 million tonnes) ([FAOSTAT], 2014).

The United States Department of Agriculture Economic Research Service (USDA ERS) reports wheat as the third largest field crop in the United States, behind corn and soybean, in both planted acreage and gross farm revenue (USDA ERS - Wheat, 2016). According to the USDA National Agricultural Statistics Service (USDA NASS), 2016 wheat production was led by Kansas (467 million bushels), followed by North Dakota (333 million bushels), and Montana (213 million bushels) (USDA NASS, 2016). Approximately 40 % of the total wheat produced in the United States is classified as hard red winter, which is grown primarily throughout the Great

Plains from Texas to Montana (USDA ERS, 2016). Five states in the Great Plains region of the United States (Texas, Oklahoma, Kansas, Colorado, and Nebraska) form the largest contiguous transect of winter wheat in the world, accounting for 8 million hectares harvested in 2013 (Tack et al., 2015).

Effects of high temperature on wheat growth and yield

Heat Stress and Winter Wheat

Environmental or abiotic stresses such as extreme heat, drought, and freezing temperatures pose a significant negative impact on wheat yields globally. Across the Great Plains, variations in temperature throughout a given cropping season are a defining factor for winter wheat yields. Freezing temperatures in the fall prior to dormancy and high temperatures in the spring during the reproductive and grain filling stages are key contributors to yield loss in this region (Tack et al., 2015).

The Intergovernmental Panel on Climate Change (IPCC) has predicted an increase in average global mean surface temperature varying between 0.3°C to 4.8°C by the end of the 21st century (IPCC, 2014). Both climate and crop models project that climate change will have a significant impact on rainfed wheat production in the Great Plains (Tubiello et al., 2002). These models predict hard winter wheat yields could decrease by 4 to 30 % across the southern Great Plains (Colorado, Kansas, Oklahoma, and Texas) by 2090, primarily due to warmer temperatures and drought stress (Tubiello et al., 2002). Although genetic potential for increased yield has been achieved through breeding, wheat yield will continue to be limited under stressful environmental conditions. Thus, addressing abiotic stress factors like heat and drought is pertinent for wheat improvement (Bahar et al., 2011).

In an attempt to bridge the gap between scientific research results and projected real world yield losses due to warming temperatures, Barkley et al. (2014) compiled 26 years of historical weather data with wheat variety performance testing yield results from 11 Kansas locations. Their results found that warmer temperatures have a significant negative impact on yield. By using regression based models they reported a drastic 21% reduction in grain yield for every 1˚C increase in projected mean temperature (Barkley et al., 2014). A similar study performed by Tack et al., (2015) found that for each additional growing degree day beyond the critical threshold of 34˚C for the spring growth period, a 7.6% reduction in grain yield is possible.

Post-flowering Heat Stress in Wheat

The optimal temperature for wheat grain filling is 21.3°C ± 1.27°C, according to a comprehensive review by Farooq et al. (2011) including 12 studies related to post-flowering heat stress. Temperatures in Kansas and across the southern Great Plains commonly reach 25 to 30°C (U.S. Climate Data, 2017) and intermittently exceed 32°C during the period often associated with wheat grain filling - late April through June. Wheat's heat stress can be categorized into two temperature scenarios: critical high temperature stress (chronic stress above the optimum temperature) ranging from upper 20's to 32°C, and heat shock which occurs when temperatures exceed 32°C during the grain fill period (Wardlaw and Wrigley, 1994).

Measures closer to critical high temperatures during post-flowering stages primarily impact yield by reducing kernel size and weight (Wardlaw and Wrigley, 1994). However, short term heat shock or extreme high temperatures can lead to early leaf senescence, inhibit kernel development, and alter starch and protein composition (Wardlaw and Wrigley, 1994). Numerous studies have focused on quantifying the level of yield reduction that can be attributed to heat

stress. In a study designed to measure the effect of heat shock, temperatures of 40°C imposed for a period of three days during early grain fill resulted in up to 23% reduction in kernel weight (Stone and Nicolas, 1994). Final grain weight at maturity decreased by 5% for each 1°C rise in temperature above a base temperature of 21/16°C day/night temperature (Tashiro and Wardlaw, 1989). After reviewing data from 75 Australian wheat cultivars, Stone and Nicolas (1995) concluded that short periods of heat stress adversely affect grain yield and quality, but genetic diversity for tolerance exists.

The primary determinants of wheat yield can be attributed to three factors; spikes per area, kernels per spike, and weight per kernel. Due to a highly variable regional climate, wheat grown in Kansas and across the Great Plains is routinely subjected to high temperatures at the time of grain fill. As a result, the rate of grain filling is accelerated and the grain fill duration is drastically shortened (Sofield et al., 1977) which hastens physiological maturity and results in lower kernel weight and reduced yield (Dias and Lidon, 2009). Yin et al. (2009) reported a 15-40% shortening of grain filling duration by 15-40% for six genotypes when temperature increased from 20 to 25°C. Post-flowering heat stress, which is responsible for a shortened grain fill period, greatly affects kernel weight as all other grain yield parameters have been established prior to this phase (Yang et al., 2002). Sharma et al. (2008) have suggested thousand kernel weight under terminal heat stress could be used as an indirect indicator for selecting high yielding, resilient cultivars. A typical genotypic response to high temperatures during grain fill is an increase in the grain filling rate. However, this does not alleviate the effect that the reduced grain fill duration has on decreasing kernel size under post-flowering heat stress under growth chamber conditions (Prasad et al., 2006). A study conducted by Gibson and Paulson (1999)

concluded that further research is necessary to understand the significance of wheat kernel development under high temperature stress.

Impact of heat stress on grain quality

Studies have shown that post-flowering heat stress may alter cell formation and development of the endosperm tissue in grain (Stone and Nicolas, 1994; Wilhelm et al., 1999). Reduced starch deposition in harvested grain has been documented as a result of smaller endosperm cells in wheat plants exposed to critically high temperatures, additionally, heat shock conditions have been known to cause kernel deformation (Wardlaw and Wrigley, 1994). Starch accumulation is significantly impacted by elevated temperatures and has been found to be more sensitive to heat stress than protein synthesis (Bhullar and Jenner, 1985; Sofield et al., 1977). Stone and Nicolas (1995) found that temperatures between 30 and 40°C caused more than 30% starch accumulation reduction in grains. Literature suggests that 10-15 days post-anthesis appears to be the most sensitive stage of grain fill in regards to yield reduction due to high temperature stress, as this correlates to the final stages of cell division and enlargement in the endosperm (Wardlaw and Wrigley, 1994). Decreased kernel size is not only detrimental to producers in terms of overall yield, but also in terms of reduced quality, thus resulting in a potential cash price dockage and lower revenue for the producer. Additionally, other studies have proven that above optimum temperatures at grain fill are likely to impact end-use quality by weakening the dough properties of the grain (Blumenthal et al., 1993, Blumenthal et al.,1995, Stone and Nicolas, 1994).

Effects of high temperature on wheat physiology

Physiology and Heat Stress

There are several physiological processes that are affected by high temperature stress that lead to decreased wheat grain yield. The rate of the temperature change, temperature intensity, and duration of elevated temperature interact to determine the severity of heat-imposed stress (Sung et al., 2003). Reduction in photosynthetic rate is one of the primary processes impacted by heat stress (Al-Khatib and Paulsen, 1984; Wahid et al., 2007). The decline in photosynthesis due to heat stress can be attributed to thylakoid membrane damage, disrupted chloroplast function, and reduced chlorophyll content (Al-Khatib and Paulsen, 1984; Ristic et al., 2007). At the plant level, Sharkey (2005) identified carbon dioxide fixation, photophosphorylation, the electron transport chain, and the oxygen evolving complex (OEC) as major processes susceptible to temperature-induced damage of photosynthetic machinery. Additional physiological processes which are impacted by heat stress include: canopy temperature depression, cell membrane thermal stability, photosynthetic rate, and stomatal conductance (Al-Khatib and Paulsen, 1984; Cossani and Reynolds, 2012; Fokar et al., 1998; Reynolds, 2001).

Wheat cultivars are known to primarily employ three differing mechanisms to handle heat stress, tolerance, escape, and avoidance (Levitt, 1980; Sun et al., 2017). Earlier maturity for cultivars is a key mechanism to escape terminal heat stress in many cultivars. In a study conducted across South Asia and parts of Mexico, Mondal et al. (2013) determined that earlier maturing wheat lines outperformed their counterparts under terminal heat stress. Escaping-end-of season heat shock and late-season droughts has long been recognized as an important characteristic among producers when selecting wheat cultivars to be grown in the Great Plains (Reitz and Salmon, 1959). The future outlook of climate and wheat production give reason to

believe that earlier maturing cultivars may be beneficial in the short term; however, the most promising long-term adaptation strategy remains improving cultivar tolerance to heat stress during grain filling (Gouache et al., 2012).

Although escape and avoidance mechanisms tend to be more easily identified, as they are associated with earlier physiological development and maturity, tolerance mechanisms require in-depth measurements and systematic testing to appropriately ascertain the means through which a plant is or is not combatting heat stress. One source of tolerance that has been identified in crop plants is an increased level of antioxidant enzyme activity leading to greater levels of tolerance to heat stress (Gupta et al.,1993; Rengang et al., 1995; Sairam et al., 2000). A study comparing heat tolerance in sorghum to wheat (Blum and Ebercon, 1981) found that cell membrane stability, or the rate of injury to cell membranes, could be used to accurately measure levels of heat tolerance in wheat cultivars. Understanding how wheat plants cope with above-optimum temperatures on a cellular level is of utmost importance if superior heat-tolerant wheat genetics are to be developed. Of equal importance in this progression is recognizing how leaf and canopy characteristics are related to yield in stressful environments. Fischer et al. (1998) concluded that stomatal conductance, maximum photosynthetic rate, and canopy temperature depression are closely and positively correlated with the mean yield based on a six-year study conducted in Mexico in collaboration with the International Maize and Wheat Improvement Center (CIMMYT).

Chlorophyll Content

Chlorophyll is the most abundant photosynthetic pigment in the plant and is closely related to plant health and its degradation leads to leaf senescence. Chloroplasts are home to photosynthetic activity in the plant and contain membranes that contain both chlorophyll *a* and *b*

pigment molecules as well as accessory pigments (Emerson and Arnold, 1932). Within these membranes, the resources required for grain development are formed by the photosynthetic reaction in which carbon dioxide (CO_2) is fixed and converted to sugars (Al-Khatib and Paulsen, 1990; Evans, 1975). Photosynthesis is dependent upon chlorophyll for the absorption of sunlight. Thus, a reduction in leaf chlorophyll content, or an increased rate of senescence preceding physiological maturity, has been shown to negatively influence yields (Lopes and Reynolds, 2012). To combat premature senescence due to heat stress, the "stay green trait" is generally recognized as the plant's ability to retain chlorophyll under stressful conditions (Reynolds, 1994; Thomas and Howarth, 2000). Although Borrill et al. (2015) reported non-significant correlation between grain yield and length of flag leaf senescence in transgenic wheat lines under optimal growth conditions, others have shown that pursuing stay-green phenotypes has the potential to aid breeding programs which are seeking ways to improve yield under adverse environmental conditions (Jagadish et al., 2015). Testing different wheat cultivars, Zhao et al. (2007) determined that a reduced chlorophyll content in flag leaves due to heat stress during grain filling is directly linked to a reduction in the duration of active stomatal regulation. Attesting to the theory of chlorophyll content impacting grain fill performance and thus yield, a study performed on Mexican wheat landraces showed a positive and highly significant correlation between seed weight and chlorophyll content in plants exposed to heat stress (Hede et al., 1999).

Photosynthesis

Photosynthesis is one of the plant processes most sensitive to heat stress (Al-Khatib and Paulsen, 1984; Fischer et al., 1998). Above optimum temperatures are damaging to the structure and function of the different apparatuses that carry out photosynthesis at the cellular level (Mathur et al., 2011), which ultimately leads to decreased photochemical efficiency and yield. A

review of literature found that even if heat stress damage is minimized, it is expected that photosynthesis would decline with temperature rise due to the established fact that, as temperatures rise, photorespiration increases at a greater rate than photosynthesis (Sharkey, 2005; Schuster and Monson, 1990). Consequently, temperatures between 35-40°C have been shown to reduce the photosynthesis rate at an increased level; this can also be attributed to reduction associated with accelerated photorespiration (Sharkey, 2005). It was previously thought that damage to photosystem II (PSII) was a factor in photosynthesis reduction at high temperatures (Enami et al., 1994; Santarius, 1976), but it has otherwise been proven that damage to PSII does not generally occur at temperatures less than 45°C (Čajánek et al., 1998; Gombos et al., 1994; Terzaghi et al., 1989; Thompson et al., 1989; Yamane et al., 1998). Photosynthetic activity however, is inhibited at less extreme temperatures than those needed to harm PSII (>40°C) (Al-Khatib and Paulsen, 1999). For these reasons, PSII is not responsible for the declining photosynthesis rate observed at temperatures of 35 to 40°C (Sharkey, 2005).

Membrane damage and Reactive Oxygen Species (ROS)

Wise et al. (2004) noticed that high temperatures primarily impact the thylakoid membrane and carbon metabolism in the stroma which leads to reduced photochemical efficiency. The thylakoid membrane serves an essential role in the photosynthetic process as it houses integral membrane proteins including: antenna pigment protein complex 18 (carotenoid, chlorophyll a, and chlorophyll b), reaction center and electron carrier proteins (cytochrome b, cytochrome f, and ferredoxin) (Taiz and Zeiger, 2006). In a study conducted by Al-Khatib and Paulsen (1990), heat stress induced electrolytic leakage in thylakoid membranes, significantly reducing photosynthetic rate, however, the extent of reduction in photosynthetic rate was variable amongst different wheat species. Dias et al. (2011) verified earlier results that under

high temperature stress, durum wheat cultivars have a greater photosynthetic performance compared to bread wheat cultivars.

Chlorophyll Fluorescence

Chlorophyll (Chl) fluorescence is a measurement routinely used in plant stress physiology studies, providing critical insight on the primary response of photosynthesis (Mathur et al., 2011; Sayed, 2003). As leaves and other plant tissues are exposed to solar radiation, Chl molecules within the plant absorb the light energy. The energy processed by Chl molecules is used to fuel photosynthesis within the plant. Excess energy is either dissipated in the form of heat or is re-emitted as light – a process called Chl fluorescence (Maxwell and Johnson, 2000). Maxwell and Johnson (2000) also noted that these processes occur competitively within the plant, thus, measuring Chl fluorescence allows insight on the performance of photosynthesis and heat dissipation. Associations between heat tolerance and low fluorescence signals have been identified in wheat (Moffatt et al., 1990), making it a useful tool in heat screening.

Chl fluorescence is an efficient, non-destructive measure for determining damage to PSII activity caused by environmental stresses. The non-destructive nature allows for multiple measurements on the same plant or leaf to be taken throughout the experimental period, producing desirable data for further research. In a study measuring heat-induced effects on PSII in wheat plants, Lu and Zhang (2000) found that the impact of heat stress on PSII could be categorized into two temperature regimes: moderately elevated temperatures (30-37.5°C) and severely elevated temperatures (>37.5°C). The results from Lu and Zhang (2000) indicated that a decrease in quantum yield of PSII electron transport is reversible under moderately elevated temperatures due to a significant increase in non-photochemical quenching, but remained irreversible under severely elevated temperature. Similar research conducted on wheat by Sayed

(1992) concluded that plants appear to exhibit signs of acclimation to warm conditions (30/25°C day/night), increasing the performance of PSII and whole-chain electron transport.

Canopy temperature depression and stomatal conductance

Canopy temperature depression (CTD) is often used as an estimate of heat stress in wheat and is a well-tested measure for selecting physiologically superior lines in warm environments (Reynolds, 2001). Traits such as leaf chlorophyll content, leaf conductance, spike number, and biomass are known to be well associated with crop performance, however, Reynolds et al. (1997) proposed that CTD was the single most effective trait associated with yield. Ayeneh et al. (2002) examined 13 spring wheat genotypes to determine the level of correlation between organ temperature depression (TD), CTD, and grain yield. Their results found that genetic variability for organ-TD exists, as well as a strong positive correlation between organ-TD, CTD, and grain yield.

Replicated research has confirmed that there is a linear relationship between air temperature, canopy temperature, and stomatal conductance (Hatfield et al.,1987; Jackson et al., 1981; Pinter et al., 1990). Moreover, numerous studies have shown a complementary relationship between stomatal conductance and an increased grain yield under irrigated conditions (Amani et al., 1996; Fischer et al., 1998; Reynolds et al., 1994). When water is a non-limiting factor, Pinter et al. (1990) discovered that wheat cultivars with a warmer canopy temperature consumed less water and had a lower stomatal conductance when compared to cultivars that exhibit a cooler canopy temperature. Under the well-watered conditions both the warm and cool canopies maintained similar yield, however when exposed to water deficit regimes the cultivars with warmer canopies maintained higher relative yields (Pinter et al., 1990).

Screening for Heat Resilience

The majority of scientific studies monitoring wheat exposed to heat stress conditions have been conducted in controlled environment settings such as growth chambers or greenhouses. Heat stress experiments conducted in natural field conditions are limited due to the difficulty of imposing heat stress in a consistent manner outdoors and also by limited access to field-based phenotyping facilities. One common way to ensure heat stress is imposed on field trials is by conducting the research in naturally warm environments, similar to Hede et al. (1999), where landraces in Mexico were tested for heat tolerance under natural environmental conditions in which air temperatures exceeded 35°C. A second popular alternative for imposing heat stress in the field is achieved via delayed planting to ensure later maturity, thus increasing the probability of late season heat stress exposure (Moffatt et al., 1990). In contrast, a relatively new and innovative design for imposing heat stress on field trials has been developed using structures designed with the intent of being placed over the growing crop. These structures, as outlined by Prasad et al. (2015) and Sunoj et al. (2017), raise the temperature inside the structure by capturing solar radiation.

An advantage of conducting heat stress experiments in controlled environment settings compared to field settings is their consistency in maintaining the desired temperature. In a study using controlled growth chambers to impose heat stress during flowering in sorghum, Prasad et al. (2015) deduced that mean daytime and nighttime temperatures in both optimum and high temperature treatment chambers were within ± 0.5°C of the targeted temperature. Furthermore, a study conducted by Pradhan et al. (2012) set out to measure variability within and between controlled growth chambers for a better understanding of their uniformity. The study involved growing a selected spring wheat cultivar in eight different growth chambers and randomly

moving plants within each respective chamber every seven days. Plants were removed from the chambers at flowering, and multiple growth trait measurements were collected. Statistical analysis of the results concluded no significant difference among growth traits in plants grown in different chambers; this data was supported by a consistent air temperature exhibited among the different chambers as indicated by temperature data loggers (Pradhan et al., 2012).

Environmentally controlled growth chambers perform well in consistently regulating air temperature for targeted intervals – a critical aspect in heat stress experiments. However, limitations exist when attempting to correlate results from a controlled setting to authentic outcomes under field grown conditions. Oftentimes relationships do exist between plant performance in controlled environments and field conditions (Prasad et al., 2015), yet differences such as plant density and microclimate may confound results between the two approaches. While explaining plant-environment interactions, Jones (2013) notes field experiments can be limited by lack of environmental control but acknowledges these conditions are nevertheless prone to being closer to natural than their controlled environment chamber research counterparts. Thus, results from field testing are generally more likely to reflect plants' responses under natural conditions.

Physiological Approaches in Breeding for Heat Resilient Lines

Wheat producers who are considering strategies for managing late season heat stress have limited agronomic options. Currently, cultivar selection is considered the best management practice for producers who need to hedge their risk against heat stress during the grain fill period. Thus, making improvement through plant breeding and genetic alteration is an effective way to stabilize wheat yields subjected to post-flowering heat stress. Unfortunately, improving genetics

related to heat tolerance poses a unique challenge to breeders due to the fact that traits associated with heat tolerance are often quantitative (Green, 2016). To further complicate matters, Paulsen (1994) concluded that wheat cultivars that have been bred and selected for growing in stressful environments such as the Great Plains, already possess a base level of tolerance. More recently however, Tack et al. (2015) have shown evidence that yield potential and heat tolerance are negatively correlated. The same study recognized that, while modern varieties adapted to the Great Plains may have a greater yield potential, they are also more susceptible to heat stress compared to older varieties.

It has been well established in previous studies that wide genetic diversity for heat tolerance exists in wheat accessions (Midmore et al., 1984; Rawson, 1986; Wardlaw et al., 1989). However, the genetic basis of heat adaptation in wheat has eluded breeders and is not well understood (Cossani and Reynolds, 2012). As a result, breeders currently focus on physiological traits related to heat resilience as their primary strategy for improving wheat's genetics in an attempt to maintain, and ideally improve, its performance under heat stress. Among the scientific measurements relied upon to study heat stress in crop plants, Reynolds et al. (2001) suggested that canopy temperature depression, stomatal conductance, and membrane thermostability offer the greatest benefit to breeders who are screening genetic material for heat adaptations in their respective breeding programs.

Although these measurements play a critical role in determining levels of heat tolerance among wheat cultivars it should be noted that they are primarily focused on leaf and canopy traits. However, as Blum (1997) points out, grain filling in wheat coincides with increasing temperatures and loss of leaf area due to senescence, leading to decreased photosynthetic capacity. Therefore, the translocation of carbon from stem reserves to developing grains is

something of importance, especially when photosynthesis becomes inhibited by heat stress during the grain filling stage (Blum, 1997). Together, canopy respiration and grain dry matter accumulation used more photosynthates than could be contributed by canopy photosynthesis during the later stages of grain filling, thus leading to the conclusion that stem reserves are essential for the completion of grain filling (Gent, 1994). The most abundant carbohydrate reserve in wheat stems is fructan, however other carbohydrates such as glucose, fructose, sucrose, and starch are also stored as reserves (Dubois et al.,1990; Wardlaw and Willenbrink, 1994). Additionally, remobilization efficiency of carbohydrates is significantly correlated with grain yield, grain weight, harvest index, and grain filling duration (Tahir and Nakata, 2005). This illustrates the importance of remobilization of carbohydrates from stem reserves during the grain filling process when wheat is exposed to heat stress. Based on the extensive literature review on heat stress impacts on wheat, it can be summarized that breeders must take into consideration key physiological aspects such as CTD, efficient assimilate translocation etc. when developing heat stress resilient wheat varieties.

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Chapter 2 - Response of Prominent Winter Wheat Varieties to Post-Flowering Heat Stress under Controlled Chambers and Field Based Heat Tents

Abstract

Post-flowering heat stress shortens grain filling duration and limits resource allocation to grains leading to lower productivity in wheat. Wheat grown in Kansas is often exposed to temperatures of 30^{o}C during grain filling, leading to lower productivity compared to the national average. Therefore, characterizing widely grown and newly released varieties for post-flowering heat stress will define the gap in resilience that needs to be addressed through breeding. In the present study, seven Kansas varieties were phenotyped for heat tolerance in a controlled environment growth chamber study and in two field experiments. To impose heat stress in the controlled growth chambers, plants grown at 25^{o}C were transferred to high day temperature (35^{o}C) chambers ten days after the first sign of anthesis. Under field conditions, custom built "heat tents" were placed over the wheat plots ten days after first flowering and remained until maturity. Plants grown under heat stress exhibited early senescence, indicating a shorter grain filling period compared to the controls. Early-maturing varieties recorded greater percent reductions in grain yield under heat stress. Post-flowering heat stress induced significant reductions in thousand kernel weight, grain number, harvest index, and grain yield. Percent reduction in yield ranged from 6 to 51% under severe heat stress exposure in controlled environments, and 2 to 27% with heat stress exposure induced by heat tents on field plots. Among the varieties tested, SY Monument and Larry performed well under both conditions, suggesting that they are relatively better suited for locations that face consistent exposure to heat stress during the post-flowering stages. Only SY Monument was consistently tolerant, whereas the others exhibited differing degrees of vulnerability. These results highlight the genuine need to explore wider genetic diversity, including wild wheat, to infuse greater resilience into ongoing wheat breeding programs.

Introduction

Among the ever-increasing negative impacts of upon crop production is global warming. With a predicted increase in global mean surface temperature varying between 0.3 and 4.8^{o}C by the end of 21st century, crop production will be challenged by heat stress leading to significant economic damage (IPCC, 2014; Lyman et al., 2015; Tack et al., 2015, 2017). Using a multi-model ensemble approach, Asseng et al. (2015) concluded that for every ^{o}C increase in global mean temperature, the global wheat (*Triticum aestivum* L.) production would decline by about 6%. Wheat, one of the important staple cereals and a major source of calories for humans (FAO, 2015), is very sensitive to heat stress during the reproductive and grain filling phases (Wollenweber et al., 2003; Farooq et al., 2011). Optimum temperature for normal growth and development in wheat ranges between 12 and 24^{o}C, and temperatures >30^{o}C are shown to induce significant yield losses (Saini and Aspinall, 1982; Farooq et al., 2011). The United States ranks fourth in world wheat production, accounting for approximately 55 million metric tons of wheat produced from a harvested area of approximately 19 million hectares (USDA-NASS, 2017; USDA-FAS, 2016). The majority of wheat grown in the United States is winter wheat, with a large proportion (~57 %) produced in the Great Plains (USDA-NASS, 2017). Among states, Kansas is the leader both in terms of total wheat area and production. However, grain yield per unit area (productivity) in Kansas (mean yield of 41 bushels per acre, from 2014 -2016) is lower than the national average for winter wheat (USDA-NASS, 2017), owing to its extreme weather conditions including high temperatures. The primary reasons for low productivity include limited water availability and warm conditions during the grain- fill period. Winter wheat grown in Kansas is often exposed to temperatures ≥30^{o}C during May and June – the typical grain filling phase – which is well beyond the optimum temperature identified for grain filling. Such

scenarios are predicted to worsen with increased frequency and magnitude of heat stress exposure associated with a changing climate, which could lead to increased economic loss for wheat growers. Thus, determining the level of post-flowering heat tolerance in prominent varieties during the grain filling phase is crucial and timely.

Heat stress at the grain filling phase induces significant grain yield and quality losses in wheat (Bhuller and Jenner, 1985; Blum et al., 1994; Viswanathan and Khanna-Chopra, 2001). Grain weight is a product of rate and duration of grain filling (Gallagher et al., 1976), wherein temperature is a key environmental driver that determines the rate and duration dynamics. High temperatures are known to reduce grain filling duration, thereby reducing the window for translocation of the stored or currently synthesized assimilates into grains, leading to lower grain weight and yield. For every 1^{o}C increase in temperature above the optimum growth temperature, the grain filling duration is shown to decline by 2.8 days (Chowdury and Warlaw, 1978; Streck, 2005). Additionally, heat stress during grain fill negatively affects many physiological and biochemical processes including photosynthesis (Blum et al., 1994), membrane integrity, and quantum yield of photosystem II (Bhullar and Jenner, 1985). Almost all previous studies quantifying the impact of heat stress during grain filling in wheat have used controlled environment facilities (Stone and Nicolas, 1994; Gibson and Paulson, 1999; Spiertz et al., 2006). In addition, the cultivars tested are generally not commercially relevant and hence not widely grown, leaving a critical gap in researchers' and wheat producers' understanding of the need for enhancing resilience and extent of improvements necessary in prominent varieties. Due to a lack of field-based phenotyping facilities, oftentimes a staggered sowing approach is used to expose crops to heat stress during critical developmental stages in field conditions (Viswanathan and Khanna-Chopra, 2001). Although this approach provides an opportunity to have the flowering or

post-flowering stage of the crop exposed to stress, the overall agronomic performance of the varieties are seriously affected due to their exposure to significantly different environments as compared to the target conditions under which they were bred (Bahuguna et al., 2015).

Considering the major knowledge gaps and limitations highlighted above, seven prominent Kansas winter wheat varieties were chosen to be exposed to heat stress during the post-flowering phase using controlled environment chambers and unique field-based heat tents. One growth chamber and two field experiments were conducted over the span of two years to investigate these specific objectives: 1) Determine the level of genetic variability for post-flowering heat tolerance in prominent and recently released winter wheat varieties grown in Kansas; 2) Assess the physiological and agronomic response during post-flowering heat stress exposure in prominent varieties in controlled chambers and field-based heat tent environments; and 3) Identify the most suitable grower preferred winter wheat varieties for the warmer conditions observed in the Great Plains region of the United States.

Materials and Methods

Field Experiment

Research was conducted in the 2015-2016 and 2016-2017 growing seasons at Kansas State University (KSU), Agronomy Research Farm at Manhattan (39 11'N, 96 35'W). Soil type was a Kennebec silt loam. Soil samples were collected at the 0-15 cm and 15-60 cm depths prior to sowing in October 2015. Samples were analyzed for: organic matter (OM), pH, P, K, N [ammonia (NH_3) and nitrate (NO_3)], S, and Cl. Each sample was composed of 15 individual soil cores representing the experimental area. Soils contained 2.3% OM, 16.5 ppm of Melich-P, 303

ppm K, 7.8 ppm of NH_4-N and 12.2 ppm of NO_3-N and had a pH of 5.5. The experiment included seven commercial varieties, four of which are commonly grown across Kansas and three which were released in the past two years. (Table 2.1). All seven varieties were grown in two temperature treatments (control and heat stress) with four replications.

Plot preparation prior to planting included multiple tillage passes of a disc, cultivator, and harrow in the summer/fall of 2015 and 2016 to prepare the seedbed for planting. Below average precipitation in September and October 2015 resulted in dry soil conditions prior to planting the 2016 experiment. Multiple irrigation applications with garden hose and fan sprinkler occurred prior to planting in fall 2015 to ensure adequate emergence. In the 2016 experiment the wheat plots were planted using a hand pushed single row seeder (Rowseed 1R, Wintersteiger, Ried im Innkreis, Austria) on 26 Oct 2015. Each plot was four rows wide and four meters in length. An additional irrigation application of 12 mm was applied two days after planting (28 Oct 2016) with a fan sprinkler attached to a garden hose. The 2017 experiment was planted on 27 Oct 2016 using a tractor (5055E, John Deere, Moline, IL) equipped with Real Time Kinematic (RTK) guidance (Trimble FMX, Trimble Inc., Sunnyvale, CA) and a grain drill (3P605NT, Great Plains Mfg., Salina, KS) modified for research plots (Kincaid Equipment Mfg., Haven, KS). Di-Ammonium Phosphate (18-46-0) was applied at a rate of 14.5 kg N ha^{-1} and as 39 kg P_20_5 ha^{-1} as an in furrow starter at planting. Each plot was six rows wide and 1.22 meters in length. The seeding rate for both experimental years was 60 seeds per meter with a row spacing of 19 cm.

Weed control in the wheat plots was managed using the labeled rate of a post-emergence herbicide along with hand weeding as necessary to minimize weed pressure throughout the growing season. Herbicide was applied with an all-terrain vehicle mounted boom sprayer at recommended carrier volume rates. Plots received 0.75 oz/ac FINESSE [2-Chloro-N-[(4-

methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide] [4,5-Dihyd ro-3-methoxy-4methyl-5-oxo-N-[[2-(trifluoromethoxy)phenyl]sulfonyl]-1H-1,2,4-triazole-1-carboxamide, sodium salt] on 10 Dec 2015 and on 9 Dec 2016 as a post-emergent treatment.

The recommended rate of 56 kg N ha^{-1} was top dressed as urea (46-0-0) to the wheat plots in both years on 29 Feb 2016 and 3 Mar 2017, respectively, using a variable rate drop spreader (Gandy Company, Owatonna, MN). Fungicide was applied to the plots at three different growth stages in 2016: at spring greenup, flag leaf (Feekes 10), and mid grain fill (Feekes 11.1). The first application was applied with a tractor and three-point mounted boom sprayer; the second and third applications were applied with a handheld spray boom and backpack sprayer. In 2017, two applications of fungicide were applied with a handheld spray boom and backpack sprayer at flag leaf (Feekes 10) and mid-grain fill (Feekes 11.1). All applications were applied at recommended carrier volume rates. In 2016 and 2017, plots received a total of 9 fl oz/ac of TWINLINE [pyraclostrobin: (carbamic acid, [2-[[[1-(4-chlophenyl)-1*H*-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-,methyl ester) metconazole: 5-[((4-chlorophenyl)methyl]-2,2-dimethyl-11(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol].

In order to impose post-anthesis heat stress on the field experiment, heat tents were manually placed on the plots ten days after approximately 50% of the wheat varieties had begun anthesis (Feekes 10.5.1) (28 April 2016, and 12 May 2017). The heat tents were constructed on a galvanized steel framework covered with a clear polyethylene film and a moveable overhead flap (0.6 m) at the roof peak. The overhead flap could be opened to avoid excessive heating. Each heat tent was 5.4 m wide, 7.2 m long, and 3.0 m high at the apex; each had 15 cm clearance on its four sides to allow for air circulation within the heat tent. The air temperature inside the heat tents was dependent upon solar radiation and was partially regulated by a thermostat set at

35°C for experimental purposes. When the temperature inside the heat tents rose above the desired temperature (35°C), actuators powered by a solar charged battery worked to automatically open the overhead flaps, allowing for open air circulation and temperature moderation. Once the temperature inside the heat tent fell below the desired temperature (35°C), the thermostat automatically triggered the actuators to close the overhead flap (Prasad et al., 2015; Sunoj et al., 2017).

Air temperature and relative humidity were recorded at 15 minute intervals for the duration of the experiment inside all four heat tents as well as an outside recording of the ambient (control) conditions using WatchDog 1650 Micro Station sensors (Spectrum Technologies, Aurora, IL). Incoming photosynthetic active radiation (PAR) was measured using PAR sensors (LightScout Quantam Light Sensor, Spectrum Technologies, Aurora, IL). The data loggers were mounted on metal posts with appropriate shields to protect from direct sunlight and placed 5 cm above the canopy level. The PAR sensors were attached near the top of the metal posts and connected to the data loggers via cable.

Data Measurements

2016 Field Experiment

Physiological traits

Physiological traits were recorded beginning ten days following anthesis (Feekes 10.5.1), and until physiological maturity (Feekes 11.3). The yellowing of the peduncle below the spike was used as the indication of physiological maturity. All measurements were taken from flag leaves of the main tiller on two plants per wheat variety within each plot that were representative of the entire plot.

Chlorophyll index and chlorophyll fluorescence traits were recorded every third day beginning ten days after commencement of stress imposition. Chlorophyll index was measured at three points along the length of the leaf blade (near culm, mid-sheath, and near the tip) on the adaxial surface of the leaf (Green, 2016) using a handheld self-calibrating SPAD chlorophyll meter (Model 502 Plus, Spectrum Technologies, Konica Minolta Incorporated, Japan) between 1000 and 1200 hours on each day measurements were recorded . Fluorescence measurements were recorded between time 1000 and 1200 hours with a hand held chlorophyll fluorometer (Model B/OS-30p, Opti-Sciences Incorporated, Hudson, New Hampshire) and settings as follows: light pulse intensity of 3000 mmol $m^{-2}s^{-1}$ and pulse duration of three seconds. Fv/Fm ratio was measured from the same flag leaves following 30 minutes of dark adaptation; leaf clips equipped with an open and close shutter were placed one-third up from the leaf base on the adaxial surface.

Agronomic traits

The wheat was hand harvested upon kernel ripeness (Feekes 11.4) for each of the varieties with harvest ranging from 15 to 22 June 2016. Two areas measuring 1.5 m in length were hand harvested from the centermost rows (two rows from the four row plots) in order to account for border effects. This resulted in a harvest of four, 1.5 m lengths totaling 6 m per wheat variety. Harvest index was measured by first weighing the whole plant sample (grain and biomass). Samples were threshed using a laboratory thresher (LD 180, Wintersteiger, Ried im Innkreis, Austria). Chaff and any foreign material were removed from the seed using a large column blower (CB-2A, Agriculex, Guelph, Ontario, Canada). Grain samples were

weighed and subtracted from the whole plant sample weight. Harvest index was then calculated as the ratio of harvested grain to total above-ground biomass.

2017 Field Experiment

Physiological traits

Physiological measurements were taken temporally (three times per week on alternate days) beginning four days after heat stress imposition (Feekes 10.5.4) until complete flag leaf senescence, which preceded physiological maturity (Feekes 11.3) in all lines. Measurements were taken from flag leaves of the main tiller on two plants that were representative of each variety within each plot. Chlorophyll index was measured using a hand held self-calibrating SPAD chlorophyll meter (Model 502 Plus, Spectrum Technologies, Konica Minolta Incorporated, Japan). All chlorophyll index measurements were recorded as an average of three points along the flag leaf of the main tiller (near culm, mid-sheath, and near the tip) on the adaxial surface of the leaf (Green, 2016).

Spike and flag leaf temperature were collected with a FLIR Vue Pro R thermal camera (FLIR Systems, Wilsonville, OR). Thermal images were taken temporally on days 6, 10, 14, and 18 after stress was imposed. The FLIR Vue Pro R was equipped with a 13 mm lens and 640x512 resolution; it was mounted to a pole and consistently held one meter above the canopy during thermal data acquisition. Individual spike and flag leaf temperature were extracted using FLIR TOOLS software.

Agronomic traits

The wheat was hand harvested upon physiological maturity (Feekes 11.3) on 15 and 16 June 2017. The plants were dried in a forced-air dryer at 45˚C for 72 hours and subsequently weighed for total above ground biomass. Two 1.0 m lengths of row were harvested from two innermost rows (six row plots) of each of the seven varieties. Harvest index was initially measured by weighing the whole plant sample - grain and biomass. Samples were threshed using a LD 180 laboratory thresher (Wintersteiger, Ried im Innkreis, Austria). Chaff and any foreign material was removed from the seed using a large column blower (CB-2A, Agriculex, Guelph, Ontario, Canada). Grain samples were weighed and subtracted from the whole plant sample weight. Then, harvest index was calculated as the ratio of harvested grain to total above ground biomass. Yield was calculated as grams/m^2 and converted to kg/ha^{-1}.

Controlled Environment

The study's other portion was carried out in controlled environment chambers at the Department of Agronomy, Kansas State University, Manhattan, Kansas in 2016. This experiment involved seven prominent Kansas winter wheat varieties (Table 2.1) grown in two temperature treatments (control and heat stress). Seeds of each of the seven varieties were sown in 30.5 x 61 cm flat seed trays filled with Sunshine Metro-Mix 380 potting soil (Sun Gro Horticulture, Agawam, Massachusetts) and placed in a greenhouse at room temperature. After most seeds had germinated, the seed trays were transferred to a vernalization chamber maintained at 5°C for 6 weeks. Following vernalization, 40 plants of each variety were transplanted into individual 1.6 L pots (10x24 cm, MT49 Mini-Treepot) and filled with Sunshine Metro-Mix 380 potting soil. Each pot received 5 g of Scotts Osmocote classic (14-14-14 of N-P-

K) and 0.5 g of Scotts Micromax Micronutrients (Hummert International, Topeka, Kansas) at the time of transplanting. Pots were kept in trays and moved to controlled environment chambers maintained at 25/15°C maximum day/minimum night temperature. To avoid confounding effects of water stress, the plants were kept in well-watered conditions by maintaining a water layer of 1 cm in the tray placed below the pots for the entirety of the experiment.

The main tiller and two subsequent tillers (considered primary tillers) of each plant were tagged on the day anthesis began. Ten days after the start of anthesis, 20 of 40 plants from each variety were transferred to high day temperature (simulation of heat stress) growth chambers set at 35/15°C day/night temperature. The remaining 20 plants stayed in the control growth chambers and maintained at an optimum 25/15°C day/night temperature. Both heat stress and control growth chambers were maintained at 16/8 hour photoperiod, with 900-1000 µmol m^{-2} s^{-1} light intensity at 5 cm above the canopy, and 70% relative humidity (RH). Maximum day and minimum night temperatures were maintained for seven and eight hours, respectively in all chambers; each day/night transition occurred in 4.5 hour periods (Supplementary fig. S1). Temperature and RH were recorded every 15 minutes using HOBO UX 100-011 and temperature/RH data loggers (Onset Computer Corp., Bourne, MA) in all growth chambers.

Data Measurements

Physiological traits

The same physiological measurements taken for the field experiments were also used in the growth chamber experiment. Physiological measurements of gas exchange, chlorophyll index, and chlorophyll fluorescence were taken periodically following anthesis (Feekes 10.5.1)

until physiological maturity (Feekes 11.3). The yellowing of the peduncle below the spike was used as the indication of physiological maturity. Measurements were taken from flag leaves of the main tiller on five individual plants of each variety that were tagged the day anthesis began. Gas exchange was taken with a LI-6400XT Portable Photosynthesis System (Licor, Lincoln, Nebraska) set at a block temperature of 25 and 35^{o}C in control and heat stress treatments, respectively. The leaf chamber CO_2 was fixed at 400 ppm at a flow rate of 500 µmol s^{-1} and a light intensity of 1000 µ mol m^{-2} s^{-1} of PAR supplied by red-blue light emitting diode. Chlorophyll index was measured at three points along the length of the leaf blade using a hand held self-calibrating SPAD chlorophyll meter (Model 502, Spectrum Technologies, Plainfield, Illinois). Fluorescence measurements were taken with a handheld chlorophyll fluorometer (Model B/OS-30p, Opti-Sciences Inc., Hudson, New Hampshire) by clamping clips (one-third up from the leaf base) on the adaxial surface of 30-minute dark adapted leaves. The photochemical efficiency of PS II (Fv/Fm) was recorded from dark adapted flag leaves at a light pulse intensity of 3000 µmol m^{-2} s^{-1} and pulse duration of 1 second (Sunoj et al., 2017). Fv/Fm ratio was measured from the same flag leaves after thirty minutes of dark adaptation, by placing clips one-third of the way up from the leaf base on the adaxial surface. Gas exchange measurements were taken 5 and 15 days after the high temperature stress was initiated. Chlorophyll index and chlorophyll fluorescence observations were taken every third day beginning the day high temperature stress commenced.

Agronomic traits

Upon kernel ripeness, wheat was hand harvested and harvest measurements were collected. Number of tillers and spikes per plant were recorded prior to harvest. Spikes were

separated from the plant tissue and categorically grouped: main tiller spike, two primary tiller spikes, and remaining spikes. The spikes were dried in a laboratory oven for 96 hours at 40°C. After drying, spike weight and spike length were recorded before threshing. Spikes were threshed using a wheat head thresher (Precision Machine Company., Lincoln, Nebraska). Number of seeds per spike was counted using an electronic seed counter, and seed weight per spike was also recorded. Finally, the biomass was dried in a forced-air dryer at 65℃ for 72 hours prior to weighing.

Statistical analysis

The experiments were organized in a split-plot randomized complete block design with temperature as the main plot factor and genotype as sub-plot factor; five replications in controlled environment chamber experiment, four replications in field 2016, and three replications in field 2017. Different sampling times were considered as sub-sub plot factors for chlorophyll index in the field experiments. Analysis of variance for all the measured parameters was performed using PROC GLM procedure in SAS software (Version 9.4, SAS Institute Inc., Cary, NC). Means were separated using least significant difference (LSD) test at $p = 0.05$.

Results

In field 2016, daytime mean temperature was similar within and outside the heat tents from the start of the heat stress period, until approximately 30 days after stress (DAS) initiation (Fig. 2.1A). From 30 DAS until physiological maturity, the daytime mean temperature inside the heat tents was 4^{o}C warmer than ambient outside temperatures (Fig. 2.1A). In comparison, field

2017 daytime mean temperature inside the heat tents was 6ºC warmer than ambient outside temperatures beginning from ten DAS and until physiological maturity (Fig. 2.1B). In contrast, nighttime mean temperature inside and outside the heat tents were similar throughout the experimental period in both seasons (Fig. 2.1A and B). This indicated that the field based heat tents increased only daytime temperatures, inducing high day temperature stress, and not confounded by higher night temperatures; these finding are similar to what was found in a similar study (Sunoj et al., 2017). Ambient daytime mean temperature during the field experiment 2017 were 2 to 16ºC warmer than ambient temperatures in the 2016 field experiment, indicating large inter-annual temperature variability. Experimentally applied heat stress induced early maturity by reducing grain filling duration when compared to the control counterparts in almost all varieties. The only exception was the shorter duration variety, WB-Cedar, in 2017 (Fig. 2.2). Among the varieties, applied heat stress resulted in reduction of grain filling duration by five days in Larry and Zenda followed by four days in WB4458 and Joe (Fig. 2.2).

Grain yield and related traits

In the controlled chamber experiment, grain yield was significantly affected by temperature, variety, and temperature-variety interaction (Table 2.2). Grain yield across varieties was reduced by an average 37% under heat stress conditions as compared to the control. Among the varieties Larry recorded the smallest percent reduction in grain yield under heat stress compared to control, followed by SY Monument. In contrast, WB-Cedar and Everest both recorded near 50% reduction in grain yield (Fig. 2.3A). However, in both of the field experiments (2016 and 2017), grain yield varied significantly between treatments ($p < 0.001$) and varieties ($p < 0.05$), but not with treatment-variety interaction effect (Table 2.3). The average grain yields under heat stress were 16% and 13% lower than the control in 2016 and 2017 field

experiments, respectively. Among the varieties in the 2016 field experiment, SY Monument recorded the smallest percent reduction (2%) in grain yield when exposed to heat stress compared to the control, followed by Everest (6%) and WB4458 (13%) (Fig. 2.3B). In the 2017 field experiment, the lowest yield reduction with heat stress exposure as compared to control was recorded in Zenda (4%), followed by Larry (7%), and Joe (7%) (Fig. 2.3C). WB-Cedar recorded highest yield reduction under heat stress compared to control in both of the field experiments (Fig. 2.3 B and C). Grain yield in the controlled chamber experiment correlated significantly with grain yield from the 2017 field experiment under both control ($r=0.74$, $p=0.058$) and heat stress ($r=0.79$, $p=0.034$) conditions. However, significant correlations were not obtained for grain yield between the controlled chamber experiment and 2016 field experiment or between field experiments.

Harvest index (HI) varied significantly for temperature, variety, and temperature-variety interaction ($p<0.001$) in controlled chamber experiment, with heat stress inducing an average 22% reduction in HI versus the control (Table 2.2). Heat stress induced significant reduction in HI in all varieties compared to control, except in SY Monument (Table 2.4). In the controlled chamber experiment, SY Monument recorded the smallest percent reduction (8%) in heat stress compared to control, followed by WB4458 (11%) (Table 2.4). In field experiments, HI was significantly affected by temperature ($p<0.05$) and variety ($p<0.01$), but not temperature-variety interaction (Table 2.3). Among the tested varieties, Joe and Larry recorded significantly lower HI in heat stress compared to control in the 2016 field experiment. However, in 2017, four varieties including WB-Cedar, Joe, Zenda, and Everest recorded significantly lower HI in heat stress compared to control (Table 2.4). Thousand kernel weight varied significantly between temperatures, genotypes, and temperature-genotype interaction in both controlled chamber and

field experiments. On average, heat stress induced 28% and 4-5% reductions in single kernel weight under controlled chamber and field experiments, respectively. Among those included, all genotypes recorded significantly lower thousand kernel weight under heat stress compared to the control in the controlled chamber experiment with the exception of SY Monument (Table 2.4). In both of the field experiments, Zenda and SY Monument did not record significant reduction in thousand kernel weight in heat stress compared to control (Table 2.4). Significant correlations were detected between field experiments for thousand kernel weight under both control ($r = 0.87$, $p = 0.011$) and heat stress ($r = 0.87$, $p = 0.013$) conditions. However, thousand kernel weight in the controlled chamber experiment did not correlate with thousand grain weight of either of the field experiments. Grain number per main and primary spikes was not significantly affected by temperature whereas, grain number in remaining spikes was significantly affected based upon variety ($p < 0.001$) and temperature-variety ($p < 0.05$) interaction in the controlled chamber experiment (Table 2.2). When averaged across genotypes, grain number per remaining spikes was reduced by 26% under heat stress compared to control. Among the varieties, WB-Cedar and Everest recorded highest reductions in grain number in remaining spikes in heat stress compared to control (Fig. 2.4).

Chlorophyll index

In the controlled chamber experiment, chlorophyll index decreased over time in both treatments with faster and greater rates of reduction present in heat stress versus control in all tested varieties (Fig. 2.5A and B). Among all varieties, WB4458 and WB-Cedar exhibited rapid reductions in chlorophyll index over time under heat stress compared to control, whereas, SY Monument maintained a similar trend under both the treatments (Fig. 2.5 A and B). Chlorophyll index in field experiments was significantly affected by temperature ($p<0.01$), variety ($p<0.001$),

and DAS ($p < 0.001$), while significant interaction effects (temperature × variety × DAS) ($p < 0.001$) were seen in the 2016, but not the 2017, field experiment (Table 2.3). Reductions in chlorophyll index were observed in both the treatments starting 30 DAS in 2016 (Fig. 2.5 C and D) and 15 DAS in 2017 (Fig. 2.5 E and F) field experiments. The naturally warmer temperatures in 2017, was evident through premature chlorophyll degradation noted in the SPAD measurements. This data supports the significant decline in yield, even under ambient conditions outside of the tents in the 2017 field experiment.

Net CO_2 assimilation rate

In the controlled chamber experiment, net CO_2 assimilation rate was significantly affected by temperature ($p < 0.001$), variety ($p < 0.001$), DAS ($p < 0.001$), and their interaction effects (Table 2.2). When averaged across genotypes, assimilation rate under heat stress was significantly reduced by 13% and 39% compared to control at 5 and 15 DAS, respectively. Adverse effects of heat stress on assimilation rate was greater at 15 DAS compared to 5 DAS, with all varieties recording increased percent reductions in heat stress compared to the control at 15 DAS (Table 2.5). Almost all genotypes showed significant reductions in assimilation rate under high day temperature (HDT) stress versus control; this was the case irrespective of DAS and experiment, with the exception of Joe at 5 DAS (Table 2.5).

Maximum quantum yield of PS II (Fv/Fm)

In controlled chamber experiments, average Fv/Fm varied significantly between treatments ($p < 0.001$) and varieties ($p < 0.001$), but not for temperature-variety interaction (Table 2.2). Heat stress induced a drastic reduction in Fv/Fm beginning five days after stress imposition in almost all the varieties (Fig. 2.6 B), while reduction in Fv/Fm in the control conditions was observed approximately ten days after the reduction was first noticed in the heat

stress treatment (Fig. 2.6 A). Among the varieties, rate of reduction in Fv/Fm was greater in Joe and Everest compared to others (Fig. 2.6 AandB). In the 2016 field experiment, Fv/Fm was significantly affected by temperature, variety, DAS, and their interaction effects (Table 2.3). All the varieties recorded greater reductions in Fv/Fm in heat stress compared to control (Fig. 2.6 C and D).

Spike and flag leaf temperatures

In the 2017 field experiment, the relationship between spike and flag leaf temperature and the measured yield components fluctuated over time. Both flag leaf and spike tissue temperatures followed the natural temperature pattern. Measurements recorded at 18 DAS exhibited maximum variation in flag leaf and spike temperature between control and stress conditions, compared to three earlier measurements at 6, 10 and 14 DAS (Fig 2.7). At 6 DAS, spike and flag leaf temperatures had a high negative correlation with grain yield and biomass, but no relationship with thousand kernel weight (Fig. 2.8). However, at 14 DAS imposition, spike and flag leaf temperatures recorded a positive correlation with grain yield, harvest index, total biomass, and thousand kernel weight when ambient air temperatures inside the heat tents were approximately 35^{o}C (Fig. 2.8). A positive relationship with a R^2 value of 0.88 was observed between flag leaf and spike temperatures (Fig. 2.9). However, the disparity between spike and flag leaf temperatures increased as ambient air temperature increased (Table 2.6). The hottest ambient temperatures (> 35^{o}C) at 18 DAS imposition induced much larger disparities between stressed and control tissue temperatures. At 18 DAS in the tents, average spike and leaf temperature across all varieties increased by 44% and 37%, respectively (Table 2.6).

Discussion

Most published studies regarding post-flowering heat stress in wheat have been conducted under controlled environment facilities (Stone and Nicolas 1994; Gibson and Paulson 1999; Spiertz et al., 2006). Hence, a comparative assessment of the response of assorted varieties across scales, particularly under field conditions will provide firsthand information about yield losses that can be expected under these realistic conditions. Simultaneously, stress impact recorded from controlled environments will aid in identifying true levels of tolerance or the ranges of susceptibility for these varieties when exposed to severe stress conditions. Information obtained across both scales utilizing the same varieties are complementary.

Currently, information regarding the impact of post-flowering heat stress in wheat is only available for genotypes that are not widely grown (Gibson and Paulsen, 1999; Narayanan et al., 2015). Additionally, there is not an indicative reference or baseline on the performance of current prominent varieties by which to ascertain the "gap in stress resilience" that may need to be filled. Filling this gap will ensure that grower-preferred varieties can be sustainably grown under current and future predicted climates. In this study, percent reduction in yield ranged from 6% to 51% when exposed to severe heat stress in controlled environments, and 2% to 27% under heat stress exposure in the field using heat tents; these results indicate high resilience in some varieties, but also presents the need for improvement in many prominent varieties. Among the prominent or recently released varieties, SY Monument and Larry performed well under both field and controlled environments, suggesting that they are suited for locations that face consistent heat stress exposure during the post-flowering grain fill stage. Under severe heat stress in the controlled environment study, the superior performance of SY Monument can be attributed to higher thousand kernel weight and grain number, whereas Larry maintained only a better grain

number. Under milder heat stress exposure in the field experiment, both SY Monument and Larry maintained a higher thousand kernel weight and maintained a lower reduction in HI. It should be noted that the lines that constitute 50% of the pedigree of both SY Monument and Larry are sister lines derived from the same cross. This suggests these materials could possess similar genetics for tolerance to high temperature stress, though the mechanisms of tolerance may be somewhat different. The data suggests SY Monument may have great stability of starch synthesis while Larry may possess greater reproductive stage resilience helping retain higher seed number under stress.

Agronomically, shorter duration varieties have been proposed as being a useful mechanism for avoiding heat stress during terminal grain filling stage (Mondal et al., 2016). WB-Cedar, a comparatively early maturation variety, recorded the highest yield decline in both growth chambers and field conditions. This demonstrates that breeding for shorter duration varieties to escape heat stress may not be the most suitable strategy, as heat stress episodes have strong inter annual variability; this was seen in the varying climatic conditions which exposed the crop to more extreme heat episodes during 2017 post-flowering grain fill period than 2016. WB-Cedar, in spite of its shorter duration, had the highest grain yield under control conditions; this could be an ideal genetic material to investigate for resource use efficiency to further enhance genetic yield potential under non-stress conditions in winter wheat.

An interesting phenomenon occurred during the 2017 field experiment, wherein the outside ambient mean temperature, especially during the early grain filling stage approached 30^{o}C, with a similar rise during the late grain filling phase. A large inter annual variability in temperatures within just two consecutive years substantiates the increasing vulnerability of wheat to heat stress in Kansas and across United States Great Plains. Furthermore, this validates

that damage caused by heat stress is already a challenge; and so much of a challenge that it must be addressed immediately. A comparatively lower reduction in yield in the 2017 field experiment compared to 2016 was primarily due to extreme heat events affecting control plots quite similarly to the plants inside the tents. (Fig. 2.1B and 2.3B, C).

Lower grain numbers in the "remaining spikes" under severe heat stress in controlled environments can be attributed to the impact of heat stress on the sensitive gametogenesis (Prasad et al., 2017). In the present study, the main spike was a primary focus for the impact of post-flowering heat stress, whereas heat stress coinciding with early reproductive organ development in the younger spikes is known to reduce pollen viability, seed set, and grain number (Prasad and Djanaguiraman, 2014). In addition, wheat florets have a tendency to flower either during early morning or late evening to escape high temperatures during the day - possibly an effective adaptive mechanism to strive for, especially under harsh upland conditions. This phenomenon of being more conspicuous under heat stress exposure has been recently demonstrated (Sun et al., 2017), providing additional support for the observed greater impact on remaining tillers compared to the main tiller in the current study. In some of the post-flowering heat sensitive varieties such as WB-Cedar, Joe, and Everest greater reductions in thousand kernel weight in heat stress versus control were recorded. These recordings suggest that post-flowering heat stress affects grain filling processes, thereby reducing grain weight and yield (Wheeler et al., 1996).

Physiologically, the rate of decline in chlorophyll index and the maximum quantum yield of PSII (Fv/Fm) were more rapid under heat stress (controlled environment) exposure during grain filling. Varieties that performed well under heat stress, Larry (controlled environment) and SY Monument (field), maintained a higher chlorophyll index compared to other varieties when

exposed to the same degree of heat stress. Overall, Fv/Fm values followed a declining trend similar to chlorophyll index. Interestingly, Joe maintained the highest Fv/Fm values in the field experiment but showed one of the most drastic Fv/Fm declines in the controlled environment heat treatment. In contrast, WB4458 exhibited some of the most stable Fv/Fm values under both treatments in the controlled environment experiment; however, under the field experiment WB4458 showed the most rapid reduction under heat stress. Amongst all varieties in both the field and controlled chamber experiments, SY Monument, Larry, and Zenda appeared to maintain the most stable Fv/Fm values when exposed to heat stress compared to the control.

Early senescence induced by heat stress was variable among genotypes. In general, the genotypes that had shorter grain filling periods and earlier maturity, such as WB-Cedar and Everest, recorded the highest percent reductions in grain yield. Across the three experiments, WB-Cedar recorded the largest percent reduction in grain yield in both the controlled environment experiment and 2016 and 2017 field study. In 2017, thermal images were taken at different times throughout the grain fill period during which individual spike and flag leaf temperatures could be extracted. It was observed that varieties that experienced lower yield reductions under heat stress, such as Larry and SY Monument, maintained spike temperatures lower than 30^{o}C under heat stress conditions of 35°C. WB-Cedar and other lower performing varieties exhibited an average spike temperature in excess of 30°C under the same conditions. One unexplained anomaly still exists: the yield reduction in Everest, which did not have an excessive spike temperature over 30^{o}C. This suggests that a spike temperature threshold for heat tolerance may be variety dependent. In addition, variability between varieties regarding disparity between flag leaf and spike temperature may be indicative to their internal mechanisms that contribute to heat stress resilience. Therefore, during parent selection, these heat tolerance

thresholds should be explored as it may restrict the suitability of particular varieties for environmental conditions similar to Kansas or the United States Great Plains.

The impact of heat stress, although mild during the first three weeks of grain filling (active grain filling stage) in the 2016 field experiment, still resulted in an 11% and 7% decrease in thousand kernel weight and harvest index, respectively. This substantiates the vulnerability of prominent Kansas varieties to moderate heat stress exposure. Considering a large inter annual climatic variability in Kansas or similar ecologies in the Great Plains, the sensitive grain filling stage is routinely exposed to warmer temperatures, leading to historically lower wheat productivity in the region. Our study provides the first report on the current resiliency in prominent winter wheat for Kansas and the United States Great Plains.

In conclusion, there exists a considerable range in heat stress response with varieties including but not limited to Larry and SY Monument being comparatively tolerant, while others such as WB-Cedar and Everest demonstrate distinct signs of greater heat stress sensitivity. Larry, a recently released variety (fall 2017), is touted for its high yield potential and its ability to tolerate heat stress; it will be an added benefit under warming temperatures. The findings of this study are similar to recent results from rice research in which just one or two prominent varieties had adequate levels of tolerance and the vast majority of the prominent varieties from Asia and Africa remain sensitive to stress, causing significant yield losses (Shi et al., 2015). While SY Monument and Larry might be better than the other varieties tested, they aren't as resilient as would be desired either currently or for future conditions. Therefore, exploration of additional sources of tolerance and pyramiding mechanisms of tolerance are important considerations for future wheat improvement.

Table 2.1 Breeding programs and characteristics of seven winter wheat varieties phenotyped in the study

Wheat varieties	Breeding programs	Characteristics
Everest	Kansas State University, Manhattan, KS	Released in 2009. Hard red winter wheat, high yield potential, resistant to barley yellow dwarf and Fusarium head scab, resistance to Hessian fly and leaf rust, drought tolerant (http://kswheat alliance.org)
Joe	Kansas State University, Hays, KS	Released in fall 2015. Hard white winter wheat, drought tolerant, wheat streak resistance, stripe and leaf rust resistance (www.agronomy.ksu.edu/research)
Larry	Kansas State University, Manhattan, KS	Released in summer 2016. Hard red winter wheat, medium–early maturity, moderately resistant to stripe and leaf rust, good acid tolerance (http://kswheat alliance.org)
SY Monument	Syngenta, Basal, Switzerland	Released in 2011.Hard red winter wheat, excellent acid soil tolerance and soil borne mosaic virus resistance, winter hardiness, medium to late maturity leaf and stripe rust resistance, drought tolerance (http://agriprowheat.com)
WB-Cedar	Monsanto, St. Louis, MO	Released in 2011. Hard red winter wheat, excellent yield potential and straw strength, shatter tolerance, early maturity to avoid heat, yellow stripe rust and Hessian fly resistance (https://www.westbred.com)
WB4458	Monsanto, St. Louis, MO	Hard red winter wheat, excellent yield potential and standability, drought tolerance (https://www.westbred.com)
Zenda	Kansas State University, Hays, KS	Released in summer 2016. Hard red winter wheat; resistance to stem, stripe, and leaf rust; good acid soil tolerance; soil born mosaic virus resistance (http://kswheat alliance.org)

Table 2.2 Probability of effects of temperature (T), variety (V), and T × V interactions on physiological and yield parameters in controlled environment experiment. Values are averages across seven Kansas winter wheat varieties for growth and yield parameters, and physiological traits

Traits	T	V	T X V	Control	Heat stress
Net CO_2 assimilation (µmol m^{-2} s^{-1})	<0.0001	<0.0001	<0.0001	15.59a	11.64b
Conductance (mol m^{-2} s^{-1})	<0.0001	<0.0001	0.0002	0.676a	0.513b
Transpiration (mmol m^{-2} s^{-1})	<0.0001	<0.0001	0.003	6.38b	11.07a
Maximum quantum yield of PS II (Fv/Fm)	0.0003	0.0025	NS	0.715a	0.642b
Chlorophyll index (spad units)	0.0001	NS	NS	46.89a	38.83b
Spike weight (g $spike^{-1}$)$_{MS}$	0.0013	<0.0001	NS	2.1a	1.8b
Spike weight (g $spike^{-1}$)$_{PS}$	0.0001	<0.0001	NS	3.51a	2.7b
Spike weight (g $spike^{-1}$)$_{RS}$	0.012	0.004	0.029	8.3a	5.5b
Grain number ($spike^{-1}$)$_{MS}$	NS	<0.0001	NS	47a	43b
Grain number ($spike^{-1}$)$_{PS}$	NS	<0.0001	NS	74a	71a
Grain number ($spike^{-1}$)$_{RS}$	0.087	0.0009	0.017	184a	136b
Kernel weight (g)$_{MS}$	<0.0001	<0.0001	NS	0.037a	0.028b
Kernel weight (g)$_{PS}$	<0.0001	<0.0001	0.01	0.037a	0.026b
Kernel weight (g)$_{RS}$	<0.0001	<0.0001	NS	0.036a	0.026b
Grain weight (g $spike^{-1}$)$_{MS}$	0.0008	<0.0001	NS	1.58a	1.29b
Grain weight (g $spike^{-1}$)$_{PS}$	<0.0001	<0.0001	NS	2.69a	1.85b
Grain weight (g $spike^{-1}$)$_{RS}$	0.006	0.01	0.01	6.28a	3.53b
Tiller number ($plant^{-1}$)	NS	<0.0001	NS	9.6a	9.8a
Spike number ($plant^{-1}$)	NS	<0.0001	NS	9.8a	9.6a
Spike weight ($plant^{-1}$)	0.004	0.0005	0.056	14.0a	10.1b
Thousand Kernel weight (g)	<0.0001	<0.0001	0.008	36.8a	26.6b
Grain number (g $plant^{-1}$)	NS	<0.0001	0.034	301.9a	254.2b
Total biomass (g $plant^{-1}$)	NS	0.0014	NS	6.95a	6.59a
Grain yield (g $plant^{-1}$)	0.0016	0.0003	0.035	10.6a	6.68b
Harvest index	0.0005	<0.0001	0.001	0.509a	0.397b

Table 2.3 Probability of effects of temperature (T), variety (V), days after stress (DAS), T × V, T × DAS, V × DAS, and T × V × DAS interactions on physiological and yield parameters in 2016 and 2017 field experiments

Traits	Variables (Pr > F)							Main effect of temperature	
	T	V	T × V	DAS	T × DAS	V × DAS	T × V×DAS	Control	Heat stress
Field experiment 2016									
Chlorophyll index (SPAD units)	<0.01	<0.001	0.243	<0.001	<0.001	<0.001	<0.01	42.4a	40.8b
Maximum quantum yield of PSII (Fv/Fm)	<0.01	<0.001	0.097	<0.001	<0.001	<0.001	0.014	0.71a	0.69a
Thousand kernel weight (mg)	0.058	<0.001	<0.001	-	-	-	-	33a	31.7b
Grain yield (g m^{-2})	<0.001	<0.05	0.660	-	-	-	-	534.6a	458.0b
Harvest index	<0.05	<0.01	0.460	-	-	-	-	0.443a	0.427b
Field experiment 2017									
Chlorophyll index (SPAD units)	0.057	<0.001	0.794	<0.001	<0.001	<0.001	0.935	37.4a	35.0b
Thousand kernel weight (g)	<0.001	<0.001	<0.05	-	-	-	-	35.5a	33.7b
Grain yield (g m^{-2})	<0.05	<0.001	0.152	-	-	-	-	526a	462b
Harvest index	<0.01	<0.001	0.061	-	-	-	-	0.440a	0.414b

Table 2.4 Harvest index and thousand kernel weight of wheat varieties grown in controlled chambers and field experiments under control and heat stress treatments

	Harvest Index		Thousand Kernel weight (g)	
Varieties	**Control**	**Heat stress**	**Control**	**Heat stress**
Controlled chamber experiment				
WB-Cedar	0.52 ± 0.01	0.36 ± 0.04	29.0 ± 0.4	22.0 ± 1.0
Joe	0.55 ± 0.01	0.43 ± 0.01	41.4 ± 0.9	30.0 ± 0.4
WB4458	0.52 ± 0.004	0.46 ± 0.01	44.0 ± 1.0	32.0 ± 0.4
Zenda	0.50 ± 0.01	0.37 ± 0.03	35.4 ± 1.0	23.5 ± 0.8
SY Monument	0.50 ± 0.01	0.46 ± 0.01	36.6 ± 0.5	30.0 ± 0.8
Larry	0.49 ± 0.01	0.42 ± 0.02	40.0 ± 1.0	28.7 ± 0.7
Everest	0.46 ± 0.02	0.27 ± 0.02	31.0 ± 0.8	20.0 ± 1.0
5% LSD (T)	0.03		2.6	
5% LSD (V)	0.04		4.9	
5% LSD (T×V)	0.05		6.9	
Field experiment 2016				
WB-Cedar	0.46 ± 0.01	0.44 ± 0.01	35.0 ± 0.4	33.1 ± 0.4
Joe	0.45 ± 0.004	0.42 ± 0.01	34.5 ± 0.4	30.8 ± 0.5
WB4458	0.42 ± 0.02	0.43 ± 0.004	34.4 ± 0.3	33.2 ± 0.6
Zenda	0.43 ± 0.002	0.43 ± 0.003	31.8 ± 0.4	31.0 ± 0.2
SY Monument	0.43 ± 0.01	0.42 ± 0.002	32.7 ± 0.3	31.8 ± 0.5
Larry	0.47 ± 0.004	0.44 ± 0.007	32.0 ± 0.4	32.8 ± 0.4
Everest	0.43 ± 0.01	0.41 ± 0.01	30.7 ± 0.8	29.2 ± 0.3
5% LSD (T)	0.01		1.42	
5% LSD (V)	0.02		0.84	
5% LSD (T×V)	-		1.18	
Field experiment 2017				
WB-Cedar	0.48 ± 0.004	0.45 ± 0.003	39.0 ± 0.7	35.9 ± 0.5
Joe	0.43 ± 0.003	0.39 ± 0.006	36.9 ± 0.5	34.5 ± 0.3
WB4458	0.43 ± 0.006	0.42 ± 0.004	36.2 ± 0.4	34.4 ± 0.4
Zenda	0.40 ± 0.006	0.37 ± 0.004	33.4 ± 0.3	32.5 ± 0.3
SY Monument	0.44 ± 0.009	0.43 ± 0.009	34.6 ± 0.3	33.8 ± 0.6
Larry	0.45 ± 0.005	0.44 ± 0.004	36.3 ± 0.3	34.7 ± 0.3
Everest	0.45 ± 0.022	0.40 ± 0.007	32.6 ± 0.1	30.2 ± 0.3
5% LSD (T)	0.01		0.23	
5% LSD (V)	0.02		0.77	
5% LSD (T×V)	-		1.09	

Table 2.5 Net CO_2 assimilation rate in flag leaf of main tiller among the seven winter wheat varieties under control and heat stress treatments in controlled chamber experiment

	5 DAS		15 DAS	
Varieties	**Control**	**Heat stress**	**Control**	**Heat stress**
WB-Cedar	14.2 ± 0.4	12.3 ± 0.4	16.1 ± 0.2	10.3 ± 0.7
Joe	14.7 ± 0.3	13.5 ± 0.4	12.9 ± 0.3	11.0 ± 0.9
WB-4458	19.5 ± 0.5	18.1 ± 0.7	18.8 ± 0.3	11.1 ± 0.5
Zenda	13.4 ± 0.6	10.5 ± 0.6	13.3 ± 0.2	9.1 ± 0.1
SY-Monument	18.6 ± 0.3	15.9 ± 0.3	17.2 ± 0.2	8.2 ± 0.1
Larry	18.4 ± 0.1	16.5 ± 0.6	17.7 ± 0.4	8.7 ± 0.3
Everest	13.1 ± 0.9	11.2 ± 0.3	10.4 ± 0.3	6.6 ± 0.4
5% LSD (T×V×DAS)	1.3			

Table 2.6 Spike and flag leaf temperature (°C) of seven winter wheat varieties under control and heat stress conditions in 2017 field experiment

	Days after imposition (d)							
	06		10		14		18	
Variety	Control	Heat stress	Control	Heat stress	Control	Heat stress	Control	Heat stress
Spike								
WB-Cedar	24 ± 0.1	20 ± 0.2	18 ± 0.2	18 ± 0.1	31 ± 0.4	33 ± 0.7	18 ± 0.2	27 ± 0.2
Joe	23 ± 0.1	19 ± 0.1	15 ± 0.06	16 ± 0.2	27 ± 0.2	29 ± 0.2	16 ± 0.2	28 ± 0.3
WB4458	24 ± 0.1	21 ± 0.1	17 ± 0.2	17 ± 0.1	30 ± 0.1	31 ± 0.1	20 ± 0.1	27 ± 0.5
Zenda	23 ± 0.06	20 ± 0.2	17 ± 0.07	18 ± 0.3	29 ± 0.03	34 ± 0.5	19 ± 0.2	25 ± 0.2
SY Monument	25 ± 0.4	20 ± 0.5	14 ± 0.3	15 ± 0.2	27 ± 0.2	27 ± 0.1	17 ± 0.6	25 ± 0.4
Larry	22 ± 0.1	21 ± 0.1	16 ± 0.1	15 ± 0.07	27 ± 0.2	30 ± 0.2	21 ± 0.4	28 ± 0.5
Everest	19 ± 0.06	21 ± 0.2	16 ± 0.1	16 ± 0.07	28 ± 0.1	29 ± 1.0	19 ± 0.2	27 ± 0.2
Flag leaf								
WB-Cedar	23 ± 0.2	18 ± 0.2	15 ± 0.3	15 ± 0.2	28 ± 0.2	26 ± 0.1	16 ± 0.2	24 ± 0.1
Joe	22 ± 0.2	16 ± 0.1	13 ± 0.2	12 ± 0.1	27 ± 0.1	25 ± 0.03	16 ± 0.1	23 ± 0.03
WB4458	23 ± 0.1	17 ± 0.1	14 ± 0.2	13 ± 0.2	30 ± 0.1	28 ± 0.3	17 ± 0.3	21 ± 0.1
Zenda	23 ± 0.2	17 ± 0.1	13 ± 0.2	14 ± 0.1	25 ± 0.06	26 ± 0.1	18 ± 0.1	23 ± 0.3
SY Monument	21 ± 0.1	18 ± 0.1	12 ± 0.03	12 ± 0.2	27 ± 0.4	26 ± 0.2	17 ± 0.2	26 ± 0.4
Larry	21 ± 0.1	17 ± 0.1	13 ± 0.2	11 ± 0.2	27 ± 0.1	25 ± 0.1	17 ± 0.2	24 ± 0.3
Everest	18 ± 0.1	19 ± 0.06	14 ± 0.4	14 ± 0.1	27 ± 0.1	26 ± 0.2	18 ± 0.1	24 ± 0.3

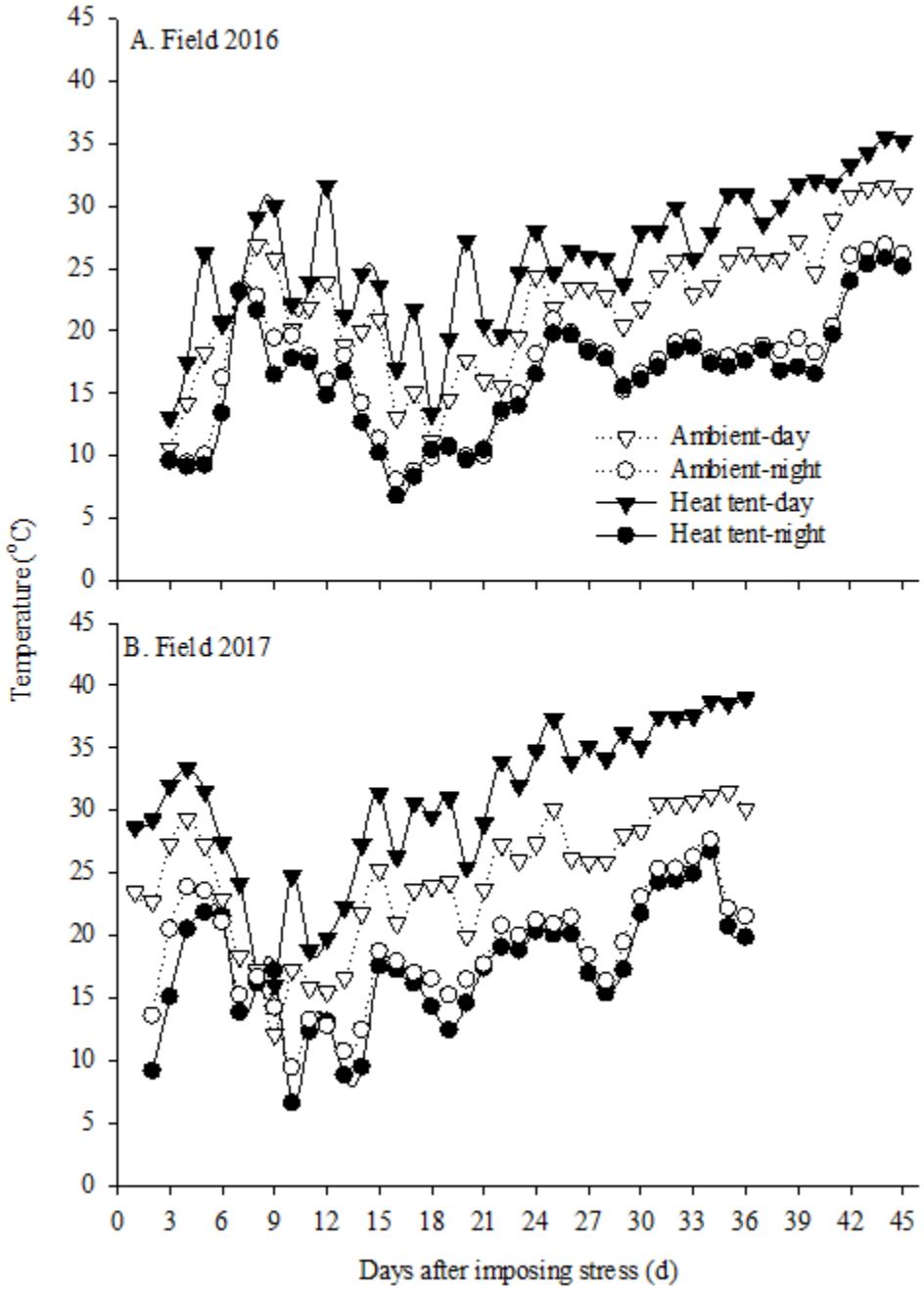

Figure 2.1 Mean day and night temperatures (°C) inside and outside (ambient) heat tents beginning from the day of heat stress imposition until physiological maturity in 2016 (A) and 2017 (B) field experiments

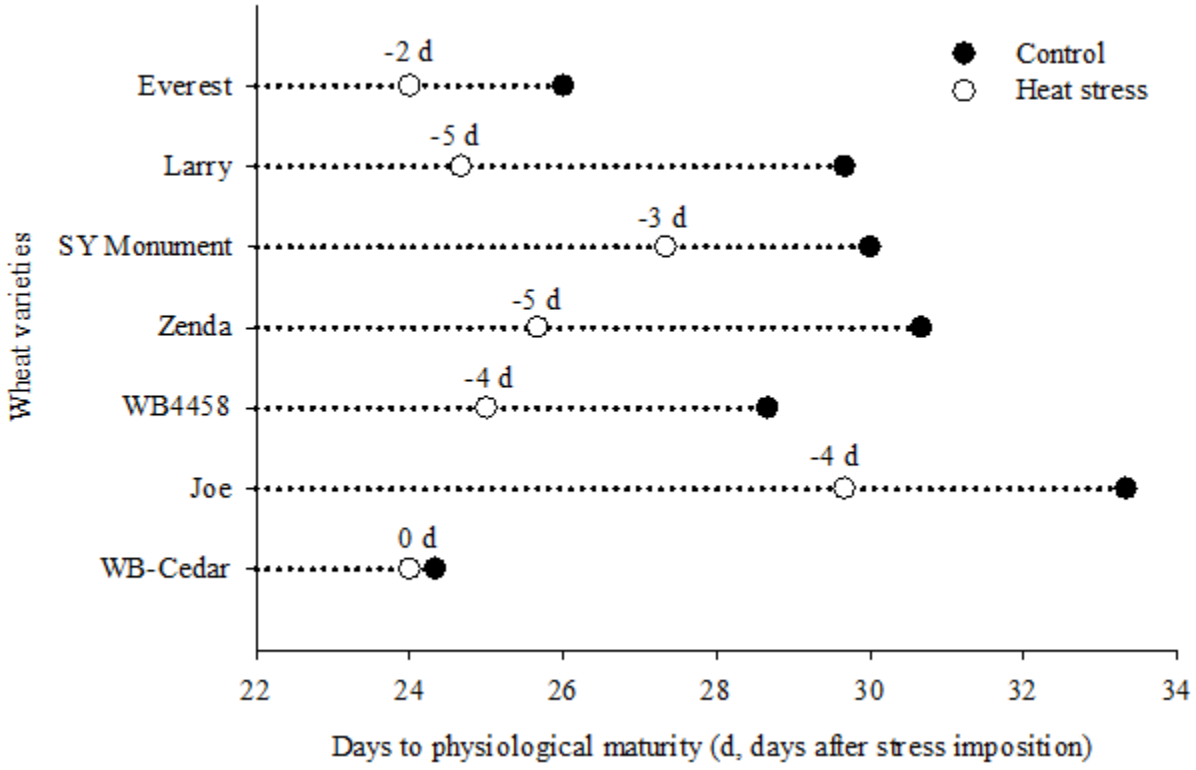

Figure 2.2 Days to physiological maturity (d) recorded from day of stress imposition until physiological maturity in 2017 field experiment

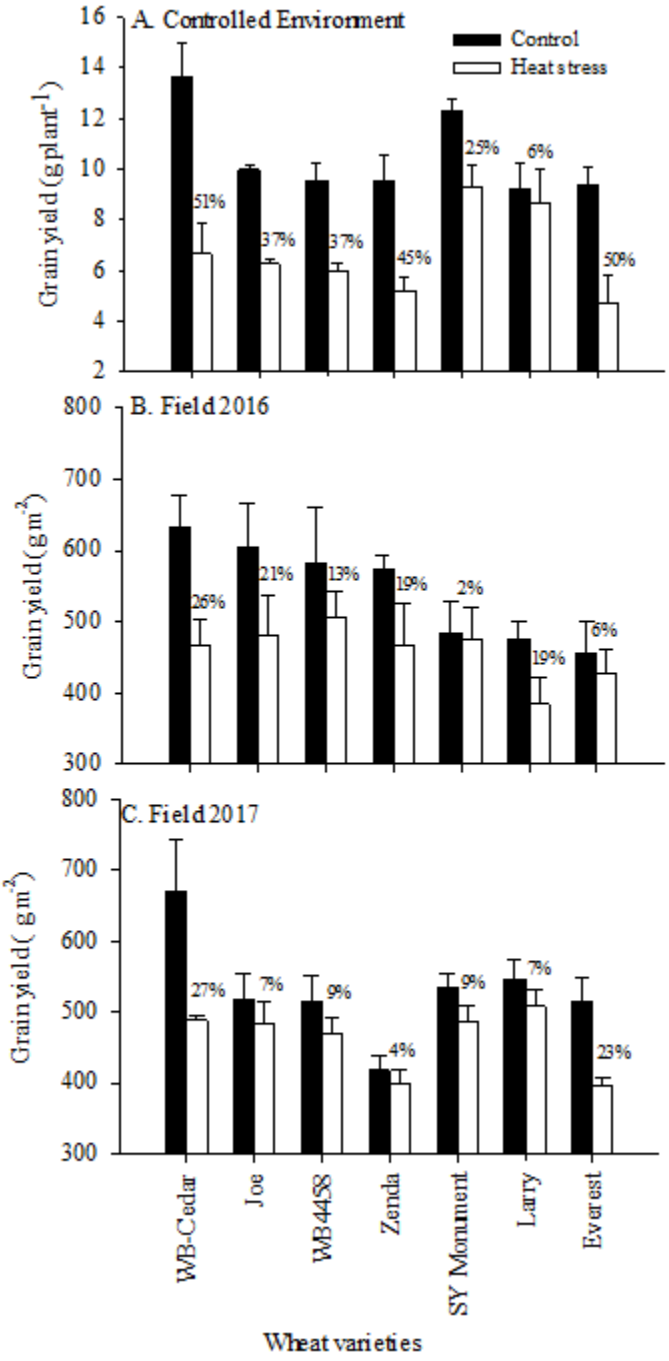

Figure 2.3 Grain yield in controlled environment (A), and field conditions (Field 2016, B; Field 2017, C) under control and heat stress treatments

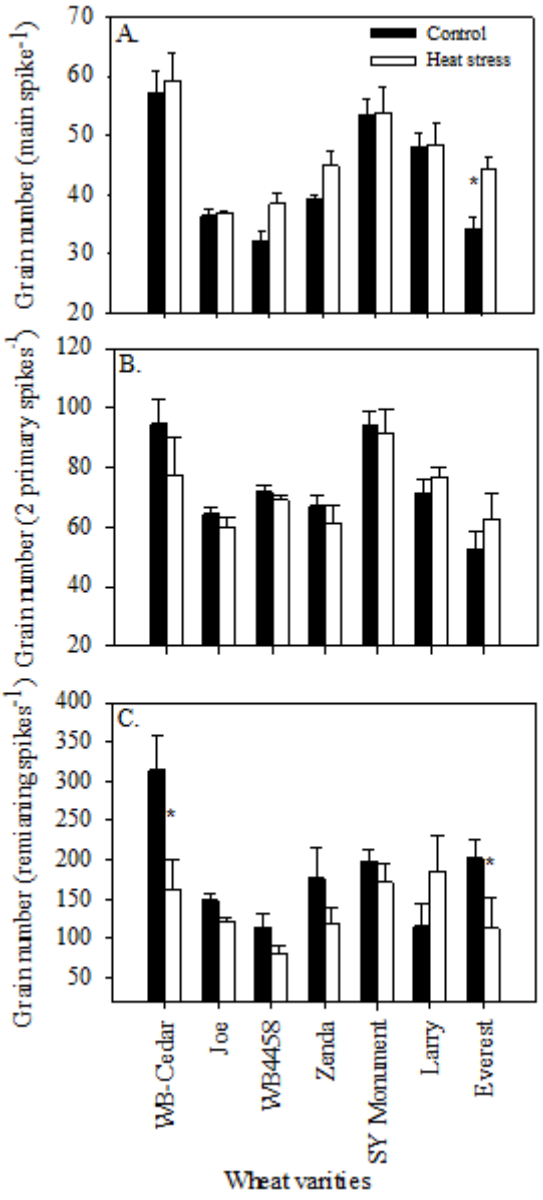

Figure 2.4 Grain number per main (A), primary (B), and remaining (C) spikes in controlled chambers under control and heat stress conditions

*indicates significant difference between the temperature treatments in a variety at 5% LSD

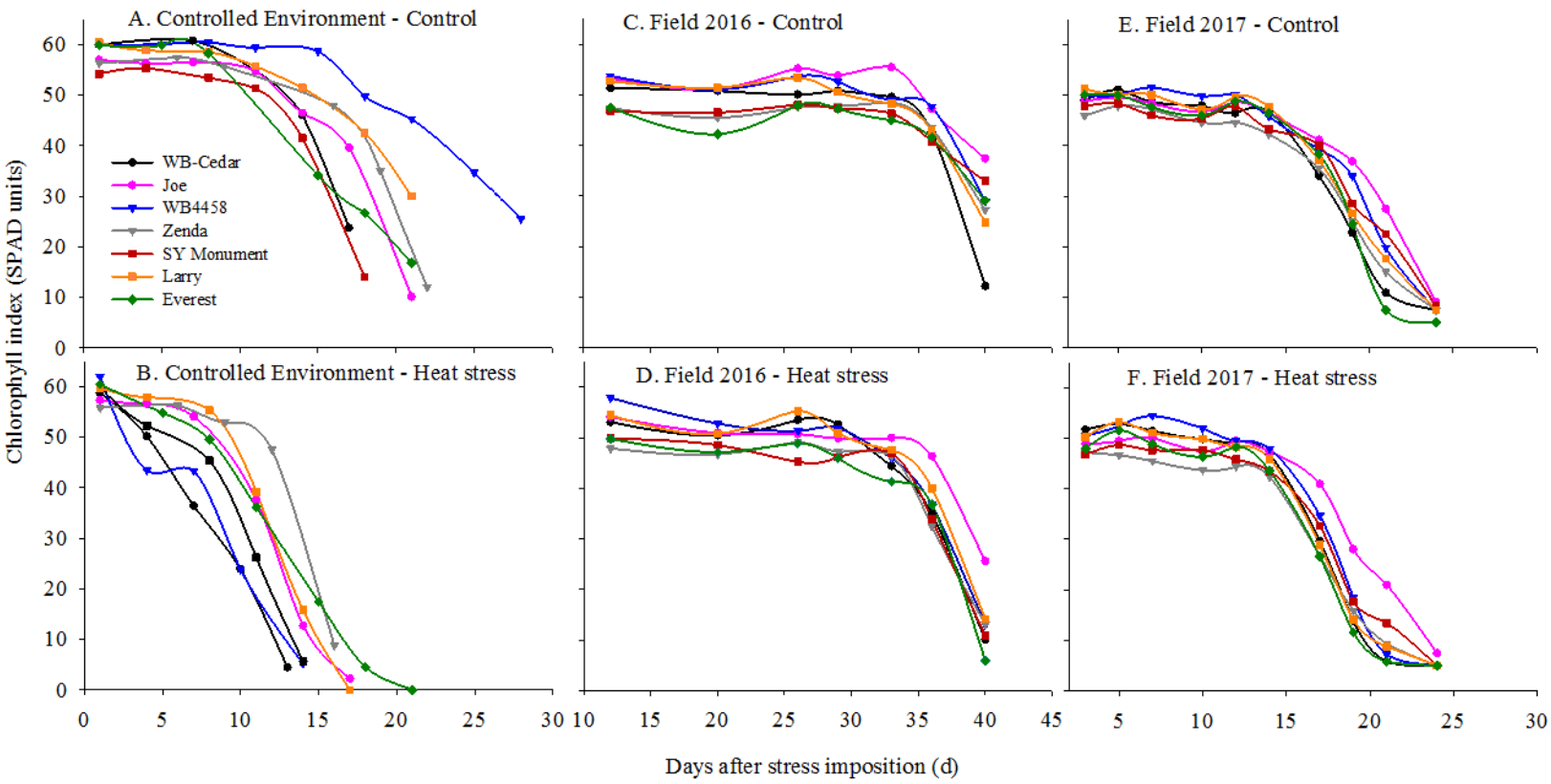

Figure 2.5 Chlorophyll index (SPAD units) in flag leaves of wheat varieties grown in controlled chambers (A. Control, B. Heat stress), and field experiments 2017 (C. Control, D. Heat stress) and 2017 (E. Control, F. Heat stress) at different time intervals from the start of heat stress imposition until physiological maturity

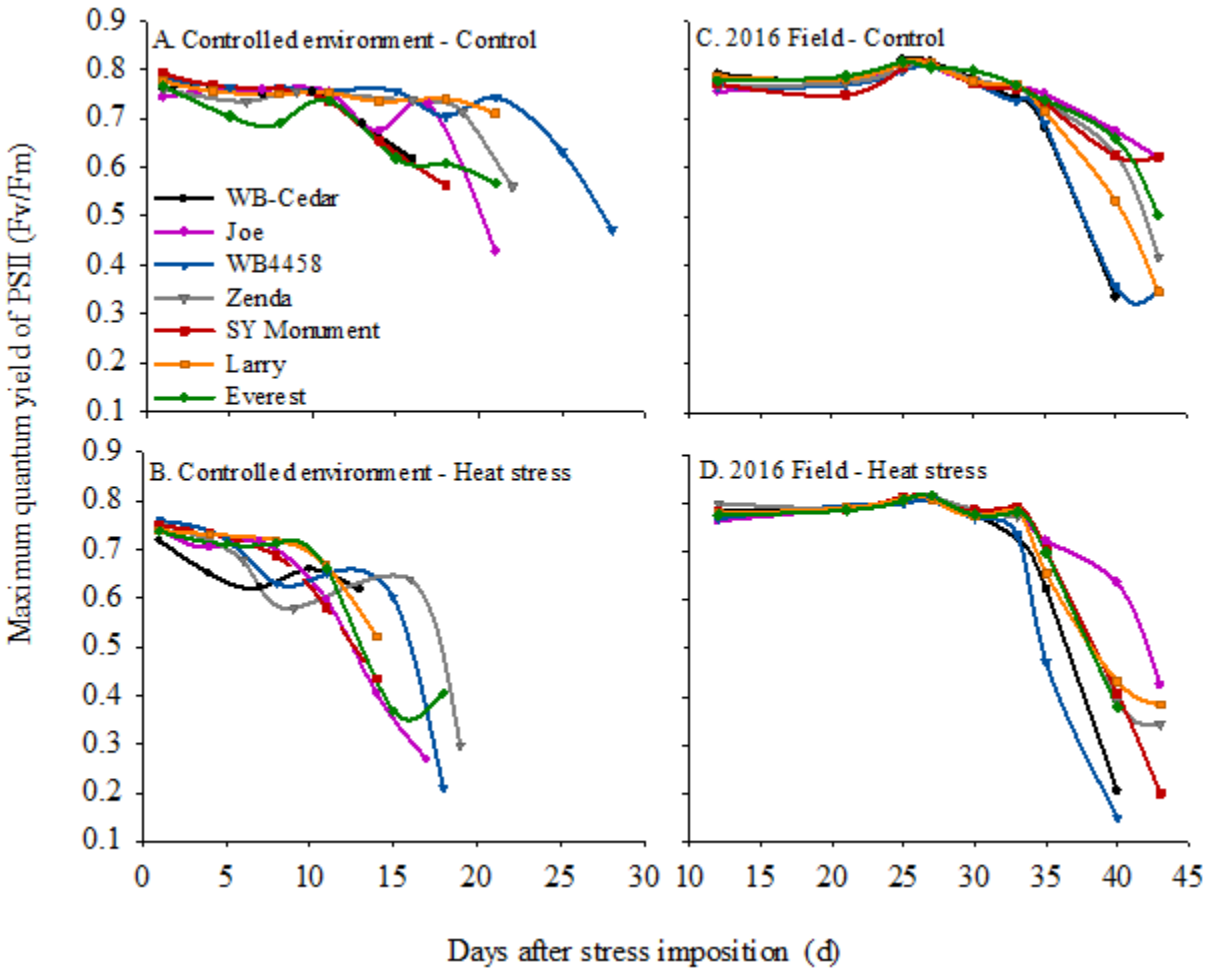

Figure 2.6 Maximum quantum yield of PSII (Fv/Fm) in flag leaves of wheat varieties grown in controlled chambers (A. Control, B. Heat stress) and 2016 field experiment (C. Control, D. Heat stress), at different time intervals following heat stress imposition until physiological maturity

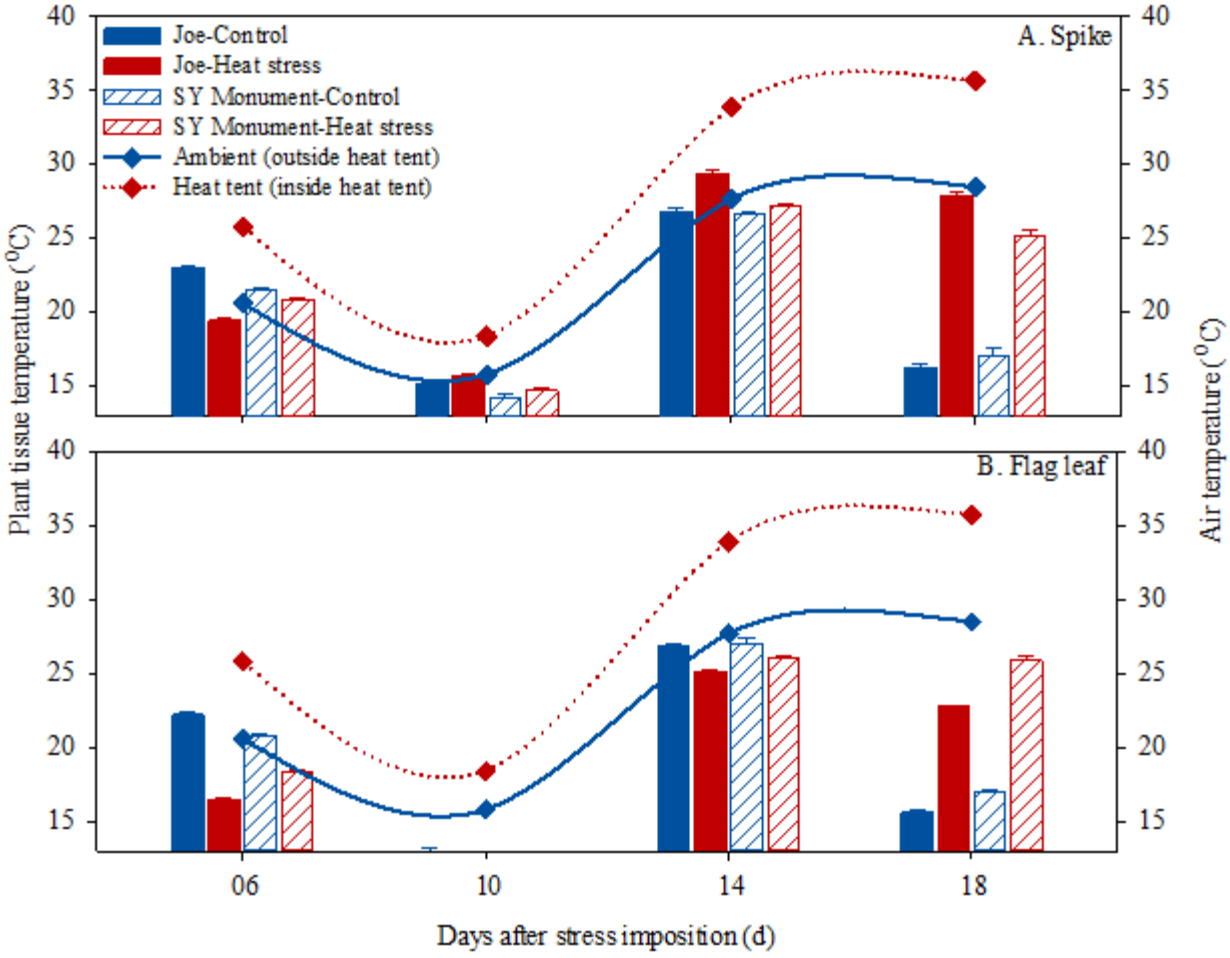

Figure 2.7 Spike (A.) and flag leaf (B.) temperature (°C) in varieties Joe and SY Monument (presented in columns) and air temperature (°C) outside and inside the heat tents (presented as lines) under control and heat stress

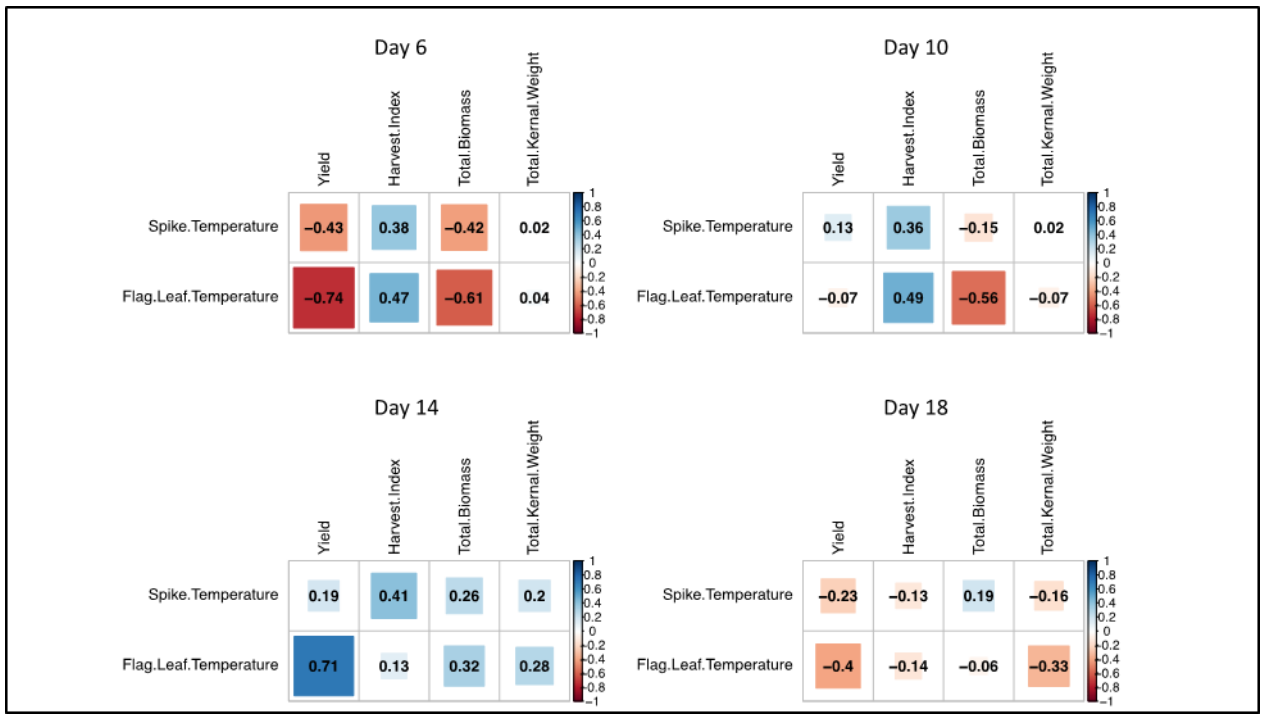

Figure 2.8 Correlation between spike/flag leaf temperature and yield components at specific days after imposition of heat stress

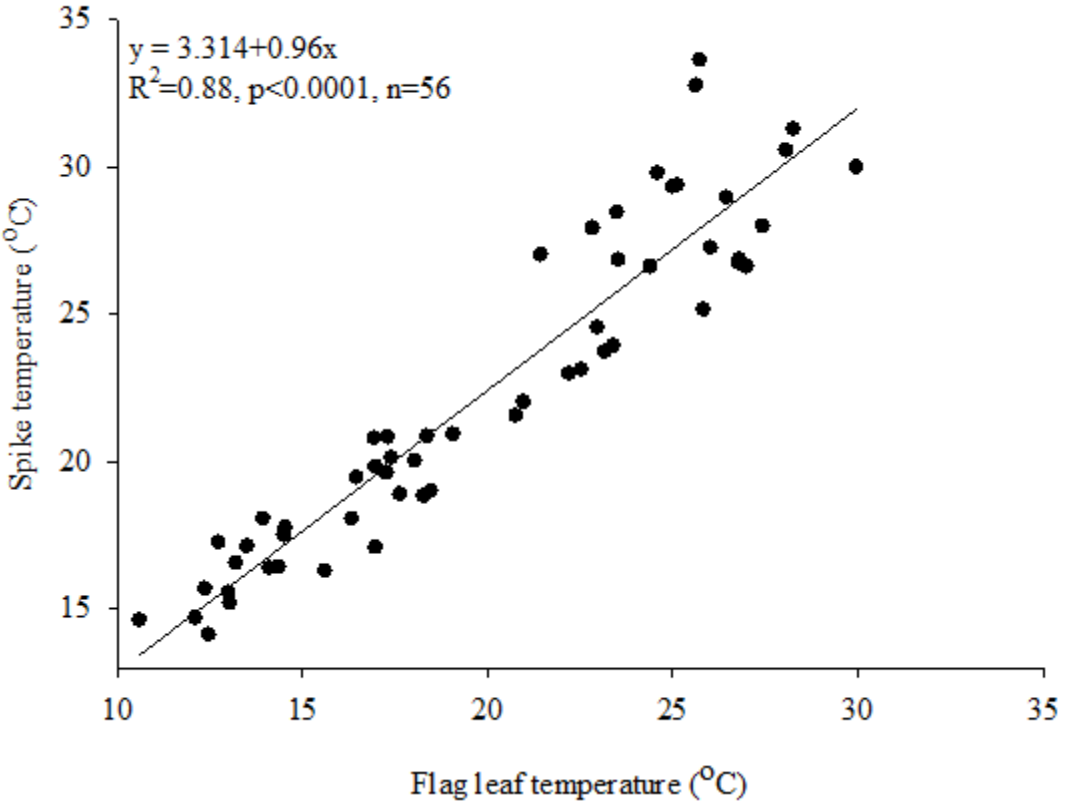

Figure 2.9 Relationship between flag leaf and spike temperature of different winter wheat varieties under both temperature treatments in 2017 field experiment

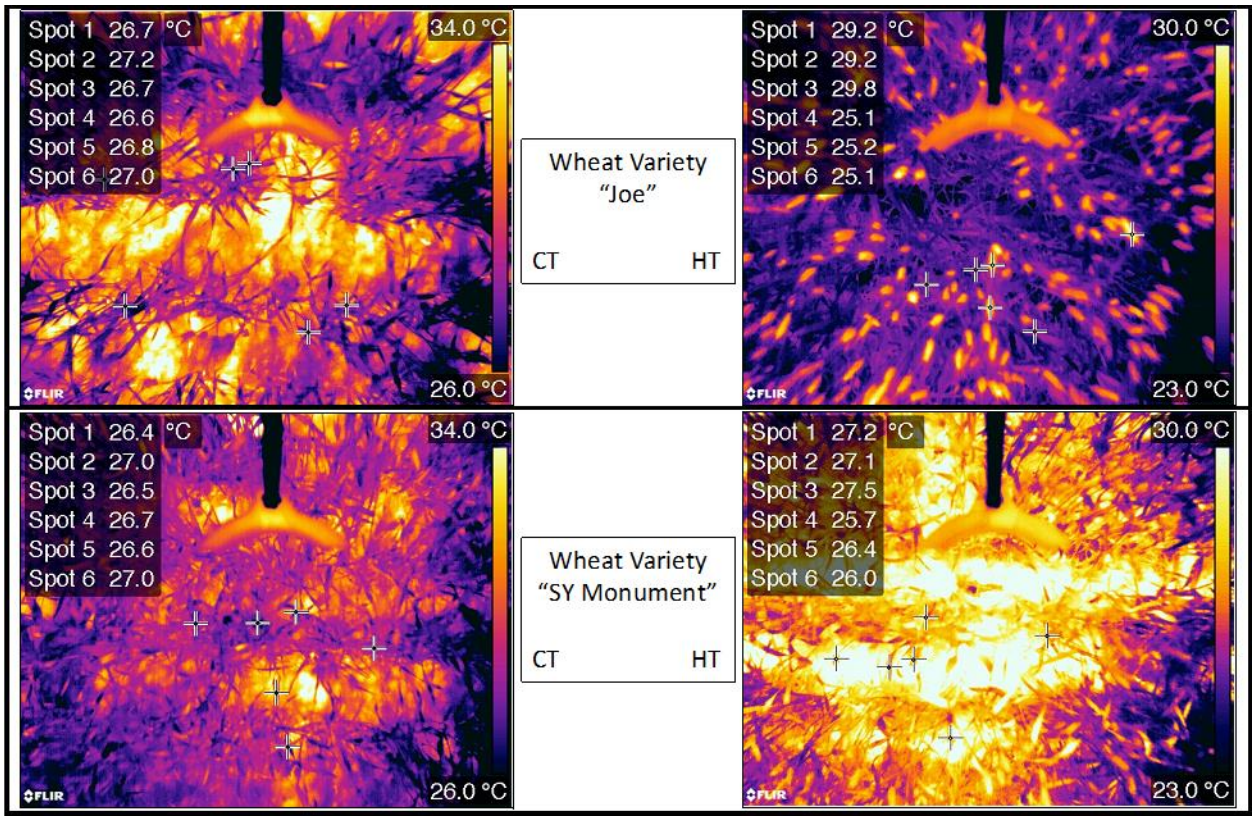

Figure 2.10 Spike and flag leaf temperature extraction

Spot 1-3 labels indicate individual spike temperature. Spot 4-6 indicate individual flag leaf temperature.

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Chapter 3 - Spike and Flag Leaf Senescence Tracked Through Chlorophyll Fluorescence Signals in Winter Wheat Exposed to Post-Flowering Heat Stress

Abstract

Among the detrimental side effects of a changing climate, an increasing temperature can negatively impact wheat production, particularly when it coincides with the reproductive and grain filling stages. Seven popular winter wheat varieties adapted for Kansas growing conditions (Everest, WB-Cedar, Zenda, Larry, SY Monument, WB4458, and Joe) were exposed to heat stress at the post-flowering stage using growth chambers [35/15°C (heat stress) and 25/15°C (control) day/night] and unique field based heat tents (daytime temperature increased 5°C compared to ambient temperatures throughout grain filling). Applicability of chlorophyll fluorescence (Chl-F) to temporally track responses of photosynthetically active wheat flag leaves and spikes were tested. Light adapted effective quantum yield of photosystem II (QY-Lss) was recorded temporally, and then compared with destructive tissue sampling for (chemical analysis of photosynthetic pigments) estimation of chlorophyll content. Moreover, estimated change point (CP) initiating leaf and spike senescence has been tested across all non-invasive measurements. The decrease of main photosynthetic pigment content during the grain filling period was measured in field conditions. Results indicated accelerated reduction under the impact of heat stress, however, the rate of decrease differed among varieties. In both growth chamber and field conditions, QY-Lss of leaves and spikes remained stable until the variety dependent CP, after which its tendency was to decrease with progressive senescence over time. The extent of this progressive decline, as well as the time elapsed to reach CP, was more significant in the case of heat stress impact. Even among varieties commonly cultivated in Kansas (which are understood to have a base level of heat tolerance), differential responses have been observed both in growth chamber and field conditions. These are discussed with emphasis upon the phenotypic approach and a potential for high-throughput phenotyping.

Introduction

Future climatic scenarios are predicted to be accompanied by increased variability in extreme weather events (IPCC, 2014). The predicted increase in frequency and magnitude of heat stress episodes can negatively affect crop production (Hatfield and Prueger, 2015), including wheat yields (Porter and Gawith, 1999). Above optimum temperatures can severely affect a range of physiological processes across different developmental and phenological stages of wheat growth (Barlow et al., 2015). The critical temperature beyond which damage is induced is typically lower for the reproductive stage compared to the vegetative stage (Barnabas et al., 2007). This suggests wheat has a greater sensitivity to heat stress during the flowering and grain filling periods. The impact of heat stress during these sensitive stages has been tested using controlled environment facilities (Liu et al., 2016; Narayanan et al., 2016; Prasad and Djanaguiraman, 2014; Shildermoghanloo et al., 2016) or field experiments (Ayeneh et al., 2002; Feng et al., 2014; White et al., 2011). A significant negative impact of heat stress on grain yield and quality in the previously referenced studies were attributed to either mobilization of stem reserves (water-soluble carbohydrates), decreased activity of key starch synthesizing enzymes, or reduced activity and duration of leaf photosynthesis and carbon metabolism. Apart from the flag leaf (Araus and Tapia, 1987), photosynthetically active portions of the spike are shown to significantly contribute to the assimilate pool during grain filling (Araus et al., 1993). Based on chlorophyll a (Chl-a), the photosynthetic capacity of spikes and flag leaves was found to be similar, while their relative contribution to grain filling varied considerably (Tambussi et al., 2007). Despite the documented contribution of the spikes to carbon assimilation (Sanchez-Bragado et al., 2016), the wide variation of results obtained across studies is primarily influenced by environmental factors (Araus et al., 2003) or genetic differences (Sanchez-Bragado et al.,

2014). Typically, greater assimilate contribution from spikes has been recorded under abiotic stress exposure, e.g., drought (Araus et al., 2008; Maydup et al., 2010; Tambussi et al., 2005; Tambussi et al., 2007). Thus, systematically assessing the photosynthetic contribution of spikes is critical, particularly under abiotic stress exposure such as heat stress, when leaf photosynthesis can be affected more than spike photosynthesis (Blum, 1985).

Photosynthesis in both wheat leaves and spikes undergoes gradual decline during the grain filling period due to natural senescence, reflecting the dismantling of the chloroplast apparatus. The senescence of both the leaf and spike is highly coordinated, made evident when a yellow coloring of the plant tissue becomes perceptible (Lim et al., 2007). Despite the invasive and destructive analytical methods, subtle color changes during initiation of senescence can be more effectively captured through plant optical signals. One of the most commonly used optical methods is chlorophyll fluorescence (Chl-F), allowing non-destructive insight into plant photochemical processes with high precision and accuracy (Baker, 2008; Krause and Weis, 1991; Maxwell and Johnson, 2000; Murchie and Lawson, 2013). Since Chl-F represents emitted light after direct excitation of chlorophyll molecules of photosystem II (PSII), its characteristics are driven by functionally rich or poor PSII. Analysis of Chl-F thus provides valuable information not only about functioning of PSII reaction centers, but also the light harvesting antenna complexes and/or both donor and acceptor sides of PSII. During senescence and/or ripening of several fruits, decline of Chl-F has been attributed to loss of chloroplast function connected with advanced maturation (e.g., apples) (Song et al., 1997) or decrease in chlorophyll content (e.g., papaya) (Bron et al., 2004).

Extending the duration of grain filling in cereals including wheat is one of the key research areas that can potentially translate to significant yield enhancement (Jagadish et al.,

2015). However, little progress has been accomplished in this direction due to the lack of standardized phenotyping approach, which can track rate of senescence during grain filling, both accurately and rapidly. Despite Chl-F as a potential tool to capture source-sink photosynthetic activity during grain filling, there has been only little attempt to demonstrate its application to capture senescence patterns across different photosynthesizing tissue of wheat. By using Chl-F imaging technique, seasonal senescence of wheat glumes and flag leaves has been investigated (Kong et al., 2015). However to date, the effectiveness of Chl-F under field conditions has only been demonstrated in rice panicles (Šebela et al., 2015). In Šebela et al. (2015), the researchers prove that effective QY-Lss of PSII efficiency can be used to track changing optical properties of rice panicles under both control and high night temperature stress. Moreover, this parameter was reliably used to estimate the elusive break-point, capturing the initiation of panicle senescence with clear genotypic variation in contrasting rice genotypes. Thus, the objectives of our studies were to detect changes of photosynthetic pigments in flag leaves and spikes in field grown plants under both control and heat stress exposure; determine the genetic differences in rate of senescence in spikes and flag leaves in prominent Kansas varieties while exposed to heat stress in controlled chambers and field based heat tents; and to estimate break-point initiating senescence and unravel association between heat stress and changes in Chl-F parameter in leaves and spikes.

Materials and Methods

Field experiment

Please refer to Chapter 2 field experiment for agronomic details.

Controlled environment experiment

Please refer to Chapter 2 controlled environment experiment for agronomic details.

Optical measurements

Chlorophyll fluorescence and chlorophyll index estimation

Šebela et al. (2015) utilized the Chl-F parameter, allowing high-throughput means to precisely estimate the dynamics captured with maturing rice panicles, to study two contrasting rice genotypes and their temporal response to high night temperature. Likewise, the same parameter has been selected and used to investigate Chl-F in both growth chamber and field experiments in this study. The Chl-F measurement of leaves and spikes of all seven varieties was performed by using a portable handheld fluorometer FluorPen (FluorPen FP 100, Photon System Instruments, Ltd., Brno, Czech Republic). Saturating light [intensity approximately 3,000 µmol (photons) m^{-2} s^{-1}] and measuring light [intensity approximately 0.09 µmol (photons) m^{-2} s^{-1}] were used to measure maximal fluorescence yield (F_M`) and actual fluorescence yield (Ft) of light adapted samples, respectively. QY-Lss of PS II was then calculated using formula QY-Lss = ($F_M$`-F)/F_M` = $\Delta F/F_M$` (Genty et al., 1989). Three measurements across the flag leaf and/or spike per tagged plant were recorded from both growth chamber and field experiment. Total recordings were as such: 15 fluorescence measurements for both flag leaves and spikes per variety. Measurements were recorded on alternate days beginning exactly (growth chamber) and approximately (field experiment) ten days post-flowering until physiological maturity. The non-invasive determination of chlorophylls (Chl a+b) in flag leaves was done by using a commercially available leaf clip meter, Dualex (Dualex 4 Scientific, Force-A, Orsay, France). Similar to Chl-F measurements, three measurements per flag leaf were determined on each tagged plant (totaling 15 readings per variety).

Pigment analyses of flag leaves and spikes

Photosynthetic pigments of flag leaves and spikes were determined using classical dimethyl sulfoxide (DMSO) - acetone extraction method (Shoaf and Lium, 1976). Despite the standardized approaches for extracting photosynthetic pigments from leaves, there are limited studies quantifying photosynthetic pigment content of spikes. Therefore, a validation component was designed during this independent experiment to verify applicability of the aforementioned extraction method using plants from the field experiment. Pigment content was determined temporally (four day interval). One representative plant from the side rows of each variety (sample including flag leaf and spike) was collected from three control and heat stress replicates, placed on ice, and immediately transported to the laboratory. In the laboratory, sample categories were separated; i.e., flag leaf (approximately 20 mg of flag leaf tissue) and spike (glumes with lemma including approximately 2 mm of surrounding awn, rachis was not included. Following tissue separation, samples were placed in glass tubes containing 10 ml solution (DMSO and 90% acetone; 1:1 v/v), placed in a dark environment at room temperature for >24 hours for complete extraction of pigments. Sufficient extraction was confirmed by white coloration of samples. Absorption spectra of extracts were subsequently measured at pre-defined wavelengths (470 nm, 645 nm, and 663 nm) using spectrophotometer U-5100 (Hitachi, Ltd., Tokyo, Japan). Chlorophyll a, chlorophyll b, total chlorophylls, and total carotenoids content were calculated using the following formulae,

$$C_a = (12.7\ A_{663}) - (2.69\ A_{645})$$

$$C_b = (22.9\ A_{645}) - (4.68\ A_{663})$$

$$C_{a+b} = (8.02\ A_{663}) + (20.2\ A_{645})$$

$$C_{x+c} = \frac{[(1000\ A470)-(1.29\ Ca)-(53.78\ Cb)]}{220}$$

where C_a, C_b, C_{a+b} and C_{x+c} are chlorophyll a (Chl-a), chlorophyll b (Chl-b), total chlorophylls (Chl_{a+b}), and total carotenoids (C_{x+c}); and where A_{470}, A_{645}, and A_{635} are values of optical absorbance at specific wavelengths (470 nm, 645 nm, and 635 nm, respectively). Pigment content was calculated and expressed in mg/g fresh weight.

Statistical analysis

For both the controlled environment and field experiments, a fitted change point (CP) of non-invasive estimation of chlorophyll content and QY-Lss of PSII of leaves and spikes was estimated according to Šebela et al., 2015. The slope and the CP for temporal Chl F and Chl index data were estimated using a plateau-linear model for the time-series non-linear regression analysis using Proc NLIN procedure in SAS 9.4 SAS software (Version 9.4, SAS Institute Inc., Cary, NC). Means were separated using LSD (least significant difference) test at p=0.05

Results

Heat stress impact on photosynthetic pigments content

Concentrations of main photosynthetic pigments (Chl-a, Chl_{a+b},C_{x+c}) and/or Chl-a to b ratio (Chl a/b) were investigated in field grown plants. This occurred temporally every four days from the initiation of the heat stress treatment (time 0), until physiological maturity (Figure 3.1). As plants progressed towards maturity, the flag leaves, and spikes were visually monitored on a daily basis in order to avoid possible discrepancies or missing physiological senescence during the four-day sampling windows. The final measurements were taken promptly on the day of physiological maturity, even if this occurred earlier than the subsequent four-day interval; in

these cases no subsequent data was obtained from these plants. At time 0 (i.e., ten days after flowering), similar trends with only small phenotypic variability were visible across all seven inspected varieties. Across varieties, concentration of Chl-a in flag leaves ranged between 2.8 mg/g (SY Monument) and 3.6 mg/g (WB4458). Spike concentrations of Chl-a ranged from 0.4 mg/g (Zenda) to 0.72 mg/g (WB4458). (Fig. 3.1).

Despite the differences in Chl-a concentrations across flag leaves and spikes, the Chl a/b ratio was the same at time 0 for both plant constituents (value approximately 3). In the flag leaves, Chl-a remained stable (> 3 mg/g for all seven inspected varieties) through the first 14 days, experiencing only slight environmentally driven changes (temperature and sunlight dropped notably during this period). Thereafter, a steep decline of Chl-a concentration and Chl a/b ratio occurred. Chl-a degradation accelerated with heat stress exposure in all seven inspected varieties (Figure 3.1), while different variety responses resulted in shorter (e.g., Everest) or longer (e.g., Joe) duration of chlorophyll retention. Everest, possessing a shorter senescence rate, also expressed a greater value of Chl a/b ratio for a short period after heat stress exposure; this generally decreased as the plants approached physiological maturity. Photosynthetic pigment content of the spikes, although a lower value than the flag leaves at the beginning of stress imposition, remained unchanged for a prolonged duration in comparison to the flag leaves across the seven tested varieties. The accelerated rate of decline in photosynthetic pigment content as observed in the flag leaves was not observed in the spikes, even under heat stress conditions. For the spikes, a more gradual decline of pigment content was observed in both control and heat stress treatments however.

Flag leaf and spike senescence

In addition to extraction of photosynthetic pigments during the field experiment, senescence was visually monitored under both controlled environment and field conditions. Tissue senescence was defined as the absolute disappearance of photosynthetic pigments (yellowing of flag leaves, and spikes). When concerns arose about the accurate detection of complete senescence during the terminal stages, Chl-F measurements were used (continuous measurements of steady state Chl-F level in light adapted state (Ft), data not shown). Tissue senescence was reached when there was no Chl-F signal, or no Chl-a content. The duration to reach senescence differed with heat stress impact across the seven varieties, in both the controlled environment and field experiments, and between flag leaves and spikes (Table 3.1).

Duration to reach senescence in leaves and spikes was longer for all inspected varieties, with the shortest duration being seen in Larry and WB4458. The extent to which senescence of leaves and spikes was accelerated by heat stress was more pronounced under growth chamber conditions versus field conditions (Table 3.1). The flag leaves, under both control and heat stress conditions, maintained longer duration of photosynthetic activity, similar to spikes. A significant correlation existed between flag leaves in chambers and field conditions for control (R^2=0.71; $p<0.05$) and heat stress treatments (R^2=0.75; $p<0.05$). On the other hand, field condition spikes retained greenness for a longer duration compared to flag leaves, but still maintained the same level of correlation between chambers and field conditions under control (R^2=0.63; $p<0.05$) and heat stress (R^2=0.71; $p<0.05$).

Optical measurements

To ascertain the impact of heat stress on the physiology of the seven prominent wheat varieties, changes in optical signals were monitored in controlled environment and field

experiment plants. Non-invasive quantitative (chlorophyll index) and qualitative (Chl-F) measurements were recorded temporally.

Non-invasive chlorophyll index

Changes in Chl were monitored in-vivo in flag leaves. Throughout the grain fill period, several changes in Chl were visible across the seven tested varieties. Values at the early stage (time 0) were consistently greater for the growth chamber, compared to field, grown plants (Figure 3.2). For both growth chamber and field grown plants however, these values remained constant until the variety-dependent break point, after which the values decreased progressively. The extent to which plants responded to heat stress differed between controlled environment and field grown plants. In both experiments, differences were observed between the control and heat stress treatments. To evaluate the trend of the curves numerically, this break point was defined as the change point (CP), and the duration to reach this point was calculated. The shape of the curve after this variety-dependent CP (Figure 3.2) has been characterized by negative values (decreasing trend), as exhibited in Table 3.2. The time to reach CP of chlorophyll index for flag leaves of control plants in growth chambers occurred between 9 days (SY Monument) and 15 days (WB4458) (Table 3.2). The same range of CP was observed in the field experiment, however, among different varieties (WB-Cedar 9 days, Joe 15 days) (Table 3.2). CP was only slightly reduced due to heat stress across the seven varieties in both experiments, ranging between 7 and 13 days in the growth chamber and between 8 and 13 days in the field experiment. It should be noted that across both experiments the varieties did not differ significantly for CP between control and stress (Table 3.2). Aside from the CP, the rate of senescence was also calculated. Heat stress-induced accelerated senescence was more pronounced in the growth chambers as lower (more negative) slope values were observed.

Amongst individual varieties in the growth chamber, WB4458 and Zenda recorded a significant increase in the rate of senescence under heat stress compared to control (Table 3.2).

Chlorophyll fluorescence measurements

To investigate the effect of heat stress on primary photochemistry of all seven selected varieties, changes in selected Chl-F parameter were measured temporally, starting the day of heat stress imposition. Effective QY-Lss of PSII was measured on alternate days for leaves and spikes. At the beginning of the experiment (time 0; ten days after flowering), the value of QY-Lss (~0.7) was identical across leaves and spikes of all varieties, for both the growth chamber and field experiments (Figure 3.3 and 3.4). Similar to the non-invasive measurements presented above (estimation of Chl index), QY-Lss tended to remain constant (~0.7) for a certain period in the case of flag leaves and spikes (Figure 3.3 and 3.4), but did vary across varieties in both field and growth chamber conditions (Table 3.3 and 3.4). Additionally, the length of the duration during which QY-Lss remained constant was noticeably shortened by the impact of heat stress. As illustrated in Tables 3.3 and 3.4, different responses are visible in control versus heat stress conditions; this is apparent by comparing CP measured quantitatively (non-invasive Chl estimation) and qualitatively (QY-Lss of flag leaves). It was noticed that flag leaves from plants grown in growth chambers had a longer duration to reach the CP for QY-Lss (Table 3.3) compared to Chl index (Table 3.2).

Across all varieties, the duration of CP from QY-Lss curves in flag leaves are more severely affected by heat stress in growth chamber cultivated plants compared to field plants. In growth chambers, the CP duration in controls ranged between 14 days (WB-Cedar) and 21 days (Larry, WB4458). When the varieties were exposed to heat stress, their CP duration was reduced between 8 (WB-Cedar) and 17 days (Zenda). However, this same range of variability was not

seen in the field experiment as five of the varieties had similar CP for QY-Lss in the flag leaves (Table 3.4). CP estimated from QY-Lss curves in spikes of field grown plants were similar across all seven varieties under both control and heat stress plants, thus no impact on time to CP was observed under the heat stress treatment (Table 3.4). In the growth chamber experiment, heat stress significantly shortened time to CP for spikes in WB-Cedar and Everest but not in the other varieties (Table 3.3).

The rate of the decline of photochemical activity (QY-Lss) after CP is presented numerically (Table 3.3 and 3.4). In growth chamber conditions, the rate of flag leaf senescence seen under heat stress conditions is characterized by lower (more negative) slope values. This decline was observed amongst all varieties being the most pronounced in Zenda and WB-4458 (Table 3.3). In field conditions, a significant decline of QY-Lss (more negative slope) was observed in three of the varieties, that is WB4458, Zenda and Larry (Table 3.4). Amongst the spikes in the growth chamber experiment heat stress induced a significant increase in the rate of senescence in all of the varieties except Joe (Table 3.3). However, in the field experiment differences in the rate of spike senescence were not observed due to heat stress (Table 3.4).

Discussion

The varieties tested in this study are commonly developed for Kansas' temperate continental climate and warm summer environments. For this reason it was expected that these varieties would exhibit a certain level of heat tolerance. However, different responses were observed. Thus, the emphasis of this experiment highlights the potential precise, high-throughput evaluation of the impact of heat stress on wheat source and sink tissue. This methodology allows for the rapid and accurate detection of photosynthetic pigments. Non-invasive optical methods

presented are faster than classical extraction methods. This allows for phenotyping large sets of accessions at high temporal frequency. However, the application of such technology on sink tissue is limited and hence was the motivation behind our studies.

Photosynthetic pigment composition during senescence

Senescence is the process leading to physiological death with visible changes in all parts of the plant. In cereals, this occurs during the development of grains. These changes have been documented across crop species including wheat and can be attributed to several physiological processes (Lim et al., 2007). The emphasis of our study, however, is primarily focused on the loss of visible greenness attributed to decline in the major photosynthetic pigments and subsequent physiological consequences. Chlorophyll is the most abundant photosynthetic pigment and a key component required for sunlight absorption and to drive photosynthesis. Changes in chlorophyll composition of leaves in field grown wheat plants during senescence can be simply governed by the developmental age (Lu et al., 2001). However, environmental stresses such as heat (Lobell et al., 2012), drought, or their combination (Lopes and Reynolds, 2012) also have a significant impact on the rate of wheat senescence. Even though a lack of correlation between grain yield and the duration of flag leaf senescence under optimal conditions has been reported (Borrill et al., 2015), it is hypothesized that developing functionally active stay-green phenotypes could help to minimze yield losses encountered by abiotic stresses (Jagadish et al., 2015).

Along with flag leaves, wheat spikes and their constituents are shown to contribute significantly to photosynthesis and assimilate supply (Reynolds et al., 2012). Moreover, their contribution is of increased importance under exposure to abiotic stresses (Araus et al., 2008; Maydup et al., 2010; Tambussi et al., 2005; Tambussi et al., 2007;). The importance of wheat

glumes, (Kohl et al., 2015; Lopes et al., 2006) lemma and palea (Lu and Lu, 2004), and awns (Robetzke et al., 2016), or their combination have been suggested as possible methods of conversion and translocation of assimilates to grain. In our study, the impact of heat stress has been investigated in flag leaves and spikes. In general, both Chl-a and Chl a/b ratio of flag leaves decreased with the progressive senescence but differed in rate across varieties (Figure 3.1). Varieties Joe and SY Monument retained photosynthetic pigments for significantly longer durations than Everest and WB-Cedar. The same trend was noted in the spikes (Figure 3.1), where the same varieties, Joe and SY Monument, retained their photosynthetic pigment longer in the spike than in the flag leaves. These findings are in accordance with existing studies, where researchers reported extended duration of chlorophyll retention before senescence in wheat spikes, compared to flag leaves, under abiotic stress (Araus et al., 2008; Maydup et al., 2010; Tambussi et al., 2005; Tambussi et al., 2007;). Chl a/b ratio was significantly greater in heat stressed flag leaves of Everest during the first few days and also towards the end compared to its control counterpart. This can be attributed to the gradual degradation of Chl-b in a variety with shorter duration to physiological senescence. This may be an attempt to reduce the risk of photo-oxidative damage by reducing the number of light harvesting complexes associated with PSII (Xu et al., 1995).

Chlorophyll index and primary photosynthetic activity (QY-Lss) remained stable until the variety-dependent break point occurred, which was different based upon exposure to heat stress and/or growing conditions (Figures 3.2, 3.3, 3.4). The rationale for this experiment was based on the work of Šebela et al. (2015), who reported the break point for senescence initiation under high night temperature stress on rice panicles. The same parameters can be used to indicate accelerated senescence of wheat leaves and spikes beyond the CP.The duration to reach CP, the

slope of the line after CP, differentiated the level of heat tolerance among varieties. The Chl-F technique allows more extensive qualitative insight into the primary photochemistry, determining the functionally rich or poor PSII. This method has been reviewed by a number of researchers however, exclusively in leaves (Baker, 2008; Krause and Weis, 1991; Maxwell and Johnson, 2000; Murchie and Lawson, 2013).

Even though contribution of photosynthetically active constituents (e.g., spikes) towards high grain yield in wheat has been suggested and mentioned above, there is limited information available regarding to Chl-F. One of the key limiting factors preventing progress could be the morphology of wheat spikes, potentially scattering Chl-F. Most currently available fluorometers are designed for leaves which are morphologically flat. However, the approach and technique used here was standardized for a more architecturally challenging rice panicle, allowing us to effectively determine QY-Lss in wheat spikes. Chl-F measurements performed by Chl-F imaging system have been previously used to investigate photochemical responses of the glumes and flag leaves of winter wheat cultivars during seasonal senescence (Kong et al., 2015).

Similar to our study, other researchers have proven that wheat spikes had greater values of QY-Lss during terminal stages of senescence (Araus et al., 2008; Maydup et al., 2010; Tambussi et al., 2005; Tambussi et al., 2007). However, in the present study, the greater value of QY-Lss in spikes compared to flag leaves during heat stress exposure suggests an improved photosynthetic efficiency of spikes even under abiotic stress (Figure 3.3, 3.4). To date, the only study related to methodological testing of the instrument was to investigate temporal changes of Chl-F signals in photosynthetically active constituents (rice panicles) with the impact of abiotic stress (Šebela et al. 2015). Here, researchers proved applicability of instrumentation and defined the best fitting Chl-F parameter (QY-Lss) to assess high night temperature induced accelerated

senescence. The same validated instrumentation and Chl-F parameter (QY-Lss) has been selected and investigated throughout the entire experiment and is presented here. In the growth chamber experiment plants tended to have a shorter duration before reaching the CP in flag leaves as seen by Chl index and QY-Lss compared to field grown plants (Table 3.2). This can be explained by natural environmental variation in field conditions, such as warmer ambient temperature and excess radiation, which increases in field conditions towards the end of the grain filling period. In contrast, temperature and light intensity remain stable in growth chambers, thus, plants retain greater photosynthetic efficiency even after the CP of Chl disappearance occurs. Additionally, in field conditions spikes tended to retain more QY-Lss compared to flag leaves, suggesting greater heat stability and delayed senescence at the later stages of grain filling (Kong et al., 2015).

Our results, combined with recent evidence of wheat spike C4 photosynthetic pathways (Rangan et al., 2016), could result in novel strategies to improve to photosynthetic efficiency of wheat to provide resilience in response to climatic factors and improve yield potential.

Table 3.1 Effect of heat stress on the duration of senescence in flag leaves, and spikes (days)

	Flag leaves						Spikes					
	GCH		Field		**GCH**	**Field**	GCH		Field		**GCH**	**Field**
	C[1]	HS[2]	C	HS	***% difference[3]***		C	HS	C	HS	***% difference***	
Joe	29	23	25	24	***-20***	***-4***	31	23	30	29	***-25***	***-3***
SY Monument	27	21	25	23	***-22***	***-8***	27	21	29	25	***-22***	***-14***
Larry	31	21	25	22	***-32***	***-12***	31	21	27	25	***-32***	***-7***
WB4458	35	25	24	21	***-28***	***-12***	33	23	29	25	***-30***	***-14***
Zenda	29	25	24	22	***-14***	***-8***	29	23	26	26	***-20***	***0***
WB-Cedar	29	23	22	21	***-21***	***-5***	29	23	25	24	***-21***	***-4***
Everest	31	25	22	21	***-19***	***-5***	29	23	25	24	***-21***	***-4***

The effect of heat stress treatment on the duration of senescence of seven selected varieties (Joe, SY Monument, Larry, WB4458, Zenda, WB-Cedar, and Everest); for controlled environment (growth chambers; GCH) and field experiments. The duration (days) is presented for control (C[1]) and heat stress treatments (HS[2]). Percentage change is calculated according to (% difference) = [(HS/(C/100)]-100; where reduction (-) or increase (+) is presented

Table 3.2 Slope, change point (CP) and their 95% confidence intervals for temporal chlorophyll concentration in flag leaf under controlled environment chamber experiment in 2016 and 2017 Field experiment

				95% Confidence limits for slope			
Variety	**Treatment**	**Slope**	**CP**	**Slope**		**CP**	
Controlled environment 2016							
WB-Cedar	Control	-3.7927	10.3974	-4.3262	-3.2593	7.8186	12.9762
	Heat stress	-4.399	7.45447	-5.1704	-3.6277	4.8142	10.0947
Joe	Control	-3.6132	11.7902	-4.246	-2.9804	8.8675	14.7129
	Heat stress	-5.6642	11.9122	-6.9125	-4.4159	9.5549	14.2695
WB4458	Control	-3.5704	15.8841	-4.1957	-2.9451	13.5871	18.181
	Heat stress	-5.9548	12.2542	-7.0998	-4.8098	10.2468	14.2617
Zenda	Control	-3.9971	12.6632	-4.5591	-3.4351	10.4053	14.9211
	Heat stress	-5.7564	13.2563	-6.7299	-4.783	11.6345	14.878
SY Monument	Control	-3.2733	9.09811	-3.9759	-2.5707	4.9657	13.2305
	Heat stress	-4.6069	9.54139	-5.7284	-3.4854	6.4226	12.6602
Larry	Control	-3.7129	13.8891	-4.1931	-3.2327	11.9776	15.8007
	Heat stress	-5.3701	10.7628	-6.573	-4.1671	8.0886	13.4371
Everest	Control	-3.3383	9.7676	-3.7408	-2.9358	7.5042	12.031
	Heat stress	-4.0409	7.71653	-4.4408	-3.6409	6.2444	9.1886
Field 2017							
WB-Cedar	Control	-3.2165	9.36904	-3.7628	-2.6702	7.2947	11.4434
	Heat stress	-4.0663	9.48405	-4.9039	-3.2288	7.3131	11.655
Joe	Control	-4.483	15.4927	-5.2558	-3.7102	14.2647	16.7207
	Heat stress	-4.1762	13.3867	-4.7294	-3.623	12.4207	14.3527
WB4458	Control	-3.6514	11.1234	-4.0997	-3.203	9.8295	12.4172
	Heat stress	-4.0918	9.84837	-4.6238	-3.5599	8.5115	11.1852
Zenda	Control	-3.0703	10.8712	-3.571	-2.5696	9.1219	12.6205
	Heat stress	-3.7554	11.2886	-4.3251	-3.1857	9.9342	12.6431
SY Monument	Control	-3.5712	13.4085	-4.1066	-3.0357	12.1062	14.7108
	Heat stress	-3.834	11.371	-4.3738	-3.2941	10.1225	12.6194
Larry	Control	-3.7994	13.0226	-4.3081	-3.2907	11.8199	14.2252
	Heat stress	-4.1308	11.0035	-4.7634	-3.4982	9.603	12.404
Everest	Control	-3.2779	10.7283	-3.9598	-2.5959	8.4739	12.9828
	Heat stress	-3.0825	7.96366	-3.678	-2.487	5.5878	10.3395

Slope or break point between the temperature treatments in a genotype are significantly different at 0.05 if their confidence intervals does not overlap

Table 3.3 Slope, change point (CP) and their 95% confidence intervals for chlorophyll fluorescence in flag leaf and spikes for the controlled environment growth chamber experiment in 2016

					95% Confidence limits for slope			
Tissue	**Variety**	**Treatment**	**Slope**	**CP**	**Slope**		**CP**	
Flag leaf	WB-Cedar	Control	-0.041	14.154	-0.048	-0.035	11.764	16.544
		Heat stress	-0.048	7.893	-0.055	-0.040	5.901	9.886
	Joe	Control	-0.045	16.573	-0.053	-0.038	14.385	18.762
		Heat stress	-0.059	11.315	-0.067	-0.050	9.905	12.725
	WB4458	Control	-0.044	20.670	-0.050	-0.037	19.232	22.108
		Heat stress	-0.060	12.686	0.066	-0.054	11.836	13.537
	Zenda	Control	-0.050	18.217	0.060	-0.041	16.077	20.357
		Heat stress	-0.097	17.183	-0.118	-0.077	16.022	18.344
	SY Monument	Control	-0.038	12.304	-0.046	-0.031	9.133	15.476
		Heat stress	-0.062	11.892	-0.074	-0.049	10.014	13.770
	Larry	Control	-0.054	20.554	-0.063	-0.045	18.931	22.177
		Heat stress	-0.068	12.613	-0.087	-0.050	10.290	14.936
	Everest	Control	-0.042	16.036	-0.047	-0.037	14.491	17.581
		Heat stress	-0.056	12.273	-0.063	-0.048	11.096	13.449
Spike	WB-Cedar	Control	-0.053	17.484	-0.060	-0.046	16.090	18.878
		Heat stress	-0.080	13.874	-0.096	-0.064	12.640	15.107
	Joe	Control	-0.036	14.873	-0.040	-0.031	13.262	16.483
		Heat stress	-0.048	12.048	-0.068	-0.029	8.987	15.109
	WB4458	Control	-0.032	15.516	-0.038	-0.025	13.046	17.986
		Heat stress	-0.097	16.427	-0.114	-0.080	15.637	17.217
	Zenda	Control	-0.052	17.757	-0.059	-0.044	16.309	19.205
		Heat stress	-0.102	15.626	-0.123	-0.081	14.552	16.700
	SY Monument	Control	-0.043	14.528	-0.050	-0.036	12.439	16.617
		Heat stress	-0.066	12.341	-0.078	-0.054	10.987	13.695
	Larry	Control	-0.041	16.348	-0.047	-0.035	14.695	18.002
		Heat stress	-0.098	15.513	-0.124	-0.072	14.121	16.906
	Everest	Control	-0.063	20.095	-0.075	-0.052	18.514	21.677
		Heat stress	-0.114	16.357	-0.136	-0.092	15.497	17.216

Slope or break point between the temperature treatments in a genotype are significantly different at 0.05 if their confidence intervals does not overlap

Table 3.4 Slope, change point (CP) and their 95% confidence intervals for chlorophyll fluorescence in flag leaf and spikes under field experiment 2017

					95% Confidence limits for slope			
Tissue	**Variety**	**Treatment**	**Slope**	**CP**	**Slope**		**CP**	
Flag leaf	WB-Cedar	Control	-0.046	9.931	-0.050	-0.042	8.906	10.956
		Heat stress	-0.056	10.007	-0.063	-0.049	8.812	11.203
	Joe	Control	-0.093	18.610	-0.106	-0.080	17.876	19.344
		Heat stress	-0.072	14.920	-0.087	-0.057	13.635	16.204
	WB4458	Control	-0.045	10.779	-0.050	-0.040	9.599	11.959
		Heat stress	-0.058	10.854	-0.065	-0.052	9.786	11.922
	Zenda	Control	-0.052	12.656	-0.058	-0.046	11.634	13.678
		Heat stress	-0.070	13.294	-0.077	-0.063	12.519	14.068
	SY Monument	Control	-0.075	17.333	-0.085	-0.064	16.507	18.159
		Heat stress	-0.064	13.047	-0.072	-0.056	12.112	13.981
	Larry	Control	-0.045	11.520	-0.052	-0.039	9.988	13.051
		Heat stress	-0.060	11.261	-0.068	-0.054	10.211	12.311
	Everest	Control	-0.047	10.730	-0.052	-0.042	9.600	11.861
		Heat stress	-0.051	9.440	-0.056	-0.045	8.279	10.601
Spike	WB-Cedar	Control	-0.037	10.958	-0.040	-0.034	9.726	12.190
		Heat stress	-0.038	9.754	-0.042	-0.035	8.242	11.265
	Joe	Control	-0.049	17.018	-0.053	-0.045	16.329	17.706
		Heat stress	-0.051	16.107	-0.056	-0.046	15.113	17.102
	WB4458	Control	-0.037	12.627	-0.041	-0.034	11.555	13.700
		Heat stress	-0.039	11.346	-0.043	-0.035	10.034	12.657
	Zenda	Control	-0.044	14.627	-0.049	-0.040	13.493	15.762
		Heat stress	-0.048	14.269	-0.053	-0.043	13.108	15.430
	SY Monument	Control	-0.041	14.459	-0.045	-0.038	13.517	15.401
		Heat stress	-0.043	13.039	-0.047	-0.039	11.970	14.108
	Larry	Control	-0.041	13.537	-0.045	-0.038	12.511	14.563
		Heat stress	-0.040	11.572	-0.043	-0.036	10.301	12.842
	Everest	Control	-0.036	11.112	-0.040	-0.033	9.680	12.543
		Heat stress	-0.041	11.106	-0.045	-0.037	9.636	12.576

Slope or break point between the temperature treatments in a genotype are significantly different at 0.05 if their confidence intervals does not overlap

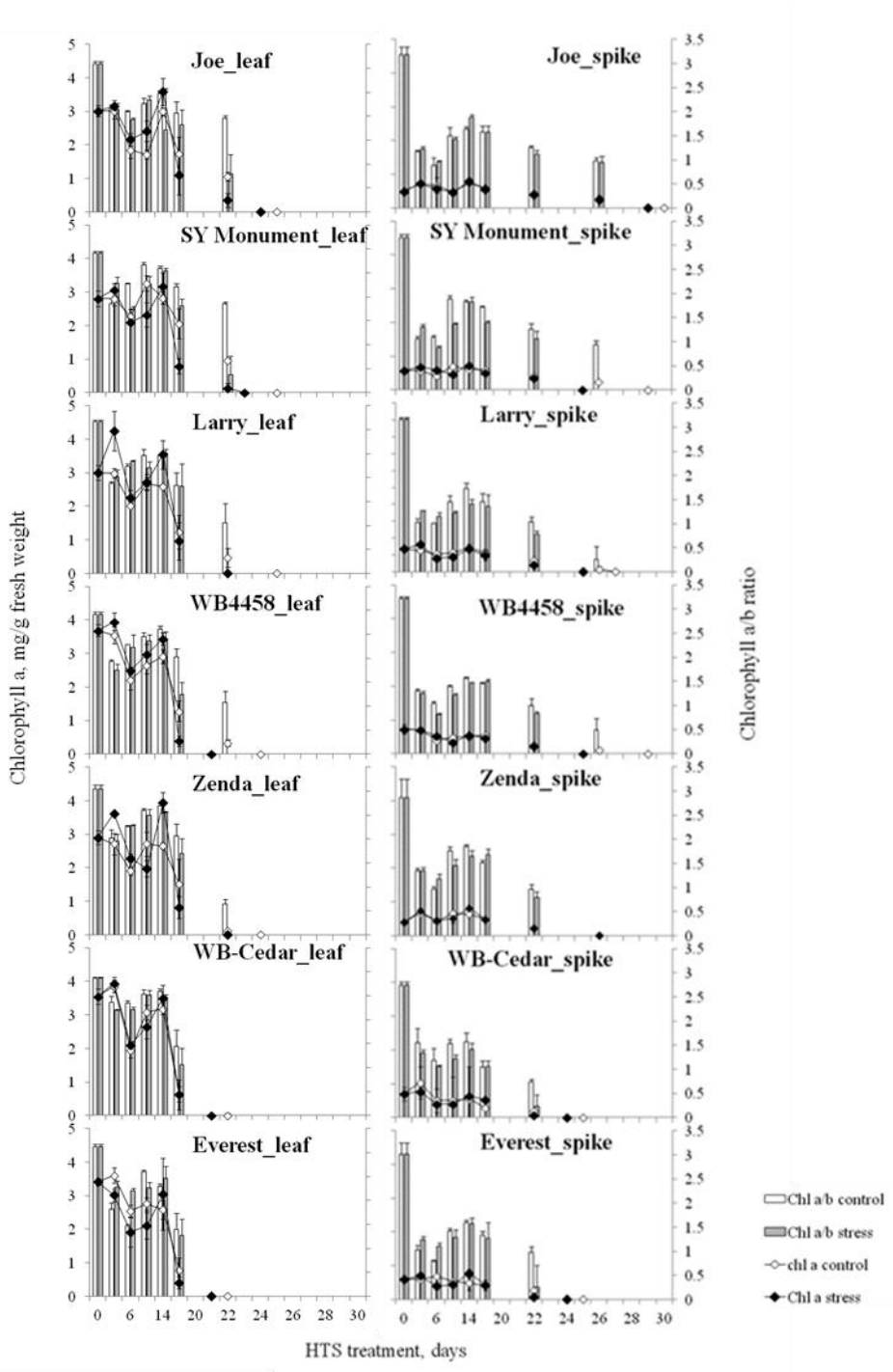

Figure 3.1 Chlorophyll-a concentration and Chl a/b ratio in field experiment

Time trend of chlorophyll-a concentration [mg/g fresh weight], and chlorophyll a/b ratio of *(a)* flag leaves, spikes for seven selected wheat varieties (Joe, SY Monument, Larry, WB4458, Zenda, WB-Cedar and Everest). Open and closed symbols represent chlorophyll-a concentration of control and HTS treatment, while white and grey columns represent chlorophyll a/b ratio for control and HTS. n=3 ± SEM.

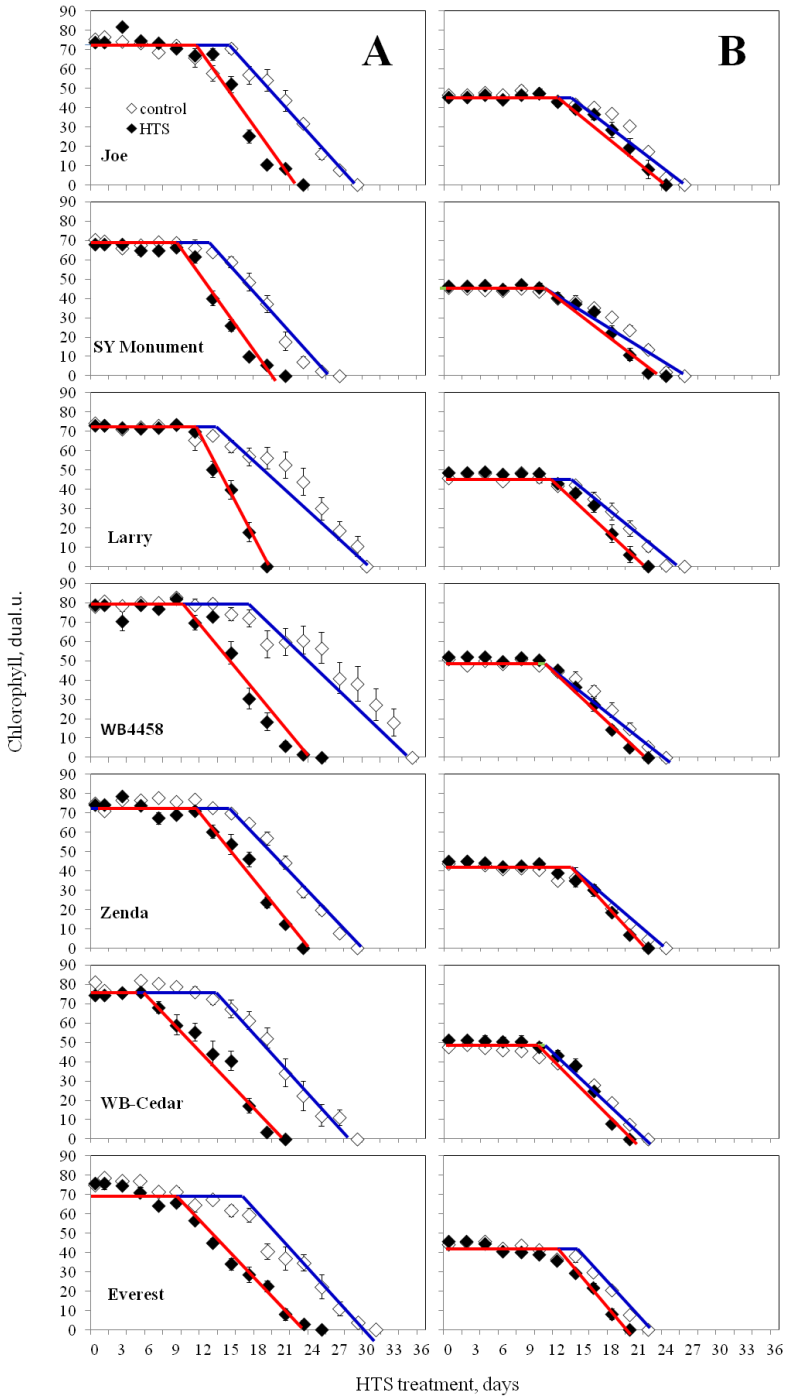

Figure 3.2 Time trend of chlorophyll concentration

Changes of chlorophyll concentration in flag leaves for seven selected wheat varieties (Joe, SY Monument, Larry, WB4458, Zenda, WB-Cedar, and Everest) for controlled environment [panel A] and field experiment [panel B], control [open symbols] and HTS [closed symbols] measured non-invasively. Interpolating line presented for change point analysis (CP), control (blue) and heat stress (red) treatment. n= 15 (controlled environment, n=60 (field) experiment ±SEM.

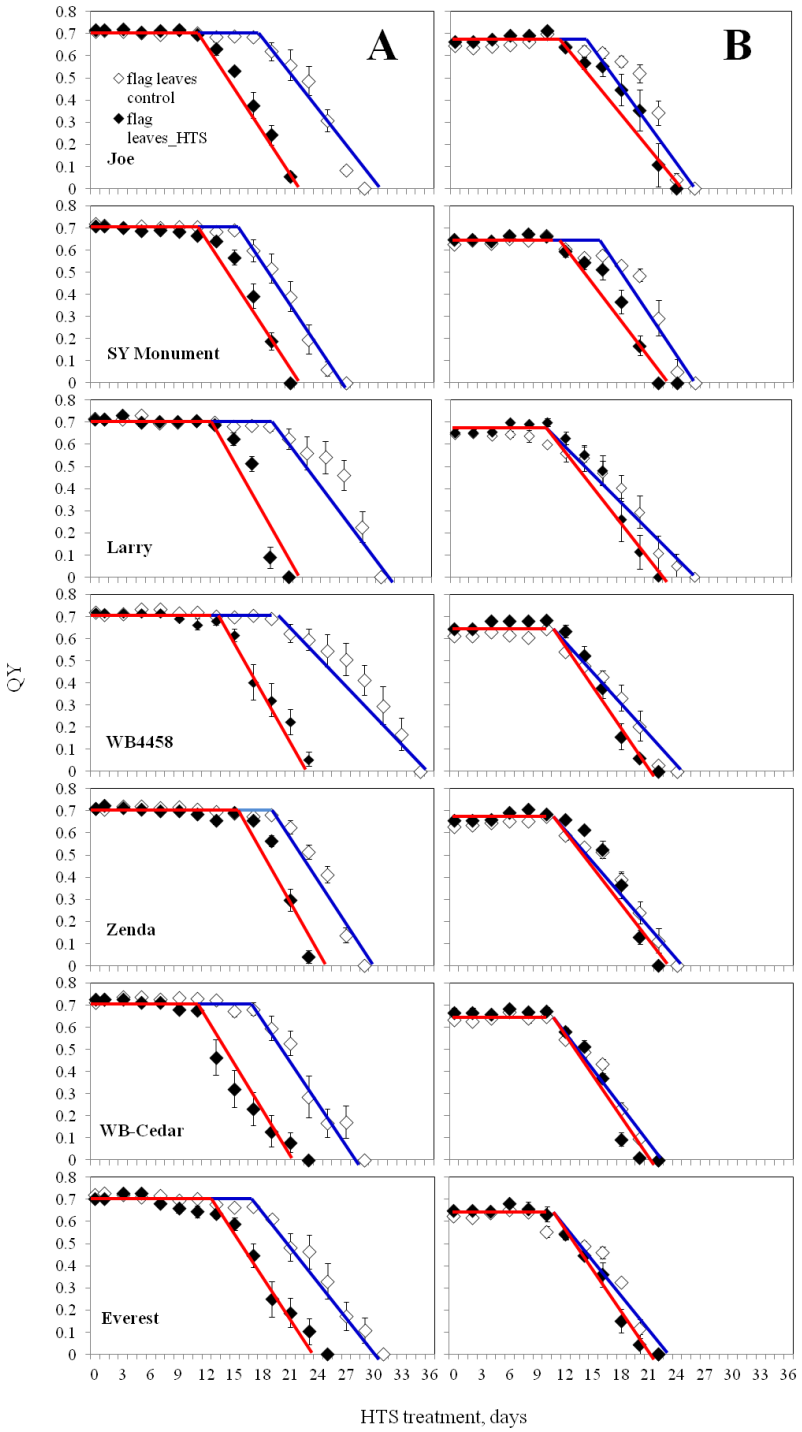

Figure 3.3 Time trend of effective quantum yield of photosystem II (QY-Lss) in flag leaves

Changes of effective quantum yield of photosystem II in flag leaves of seven selected wheat varieties (Joe, SY Monument, Larry, WB4458, Zenda, WB-Cedar, and Everest) for controlled environment [panel A] and field experiment [panel B], control [open symbols] and HTS [closed symbols]. Interpolating line presented for change point analysis (CP), control (blue) and heat stress (red) treatment. n= 15 (controllled environment), n=60 (field) experiment ±SEM.

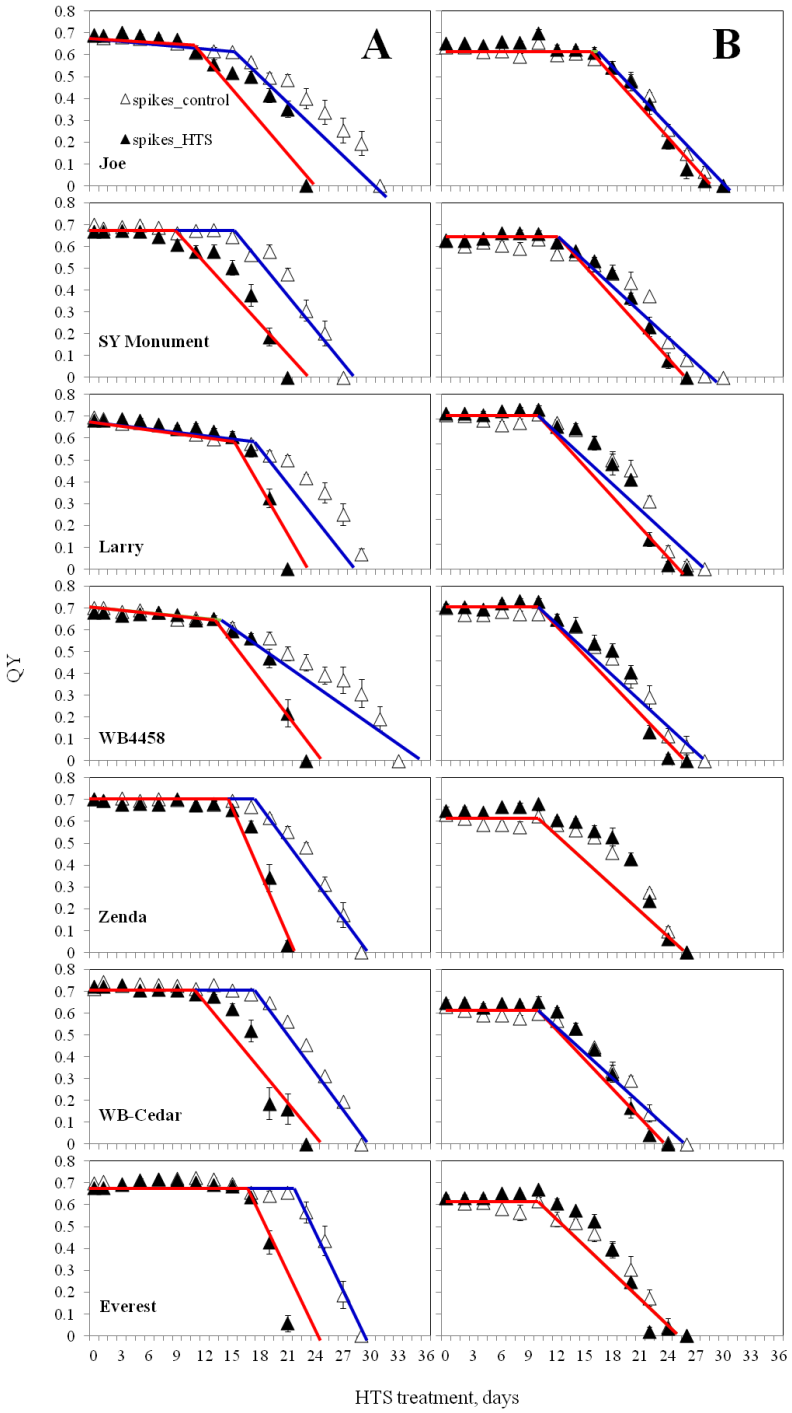

Figure 3.4 Time trend of effective quantum yield of photosystem II (QY-Lss) in spikes

Changes of effective quantum yield of photosystem II in spikes of seven selected wheat varieties (Joe, SY Monument, Larry, WB4458, Zenda, WB-Cedar, and Everest) for controlled environment [panel A] and field experiment [panel B], control [open symbols] and HTS [closed symbols]. Interpolating line presented for change point analysis (CP), control (blue) and heat stress (red) treatment. n= 15 (controlled environment), n=60 (field) experiment ±SEM

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Chapter 4 - Impact of Post-Flowering Heat Stress on Advanced Winter Wheat Breeding Lines Under Field Conditions

Abstract

Post-flowering heat stress is one of the major environmental constraints for wheat (*Triticum aestivum* L.) production in the state of Kansas, where wheat is the most widely grown grain crop. Studies have shown the optimal temperature for wheat grain development is approximately 21°C. During the grain filling stage for wheat in Kansas, it is fairly common for temperatures to reach more than 30°C and above. These scenarios have resulted in lower productivity and yield in Kansas compared to other regions of the United States. With future temperatures projected in increase, it is vital for breeding programs to improve wheat's genetic resilience to heat stress. Therefore, the purpose of this study was to explore levels of post-flowering heat stress resilience in ten breeding lines from Kansas State University's Wheat Breeding Program, under field conditions. Of the ten breeding lines used in this experiment, four are advanced breeding lines derived from materials previously reported to be tolerant of high temperatures and six are near isogenic lines (NILs). NILs were developed as part of previous work from Kansas State University's wheat breeding program to study the effect of cytoplasmic diversity on traits related to heat tolerance during the grain filling phase. In order to impose heat stress under field conditions in 2017, heat tents were placed over the wheat plants ten days after initial flowering began and remained until maturity. Temporal physiological measurements were recorded throughout the grain filling period; they were chlorophyll concentration and fluorescence. Number of days to physiological maturity was recorded as the days after stress imposition, and until maturity for both heat stress and control plots. Yield and yield components were recorded upon grain ripeness (Feekes 11.4). Chlorophyll (Chl.) index and effective quantum yield of PSII (QY-Lss) exhibited a reduction when exposed to heat stress over time compared to control plants, indicating early senescence. Both the breeding lines and NILs varied

in their performances under heat stress; some lines showed signs of heat resilience and experienced little to no drop off in heat stress conditions compared to control, while other lines showed a significant decline in yield due to heat stress. Among the breeding lines, KS070736K-1 and KS070717M-1 showed greater resilience to stress, while the NILs Stanof X060714 and Jagger X060724 were more resilient than their alloplasmic counterparts, suggesting the wheat cytoplasm is more favorable than the alien cytoplasm in these backgrounds. Genetic diversity documented through phenotyping these ten advanced wheat breeding lines and NILs for heat stress response under field conditions can aid in improving the heat tolerance and sustainment of Kansas cultivars even through future climatic changes.

Introduction

Heat stress is a major environmental constraint for wheat production and reduces both grain yield and quality (Bhullar and Jenner, 1985; Wardlaw et al., 2002). Wheat is particularly sensitive to heat stress during the reproductive and grain filling stages (Wollenweber et al., 2003) and prefers temperatures ranging from 12 to 24°C for optimum grain development (Farooq et al., 2011). Temperatures exceeding 30°C during the grain fill period have been shown to induce significant yield loss (Saini and Aspinall, 1982; Stone and Nicolas, 1994; Tack et al., 2015). Nearly all of the wheat grown in temperate regions, which accounts for 40% of global production, is affected by terminal heat stress (Reynolds, 2001). Winter wheat in Kansas is often subjected to temperatures >30°C during the grain fill period which coincides with the late spring months of May and June. Warming scenarios are predicted to worsen with increased frequency and magnitude of heat stress exposure, increasing the likelihood of greater economic losses due to reduced yield and quality for Kansas wheat producers. Thus, it is critical to explore diverse

wheat genetics for post-flowering heat resilience in order to improve current and future wheat varieties' performance under warming temperatures.

Heat stress during grain fill ultimately leads to a decline in overall yield. There are several specific physiological processes, which are impacted by heat stress, that lead to this outcome. Days to physiological maturity, flag leaf photosynthesis, leaf Chl. index, stomatal conductance, and canopy temperature depression were shown to be effected by heat stress, and thus, a lower grain yield was observed (Reynolds et al., 1994). Nearly all previous studies with the objective of quantifying the impact of post-flowering heat stress in wheat have been conducted using controlled environment facilities (Stone and Nicolas, 1994; Gibson and Paulsen, 1999; Spiertz et al., 2006). Due to lack of field based phenotyping facilities, studies performed under field conditions commonly used a staggered sowing approach to ensure that the crop was exposed to heat stress during critical developmental stages (Viswanathan and Khanna-Chopra, 2001). While this approach increases the probability of heat stress exposure during the post-flowering stage of the crop, it is limited in the fact that the crop is subjected to a significant change in environmental conditions compared to the conditions for which it has been bred (Bahuguna et al., 2015).

Our previous heat tolerance phenotyping study (Chapter 2) with seven prominent Kansas cultivars has indicated that only two of seven cultivars were found to be moderately heat tolerant while the remaining cultivars all exhibited sensitivity to heat stress. This lack of tolerance for heat stress among these recently bred varieties emphasizes the need to further explore genetic variability for heat resilience in wild wheat accessions and land races for further incorporation into ongoing wheat breeding programs. Taking into consideration the current limitations of wheat production in heat stressed environments, ten breeding lines from Kansas State

University's Wheat Breeding Program were chosen. These ten lines have shown potential for heat resilience in preliminary growth chamber studies and were selected for heat tolerance phenotyping under field based heat tents. Of the ten selected lines, four of the genotypes are advanced breeding lines while six remaining are near isogeneic lines NILS developed by transferring euplasmic nuclear genomes of different wheat lines into the alloplasmic lines in order to study the cytoplasmic effects of heat tolerance (Talukder et al., 2015). Two alloplasmic lines (PI 590259 and PI 590261) developed by Allan (1997) are the sources of cytoplasm for the NILs used in our experiment. This experiment also includes NILs developed by backcrossing euplasmic lines 'U1275' (also informally named 'Stanof') and 'Jagger' as recurrent parents (male) backcrossed with the alloplasmic lines (Talukder et al., 2015). Testing performed by Talukder et al., (2015) on the NIL population in controlled environment settings attributed increased Chl. index and QY-Lss of wheat under post-flowering heat stress to cytoplasmic variation. These results support other studies which suggest that cytoplasm plays an important role in physiological and agronomic crop responses to heat stress exposure. For example, research conducted by Roach and Wulff (1987) discovered a cytoplasmic maternal genetic effect resulted from plastid and mitochondria genomes being directly transferred from the maternal parent to the offspring during ovulation. Improved heat tolerance, among numerous other agronomic traits, is also documented as influenced by cytoplasm (Shonnard and Gepts, 1994).

In the current study, the ten breeding lines were phenotyped for post-flowering heat stress tolerance using field based heat tents to address the following objectives: 1) Determine the level of genetic variability for post-flowering heat resilience in promising breeding lines and NILs , 2) Assess the physiological and agronomic response during post-flowering heat stress exposure in

breeding lines and NILs under field based heat tents, and 3) Identify the breeding lines with the greatest heat resilience.

Materials and Methods

Two field experiments were carried out during the 2015-2016 and 2016-2017 growing seasons at Kansas State University, Agronomy Research Farm at Manhattan (39 11'N, 96 35'W). Soil type was a Kennebec silt loam. Soil samples were collected at the 0-15 cm surface and 15-60 cm subsurface prior to sowing in October 2015 in order to analyze organic matter (OM), pH, P, K, N [ammonia (NH_3) and nitrate (NO_3)], S, and Cl. Each sample was composed of 15 individual soil cores representing the experimental area. The experiments included four advanced breeding lines and six cytoplasmic NILs developed by the University's Wheat Breeding program (Tables 4.1 and 4.2); all lines were grown in two temperature treatments – control and heat stress – with four replications.

In the 2015-2016 experiment, a limited amount of seed allowed for only a single row to be planted within each treatment replication. After planting, irregular plant stands, due to poor emergence, were also observed. Physiological and yield data were obtained. However, due to inconsistent plant spacing within rows and between replications, it became difficult to obtain sound and consistent data. Thus, we deemed the data to be unreliable and it is not included as part of this chapter. Grain harvested from the control treatments in the 2015-2016 experiment was used as seed for planting the 2016-2017 experiment.

Plot preparation prior to planting included multiple tillage passes of a disc, cultivator, and harrow in the summer/fall of 2016 to prepare the seedbed. The 2016-2017 experiment was planted using a tractor (5055E, John Deere, Moline, IL) equipped with RTK guidance (Trimble FMX, Trimble Inc., Sunnyvale, CA) and a grain drill (3P605NT, Great Plains Mfg., Salina, KS)

modified for research plots (Kincaid Equipment Mfg., Haven, KS). Planting occurred on 27 Oct. 2016. Di-Ammonium Phosphate (18-46-0) was applied at a rate of 14.5 kg N ha^{-1} and as 39 kg P_2O_5 ha^{-1} as starter at the time of planting. The seed rate was 60 seeds per meter; row spacing was 19 cm and row length was 1.22 m. Three rows of each genotype were planted in each replication. The grain drill was six rows wide, thus two genotypes were planted side by side in each of the 1.22 m blocks. Experimental design is a randomized complete block design with split-plot temperature treatment structure; temperature was the main plot factor, and genotype the subplot factor.

Weed control in the wheat was accomplished using the labeled rate of a post-emergence herbicide along with hand weeding as necessary to minimize weed pressure throughout the growing season. Herbicide was applied with an all-terrain vehicle mounted boom sprayer at recommended carrier volume rates. Plots received 0.75 oz/ac FINESSE [2-Chloro-N-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl)aminocarbonyl]benzenesulfonamide] [4,5-Dihyd ro-3-methoxy-4methyl-5-oxo-N-[[2-(trifluoromethoxy)phenyl]sulfonyl]-1H-1,2,4-triazole-1-carboxamide, sodium salt] on 9 Dec. 2016 as a post-emergent spray.

The recommended rate of 56 kg N ha^{-1} was top dressed as urea (46-0-0) to the wheat plots on 3 Mar. 2017 using a variable rate drop spreader (Gandy Company, Owatonna, MN). Fungicide was applied to the plots with a handheld spray boom and backpack sprayer at flag leaf (Feekes 10) and mid grain fill (Feekes 11.2) as preemptive care for rust disease. All applications were applied at recommended carrier volume rates. Plots also received a total of 9 fl oz/ac of the fungicide TWINLINE [pyraclostrobin: (carbamic acid, [2-[[[1-(4-chlophenyl)-1*H*-pyrazol-3-yl]oxy]methyl]phenyl]methoxy-,methyl ester) metconazole: 5-[((4-chlorophenyl)methyl]-2,2-dimethyl-11(1*H*-1,2,4-triazol-1-ylmethyl)cyclopentanol].

In order to impose post-flowering heat stress in the field, custom built "heat tents" were manually placed on the plots ten days after approximately 50% of the wheat varieties had begun anthesis (Feekes 10.5.4). The heat tents were constructed from a galvanized steel framework and covered with a clear polyethylene film; a moveable vented overhead flap (0.6 m) was constructed at the roof peak (Image 4.1).

The temperature inside the heat tents was dependent upon solar radiation and was partially regulated by a thermostat indefinitely set at 35°C. When the temperature inside the heat tents rises above the desired temperature (35°C), the vented flap, which runs parallel to the peak of the roof, automatically opened to allow open air circulation and temperature moderation. Once the temperature falls below that which was desired (35°C), the flap automatically closed (Prasad et al., 2015; Sunoj et al., 2017). Temperature, relative humidity, and photosynthetic active radiation (PAR) were recorded for the duration of the experiment inside all four heat tents as well as an outside recording of the ambient (control) conditions using WatchDog 1650 Micro Station sensors (Spectrum Technologies, Inc., Aurora, IL). The data loggers were mounted on a metal post with appropriate shields to protect from direct sunlight, and placed 5 cm above the canopy level (Image 4.1).

Data measurements

Physiological measurements

Physiological measurements were taken temporally (three times per week on alternate days) in all lines, beginning four days after heat treatment commenced (Feekes 10.5.4), and until complete flag leaf senescence, which preceded physiological maturity (Feekes 11.3). Chl. index was measured using a handheld self-calibrating SPAD chlorophyll meter (Model 502, Spectrum

Technologies, Plainfield, IL). Self-calibrating SPAD chlorophyll meters measure leaf greenness and are correlated to Chl. index (Markwell, 1995). Chl. fluorescence measurements were collected using a portable handheld fluorometer, FluorPen (FluorPen FP 100, Photon System Instruments, Ltd., Brno, Czech Republic). Saturating light (intensity approximately 3,000 µmol (photons) m^{-2} s^{-1}) and measuring light (intensity approximately. 0.09 µmol (photons) m^{-2} s^{-1}) were used to measure maximal fluorescence yield (F_M`) and actual fluorescence yield (F) of light adapted samples, respectively. QY-Lss was calculated using formula QY = ($F_M$`-F)/F_M` = $\Delta F/F_M$` (Genty et al., 1989). Chl. fluorescence is a measure of active PSII receptors and is correlated to both photosynthetic leaf health and heat stress (Maxwell and Johnson, 2000; Ristic et al., 2007). Both Chl. index and Chl. fluorescence measurements were recorded as an average of three points along the flag leaf of the main tiller (near culm, mid-sheath, and near the tip) on the adaxial surface of the leaf (Green, 2016).

Agronomic traits

Physiological maturity (Feekes 11.3) was noted for each genotype per temperature treatment. Wheat was hand harvested upon physiological maturity (Feekes 11.3) on 15 and 16 Jun. 2017. The plants were dried in a forced-air dryer at 45°C for 72 hours, then weighed for total above ground biomass. In each treatment, a 1.0 m length of row was harvested from the center row of the three row plots. The harvested samples were air dried, dry weights recorded, and samples threshed using a LD 180 laboratory thresher (Wintersteiger, Ried im Innkreis, Austria). Thousand kernel weight (TKW) was counted using an electronic seed counter (Key-Mat Equipment Co., Inc. Batavia, IL) and weights were recorded. Harvest index was calculated as the ratio of dry weight of harvested grain to dry weight of total above ground biomass. Shoot weight was determined as total above ground biomass minus grain weight.

Statistical analysis

The experimental design was a split plot randomized complete block design with temperature as the main plot factor and genotype as sub-plot factor, and with four replications. Dissimilar time of measurements was considered as sub-sub plot factor for Chl. index and Chl. fluorescence data. Analysis of variance for all of the measured parameters was performed using PROC GLM procedure in SAS software (Version 9.4, SAS Institute Inc., Cary, NC). Means were separated using LSD (least significant difference) test at p=0.05.

Results

Microclimatic conditions and phenology

On average, the daytime mean temperature inside the heat tents was 6^{o}C warmer than outside ambient temperatures, beginning from ten days after stress (DAS) and until physiological maturity (Fig. 2.1B). However, nighttime mean temperatures, both inside and outside the heat tents, were similar throughout the experimental period (Fig. 2.1A and Fig. 2.1B). This indicated that the field based heat tents increased only day time temperatures inducing high day temperature stress, similar to the previous work from our lab (Sunoj et al., 2017), with results not confounded by high night temperature. Relative humidity was not significantly different between heat tents and ambient control environments for either day or night. The average relative humidity during daytime was approximately 50 and 54% inside and outside the heat tents, respectively. At night, the average relative humidity was approximately 74 and 70% inside and outside the heat tents, respectively. The average PAR transmitted inside heat tents at canopy level was about 800 µmol m^{-2} s^{-1} while ambient conditions PAR was approximately 1000 µmol m^{-2} s^{-1}. Heat stress induced by warmer temperatures within the heat tents prompted earlier

maturity in all lines by reducing the grain filling duration as compared to the control. Among all tested lines, KS 070717 M-1, KS 070729 K-26, PI 590259 X060714, and Jagger X060724 exhibited the greatest reduction in grain filling duration, each exhibiting a four day reduction (Fig. 4.1).

Grain yield and related traits

Grain yield was not significantly affected by either temperature or line (Table 4.3). On average, grain yield was reduced by 4.3% under heat stress. Highest grain yield under ambient control conditions was recorded in PI 590259 X060714, followed by Jagger X060725 and KS070725M-3. Under heat stress, KS070725M-3 recorded the highest yield among all lines, followed by KS070736K-1 and NIL Stanof X060714 (Fig. 4.2). PI 590259 X060714 and Jagger X060725 recorded the most significant decline in yield due to heat stress at 34 and 13%, respectively (Fig. 4.2). Although absolute grain yield under control conditions was comparatively lower, KS070729K-1 followed by KS070725M-3 recorded 10 and 9% greater grain yield under heat stress compared to their absolute grain yield performance in the control environment (Fig. 4.2). Harvest index (HI) expressed statistically significant variation for temperature ($p< 0.01$) and genotype ($p<0.001$), but with no treatment by line interaction. On average, a 4.5% reduction in HI was noticed among all lines exposed to heat stress. NIL PI 590259 X060714 had the highest decline in HI at 18% (Table 4.4). Thousand kernel weight (TKW) varied significantly between temperature and lines, but there was no significant temperature by line interaction (Table 4.3). Among all lines, five of the ten recorded significant reductions in TKW; and across all lines, TKW declined 2.9% under heat stress compared to control. PI 590259 X060714 exhibited highest reduction (8%) in TKW among all lines under heat stress followed by Jagger X060725, which recorded a 6.5% reduction (Table 4.4).

Physiological traits

Chl. index was significantly affected by temperature ($p<0.01$), line ($p<0.001$), DAS ($p<0.001$), and their interaction effects ($p<0.001$) (Table 4.3). Chlorophyll index maintained consistent levels among all genotypes until 12 DAS in the control and 10 DAS in the heat tents (Fig 4.3). Rapid reductions in chlorophyll index were noticed at 14 DAS in both treatments. In approximation, genotypes exposed to the heat stress treatment reached complete senescence at 19 DAS, while those exposed to the ambient temperature treatment did not reach complete senescence (with very low chlorophyll index) until 24 DAS.

QY-Lss was significantly impacted by temperature ($p<0.01$), line ($p<0.001$), DAS ($p<0.001$), and their interaction effects (Table 4.3). QY-Lss remained relatively stable among genotypes in both treatments until 14 DAS, at which time most genotypes exposed to the heat stress treatment began experiencing a rapid decline. The control treatment growing under ambient temperatures continued to show consistent QY-Lss until 16 DAS in most lines. Lines KS070729K-26 and KS070736K-1 exhibited a gradual decline and a less drastic QY-Lss reduction compared to other lines in both control and heat stress treatments compared to other lines (Fig. 4.4 B and D).

Discussion

While it is believed that current wheat varieties adapted to Kansas and the southern Great Plains possess a certain base level of heat tolerance, research conducted by Barkley et al. (2014) suggests that wheat yields in Kansas are negatively impacted by heat stress. For this reason, identifying alternate genetic sources of heat stress resilience is both crucial and timely. Most of

the published research regarding post-flowering heat stress in wheat has been carried out under controlled environment facilities (Stone and Nicolas, 1994; Gibson and Paulsen, 1999; Spiertz et al., 2006). Thus, research regarding agronomic responses of wheat genotypes grown under field conditions and exposed to heat stress during the grain fill period is lacking. Previous work by Talukder et al. (2015) on the development and research of NILs, as well as other breeding lines, has identified genotypes that display resilience to heat stress in controlled environment settings. Testing the same promising genotypes under field conditions will aid in validating the true level of heat resilience these lines possess when exposed to post-flowering heat stress under realistic field conditions.

In the present study, the impact of heat stress on grain yield did not induce significant change in many lines, but did reduce yield by 34% in one NIL: PI 590259 X060714. The large yield reduction observed in PI 590259 X060714 aligns with its performance measured by other yield parameters such as shoot weight and HI; this NIL's poor performance is also apparent as it led all genotypes in percent reduction by 9% reduction in shoot weight and 18% reduction in HI respectively under heat stress. While these reductions are extreme, it should be noted that PI 590259 X060714 statistically tied for the highest overall TKW among all genotypes under both control and heat stress treatments. This phenomenon seemed to be reoccurring in other genotypes. To further explain, the highest performing entries under control treatments are also the most detrimentally affected by heat stress, whereas middle and lower performing genotypes tended to record much lower percent reduction in TKW. Among NILs, Stanof X060714 and Jagger X060724 emerged as heat resilient lines, each recording less than a 1% change in grain yield between temperature treatments. Additionally, their performance in other agronomic traits such as TKW and HI are top-ranking when compared to all other lines studied. The source of

heat tolerance in Jagger X060724 may be from the "Jagger" in the pedigree; this is its source of cytoplasm, as Jagger is understood to be moderately heat tolerant. On the contrary, Stanof, which is the source of cytoplasm in Stanof X060714, has previously been known for its heat susceptibility.

Among the four breeding lines included in the ten genotypes, KS070736K-1 was least affected by post flowering heat stress when considering yield, TKW, and HI. This genotype's improved performance could be attributed in part to its long grain fill duration. KS070736K-1 recorded the second longest grain fill duration under both heat stress and controlled environments when averaged among all genotypes. Its reduction in grain fill duration under the heat stress treatment was three days, which is equal to the average of treatment group. Genetic background of KS070736K-1 is derived from 'Proteinka' which is a known source of heat tolerance among wheat genotypes (Ristic et al., 2007).

Early senescence caused by heat stress was variable among genotypes. Physiologically, the rate of decline in Chl. index was less drastic in the heat tolerant line KS070736K-1 as opposed to KS070717M-1, which declined rapidly and experienced large reductions in TKW and HI during heat stressed treatment. However, increased rate of decline in Chl. index cannot be used to explain the significant reduction in TKW of KS070725M-3 as it displayed a similar rate of decline in comparison to KS070736K-1 (Fig. 3). The rate of Chl. index decline was considerably less variable among the six NIL genotypes compared to the four breeding lines. Thus, determining a correlation between rate of senescence and agronomic performance proves challenging warrants further testing.

A rate of decline similar to Chl. index was noticed for the Chl. fluorescence measurement QY-Lss among lines; however, more variability exists between the wheat lines compared to Chl.

index. Among the breeding lines, the performance of KS070736K-1 and KS070729K-26 indicated increased ability to retain QY-Lss during the final seven days of the grain fill period compared to KS070717M-1 and KS070725M-3, which exhibit a more drastic decline. Now focusing on the NILs, it is interesting to note that PI 590259 X060714 – the genotypic leader in reduction under heat stress in all agronomic measurements – had the slowest reduction and maintained the highest level of QY-Lss throughout the last seven days of grain fill. However, Jagger X060725 had the sharpest decline in QY-Lss over the final four days of grain fill and was also among the lowest performing lines when considering TKW, HI, and yield under heat stress.

In conclusion, there is a considerable range of performance among the ten experimental lines tested in this field experiment. When comparing to the commercial varieties tested in chapter two, both the breeding lines and NILs had a much lower relative reduction in grain yield, harvest index and thousand kernel weight when exposed to heat stress (Table 4.5). This indicates that the breeding lines possess desirable traits and could be used as heat tolerant donors within the Kansas State Wheat Breeding Program. The experimental breeding line KS070736K-1 and KS070717M-1 exhibited promising potential as genetic sources of heat tolerance when considering both the agronomic and physiological traits. Among the NILs, Stanof X060714 and Jagger X060724 demonstrated greater resilience under heat stress, indicating their potential to be considered as heat tolerant donors for breeding purposes. The findings from this study correlate with the previous work done by Talukder et al. (2015), which examined the NILs under controlled environment settings. In order to overcome the current and future realized wheat yield losses due to post-flowering heat stress, it is of utmost importance to integrate greater heat resilient genetics into ongoing wheat breeding programs. Current efforts to enhance our future

cultivars' resilience to heat stress will help to ensure sustainability of wheat production not only in Kansas and across the Great Plains, but globally as well.

Table 4.1 Pedigree and other characteristics of four breeding lines phenotyped for heat tolerance in 2017 field experiment

Breeding Lines		
Line	**Pedigree**	**Characteristics**
KS070717M-1	KV RIL73/KS020439M~2//KS010957-9	KV: Karl/Ventnor RIL73 RIL: Recombinant inbred line Ventnor: Australian hard white winter wheat known as heat tolerant
KS070725M-3	KV RIL73/Karl92//KS06O3A~58	KV: Karl/Ventnor RIL73 RIL: Recombinant inbred line Karl 92: known to be heat sensitive, developed by Kansas State University Ventnor: Australian hard white winter, heat tolerant
KS070729K-26	Jefimija/KS010525-1-1//HV9W03-1601R-2	Jefimija: spring wheat, known to be heat tolerant
KS070736K-1	Proteinka/KS020446TM~1//KS06O3A~6	Proteinka: known to be heat tolerant

Table 4.2 Pedigree, origin, and other characteristics of six cytoplasmic near isogenic lines (NIL) phenotyped for heat tolerance in 2017 field experiment

Near Isogenic Lines				
Line	**Pedigree**	**Origin**	**Cytoplasm (female)**	**Characteristics**
PI 590259 X060714	PI590259/TAM-107//5*Stanof	X060714	PI 590259	PI 590259: Alloplasmic line developed from Stephens background (Allan 1997) PI 590259 pedigree: Aegilops juvenalis/6*CHR//9*SK(NDMI)/3/7*SPN SPN: Stephens, soft white winter cultivar widely grown in the U.S. Pacific Northwest
Stanof X060714	PI590259/TAM-107//5*Stanof	X060714	Stanof	PI 590259: Alloplasmic line developed from Stephens background (Allan 1997) PI 590259 pedigree: Aegilops juvenalis/6*CHR//9*SK(NDMI)/3/7*SPN SPN: Stephens, soft white winter cultivar widely grown in the U.S. Pacific Northwest Stanof: moderately heat susceptible (also known as U1275)
PI 590259 X060724	PI 590259/KARL 92//5*Jagger	X060724	PI 590259	PI 590259: Alloplasmic line developed from Stephens background (Allan 1997) PI 590259 pedigree: Aegilops juvenalis/6*CHR//9*SK(NDMI)/3/7*SPN SPN: Stephens, soft white winter cultivar widely grown in the U.S. Pacific Northwest
Jagger X060724	PI 590259/KARL 92//5*Jagger	X060724	Jagger	PI 590259: Alloplasmic line developed from Stephens background (Allan 1997) PI 590259 pedigree: Aegilops juvenalis/6*CHR//9*SK(NDMI)/3/7*SPN SPN: Stephens, soft white winter cultivar widely grown in the U.S. Pacific Northwest Jagger: moderately heat tolerant
PI 590261 X060725	PI 590261/6*Jagger	X060725	PI 590261	PI 590261: Alloplasmic line developed from Stephens background (Allan 1997) PI 590261 pedigree: Aegilops cylindrica/CHR//10*SK(NDM2)/3/7*SPN SPN: Stephens, soft white winter cultivar widely grown in the U.S. Pacific Northwest
Jagger X060725	PI 590261/6*Jagger	X060725	Jagger	PI 590261: Alloplasmic line developed from Stephens background (Allan 1997) PI 590261 pedigree: Aegilops cylindrica/CHR//10*SK(NDM2)/3/7*SPN SPN: Stephens, soft white winter cultivar widely grown in the U.S. Pacific Northwest Jagger: moderately heat tolerant

Table 4.3 Probability of effects of temperature (T), line (L), days after stress (DAS), T × L, T × DAS, L × DAS, and T × L × DAS interactions on physiological and yield parameters in 2017 field experiment

	Variables (Pr>F)							Main effect of temperature	
Traits	**T**	**L**	**T × L**	**DAS**	**T × DAS**	**L × DAS**	**T × V×DAS**	**Control**	**Heat stress**
Chlorophyll index (SPAD units)	<0.05	<0.001	<0.05	<0.001	<0.001	<0.001	<0.001	36.2a	34.1b
Effective quantum yield (QY-Lss)	<0.01	<0.001	0.059	<0.001	<0.001	<0.001	<0.001	0.48a	.43b
Shoot dry weight (g m^{-1})	0.858	<0.01	0.757	-	-	-	-	133.0a	138.3a
Thousand kernel weight (g)	0.05	<0.001	0.108	-	-	-	-	34.3a	33.3a
Grain yield (g m^{-2})	0.219	0.061	0.070	-	-	-	-	474.6a	453.0a
Harvest index	<0.01	<0.001	0.211	-	-	-	-	0.40a	0.39b

Table 4.4 Shoot weight, harvest index, and thousand kernel weight of wheat breeding and NILs grown in 2017 field experiment exposed to control and heat stress treatments

Field Experiment 2017						
	Shoot weight (g m^{-1})		Harvest index		Thousand kernel weight (g)	
Line	**Control**	**Heat stress**	**Control**	**Heat Stress**	**Control**	**Heat Stress**
KS070717M-1	125 ± 13	119 ± 20	0.42 ± 0.03	0.39 ± 0.02	34.5 ± 1.1	33.0 ± 0.9
KS070725M-3	135 ± 11	141 ± 8	0.43 ± 0.01	0.41 ± 0.01	39.2 ± 0.7	36.7 ± 0.2
KS070729K-26	136 ± 2	154 ± 24	0.36 ± 0.01	0.35 ± 0.01	29.6 ± 0.8	29.3 ± 0.5
KS070736K-1	121 ± 5	147 ± 7	0.41 ± 0.01	0.39 ± 0.01	35.2 ± 1.2	35.8 ± 0.3
PI 590259 X060714	165 ± 17	150 ± 12	0.39 ± 0.02	0.32 ± 0.01	39.9 ± 1.0	36.7 ± 1.3
Stanof X060714	153 ± 25	154 ± 9	0.38 ± 0.02	0.38 ± 0.01	37.1 ± 0.2	35.0 ± 0.6
PI 590259 X060724	128 ± 10	126 ± 5	0.39 ± 0.02	0.38 ± 0.01	33.9 ± 0.6	34.0 ± 0.6
Jagger X060724	119 ± 6	116 ± 9	0.42 ± 0.01	0.43 ± 0.01	31.6 ± 0.5	32.0 ± 0.5
PI 590261 X060725	108 ± 2	115 ± 2	0.42 ± 0.01	0.41 ± 0.01	29.9 ± 0.7	29.7 ± 0.4
Jagger X060725	142 ± 18	135 ± 7	0.42 ± 0.01	0.40 ± 0.01	32.5 ± 0.9	30.4 ± 0.5
5% LSD (T)		-		0.01		0.59
5% LSD (L)		21.26		0.02		1.31
5% LSD (T×L)		-		-		-

Table 4.5 Comparison of all 17 genotypes tested in the 2017 field experiment, including seven Kansas varieties and ten breeding lines, for grain yield, harvest index, and thousand kernel weight

	Grain yield (g m^{-2})		Harvest index		Thousand kernel weight (g)	
Variety	**Control**	**Heat Stress**	**Control**	**Heat Stress**	**Control**	**Heat Stress**
WB-Cedar	615.0 ± 58.1	488.2 ± 7.6	0.48 ± 0.004	0.45 ± 0.003	39.0 ± 0.7	35.9 ± 0.5
Joe	517.8 ± 35.9	483.7 ± 31.0	0.43 ± 0.003	0.39 ± 0.006	36.9 ± 0.5	34.5 ± 0.3
WB4458	514.3 ± 37.3	468.8 ± 22.8	0.43 ± 0.006	0.42 ± 0.004	36.2 ± 0.4	34.4 ± 0.4
Zenda	417.6 ± 20.1	399.2 ± 18.8	0.40 ± 0.006	0.37 ± 0.004	33.4 ± 0.3	32.5 ± 0.3
SY Monument	535.3 ± 19.7	486.2 ± 21.5	0.44 ± 0.009	0.43 ± 0.009	34.6 ± 0.3	33.8 ± 0.6
Larry	546.6 ± 26.6	508.4 ± 22.6	0.45 ± 0.005	0.44 ± 0.004	36.3 ± 0.3	34.7 ± 0.3
Everest	515.2 ± 33.4	395.3 ± 11.2	0.45 ± 0.022	0.40 ± 0.007	32.6 ± 0.1	30.2 ± 0.3
KS070717M-1	465.4 ± 1.2	470.1 ± 4.2	0.42 ± 0.03	0.39 ± 0.02	34.5 ± 1.1	33.0 ± 0.9
KS070725M-3	534.0 ± 6.0	508.1 ± 4.2	0.43 ± 0.01	0.41 ± 0.01	39.2 ± 0.7	36.7 ± 0.2
KS070729K-26	403.9 ± 1.3	441.2 ± 4.4	0.36 ± 0.01	0.35 ± 0.01	29.6 ± 0.8	29.3 ± 0.5
KS070736K-1	445.1 ± 2.0	488.1 ± 2.7	0.41 ± 0.01	0.39 ± 0.01	35.2 ± 1.2	35.8 ± 0.3
PI 590259 X060714	566.6 ± 18.7	372.4 ± 7.3	0.39 ± 0.02	0.32 ± 0.01	39.9 ± 1.0	36.7 ± 1.3
Stanof X060714	490.6 ± 9.2	486.6 ± 4.6	0.38 ± 0.02	0.38 ± 0.01	37.1 ± 0.2	35.0 ± 0.6
PI 590259 X060724	428.5 ± 11.3	413.0 ± 4.1	0.39 ± 0.02	0.38 ± 0.01	33.9 ± 0.6	34.0 ± 0.6
Jagger X060724	454.7 ± 6.3	458.7 ± 6.4	0.42 ± 0.01	0.43 ± 0.01	31.6 ± 0.5	32.0 ± 0.5
PI 590261 X060725	403.8 ± 3.9	421.0 ± 4.5	0.42 ± 0.01	0.41 ± 0.01	29.9 ± 0.7	29.7 ± 0.4
Jagger X060725	541.3 ± 14.7	471.3 ± 5.8	0.42 ± 0.01	0.40 ± 0.01	32.5 ± 0.9	30.4 ± 0.5
5% LSD (T)	24.5		0.011		0.852	
5% LSD (L)	67.5		0.025		1.416	
5% LSD (T×L)	90.6		0.031		1.785	

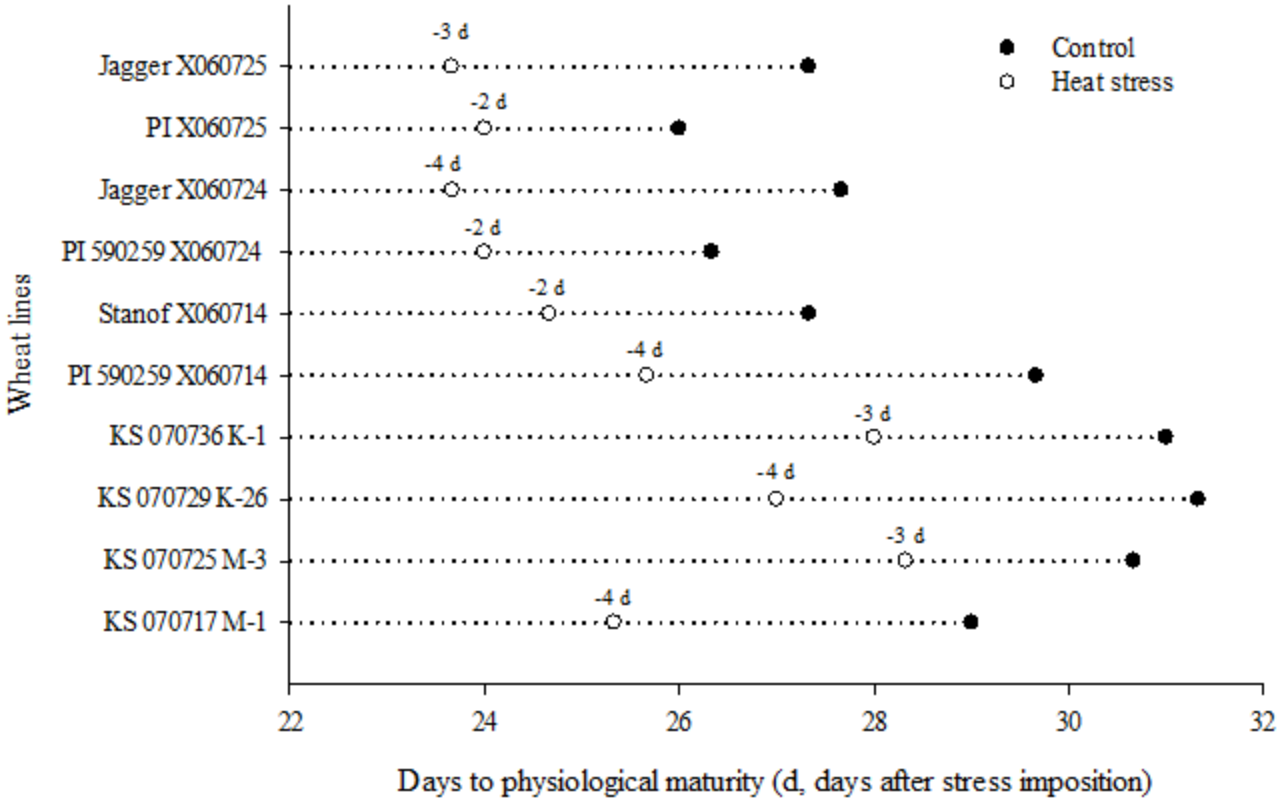

Figure 4.1 Days to physiological maturity (d) recorded from the day of stress imposition until maturity in four breeding and six cytoplasmic NILs grown in 2017 field experiment

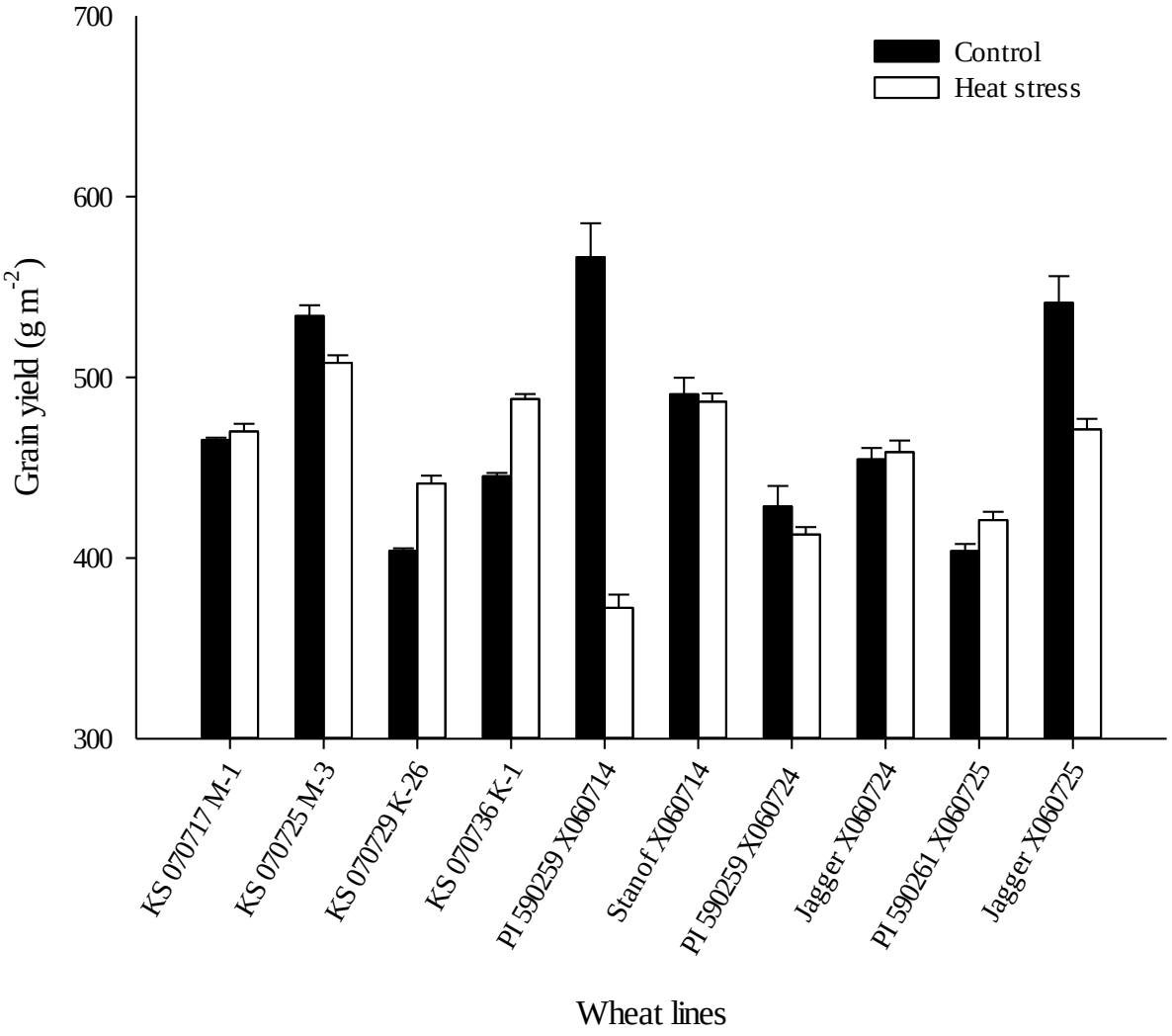

Figure 4.2 Grain yield of four breeding and six near isogenic wheat lines grown in 2017 field experiment under control and heat stress treatments

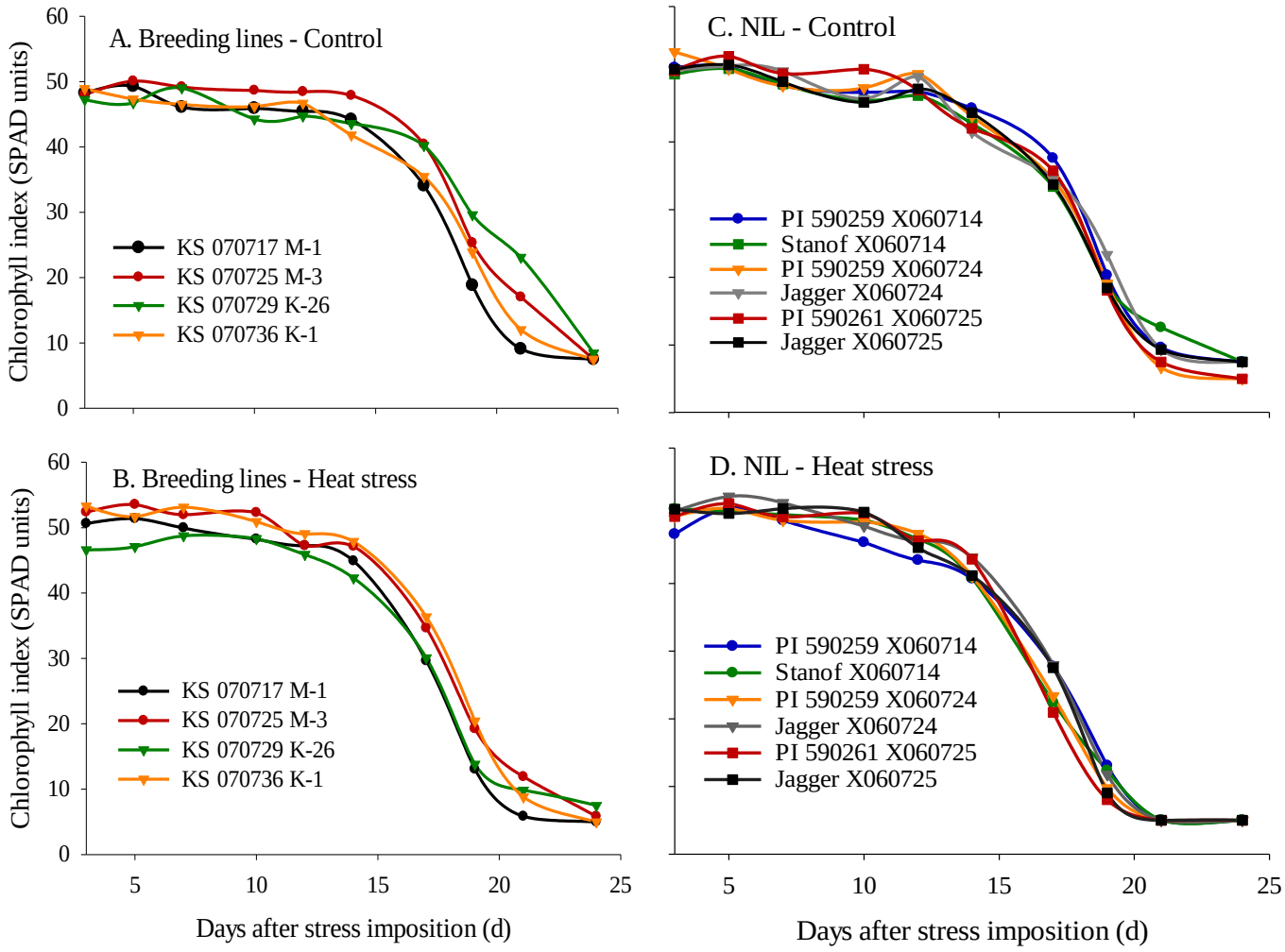

Figure 4.3 Chlorophyll index (SPAD units) in flag leaves of four breeding lines (A. Control and B. Heat stress) and six NILs (C. Control and D. Heat stress) grown in 2017 field experiment; at different time intervals following control and heat stress exposure

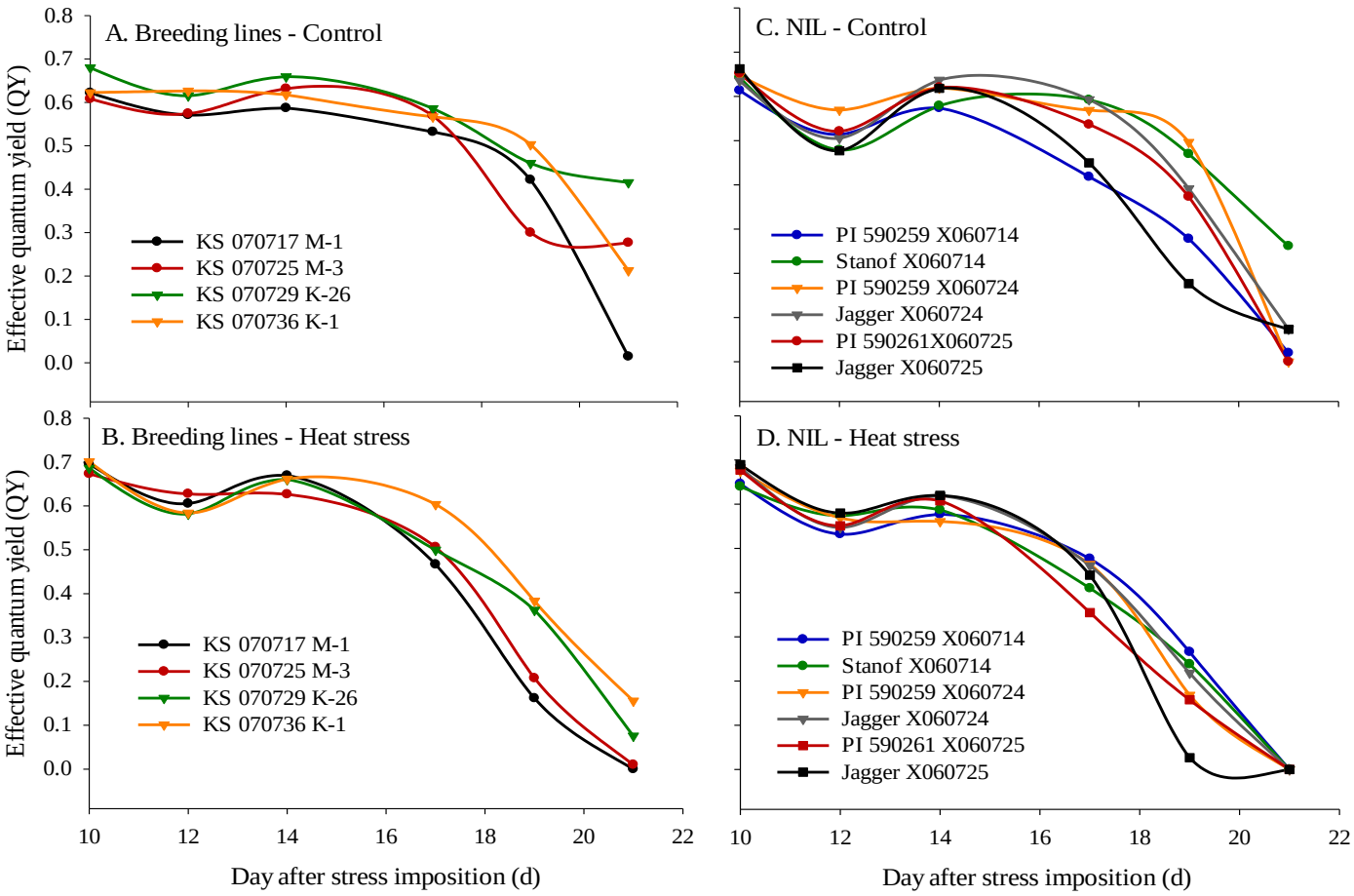

Figure 4.4 Effective quantum yield (QY-Lss) in flag leaves of four breeding lines (A. Control and B. Heat stress) and six NILs (C. Control and D. Heat stress) grown in 2017 field experiment, at different time intervals following control and heat stress exposure

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Appendix A - Field based heat tents

A.1 Unique field based heat tents placed over wheat plants to impose heat stress during grain filling phase (A) and wheat plants inside the heat tents (B)

A.2 Watchdog 1650 Micro Station placed inside white radiation shield mounted to white metal post, PAR sensor mounted at the top of metal post. Sensors were placed both inside heat tents and outside to measure ambient conditions