Physiological and agronomic characterization of post-flowering heat stress in wild wheat species and Robertsonian translocation lines

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Abstract

Heat stress during flowering and grain-fill stages is a major environmental factor affecting winter wheat production in the Great Plains of the United States. Wild emmer wheat (*Triticum diccocoides*) is an annual grass species native to the Fertile Crescent and is hypothesized to have a large genetic diversity for improving cultivated wheat. Similarly, *Aegilops speltoides* is known to possess a higher level of tolerance to abiotic stresses, including heat. The hypothesis is that the chromosomal segment from *A. speltoides* incorporated into commercial wheat varieties will help enhance heat stress tolerance in winter wheat. The potential of wild wheat species in helping address heat stress damage in cultivated wheat has not been fully explored. Therefore, the major objective of this research project was to capture the genetic variability for post-flowering heat tolerance and asses the physiological and agronomic responses in wild emmer wheat and Robertsonian translocation lines. Chromosomal segments from *A. speltoides* were incorporated into adapted wheat background, creating Robertsonian translocation lines (RobT's) (*Triticum aestivum-Aegilops speltoides*). In the first study, 28 different wild wheat entries were grown under control treatment (25°C) and transferred to high day temperature treatment (35°C) at first signs of flowering and exposed to heat stress for 21 days. Plants exposed to heat stress reached physiological maturity faster, and recorded a significant reduction in yield. Photosynthesis rate and chlorophyll fluorescence were rapidly reduced under heat stress. A moderate range in tolerance to heat stress was identified within the wild wheat with certain accessions having a comparatively higher level of tolerance to heat stress. In the second study a set of 20 RobT's, along with their parental lines were exposed to heat stress (35°C) at flowering

for 21 days. Certain RobT's outperformed the parent lines, recording a higher photosynthesis rate, maintaining chlorophyll index through an extended period of stress, as well as recording higher yield and lower heat susceptibility index. The findings indicate that the genetic potential in wild wheat, especially RobT's can be exploited to enhance terminal heat stress in winter wheat. Therefore, wild wheat needs to be explored further and genomic regions inducing greater tolerance to abiotic stresses needs to be incorporated into breeding programs to enhance resilience of popular wheat varieties to current and future warmer climate.

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Dedication

This thesis is dedicated to the Bustamante-Trejo family. To my mother Yunuen and father Jose, my brother Camilo, my Sister Maria, and my little brother Isaac. Thank you for your love, encouragement, and support.

Chapter 1 – Literature Review

Introduction - Importance and origin of wheat

World population is predicted to reach over 9 billion by 2050 and global agricultural production has to increase by 60 to 70 percent from current level to meet the increasing food demand (Silva et al, 2018). Wheat (*Triticum* aestivum L.) is the third most important crop in terms of global food production. It is considered to be a primary source of energy (carbohydrate) and in addition contain significant amount of other important nutrients including proteins, fiber, and minor components such as lipids, vitamins, and minerals that contribute to a healthy diet (Shewry et al., 2015). Annual global production of wheat was around 765 million tonnes in 2019 (FAOSTAT, 2019), with China being the world's leading wheat producing country with 134 million tonnes, followed by India (98 million), Russia (85 million), United States (47 million), and France (36 million) (FAOSTAT, 2019). In 2019, Kansas was the leading wheat producing state in the U.S. with 51.3 million metric tonnes, but has experienced a steady decline in production averaged over the last 10 years, attributed to reduced planted area and post-flowering exposure to increasing temperature and drought (Kansas Wheat Alliance, 2019). Current wheat yields are increasing at a rate of 1% per year, but in order to meet the projected food demand, wheat yields must increase by 2.4% annually (Ray et al., 2013). Empirical evidence suggests that increases in temperature in the period between 1980 and 2008 have already resulted in an average global maize and wheat yield reductions by 3.8% and 5.5%, respectively (Lobell et al., 2011).

Wheat originated in Southeast Turkey approximately 10,000 years ago during the Neolithic revolution which marked the transition from hunting and gathering food to settled

agriculture (Molnar et al., 2015). One of the first wheat cultivars were wild diploid wheat (*Triticum urartu*, genome AA) hybridized with goatgrass (*Aegilops speltoides*, genome BB) to produce wild emmer wheat (*Triticum dicoccoides*, genome AABB) (Huang et al., 2002, Dvorak and Akhunov, 2005). As early farmers continued to improve wheat, cultivated emmer wheat (*Triticum dicoccum*, genome AABB) blended with another wild goatgrass species (*Aegilops tauschii*, genome DD) leading to the creation of *Triticum spelta* which possesses the D genome (Heun et al., 1997, Avni, et al., 2017). The origin of the B genome is not fully understood. The genome *Aegilops speltoides* Tausch is most closely related to the B genome, but it is possible that the species contributing to this genome has not been discovered or has gone extinct (Kilian et al. 2007). Primitive farmers selected crops based on desirable visual traits such as grain number and size, plant biomass, and plant height. Nonetheless, native wheat cultivars experienced shattering of seed at maturity, which resulted in significant loss in grains at harvest. For that reason, an important step in wheat domestication occurred when the shattering trait was determined by mutations at the Br (Brittle rachis) locus (Nalam et al., 2006), paving the way for current wheat cultivars that avoid shattering-induced seed loss. Another important trait identified and incorporated that has helped shape the current wheat cultivars is the free-threshing trait, which eases the removal of glumes and allows farmers to obtain clean grain. The free forms arose by a dominant mutant at the Q locus which modified the effects of recessive mutations at the TG (tenacious glume) locus on Chromosome 2D (Jantasuriyarat et al., 2004).

The improvement of such traits eventually lead to the creation of Durum wheat (*Triticum durum*) and Bread wheat (*Triticum aestivum*). About 95% of the global cultivated wheat in the twenty first century is hexaploid bread wheat, with the remaining 5% being mostly tetraploid

durum wheat (Shewry, 2009). The vast majority of current wheat varieties were developed from the same ancestors that originated 10,000 years ago (Shewry et al., 2018). Part of the reason for stagnating wheat yields is the lack of genetic diversity in the gene pool of wheat germplasm used in current wheat breeding programs (Dwivedi et al., 2017). This is partially because of how wheat originated and evolved from a spontaneous cross between two wild grasses. Such an event occurred only once or twice and the reproductive isolation of wheat from its wild parents has led to narrow genetic base in wheat breeding programs (Kihara et al., 1944, McFadden & Sears, 1944).

Impact of climate change on wheat production

Feeding the world population in a changing climate presents a significant challenge and the projected yields of crops under a range of climatic scenarios are needed to asses future food security prospects (Beddington et al., 2012). Global average temperature is expected to rise as a result of climate change. By the year 2050 the global average temperature is projected to have increased between 2 and 4°C above the pre-industrial era (IPCC, 2014). Climate is an important factor affecting wheat production (Lollato et al., 2017) and understanding the specific impacts of different climate change drivers on food security is of extreme importance. The most significant challenge in food production are stagnating yields and an expected 20 to 30 percent loss in wheat production due to increasing temperatures (Zhao et al., 2017).

Crop models are helpful tools for assessing the threat of climate change to local and global food production (Challinor et al., 2014). Global wheat production is estimated to decrease by 6% for every 1°C increase in temperature, with increased variability over location and time (Asseng

et al., 2015). A large geographic variation in wheat yields across similar climatic conditions points to sizeable yield gaps indicating regional variability across wheat growing regions (Balkovic et al., 2014). Geographical and seasonal variabilities in wheat yields are critical to economy as these factors influence the global market, grain supply, and price fluctuations (Lizumi et al., 2013). Current climate conditions threaten the future of wheat production and other agriculture related industries, which altogether contribute to more than $750 billion of the gross domestic product in the USA (USDA, 2016). To mitigate the effects of climate change on staple food grain production, improving wheat with enhanced resilience to harsh environmental conditions is essential. Sustaining production under future changing climate requires an ongoing adaptation of cropping systems through breeding and appropriate agronomic strategies which can limit the impact of abiotic stresses including heat stress (Nuttall et al., 2018).

Wild wheat and heat stress responses

The domestication of wild wheat was an evolutionary process where humans cultivated plants for traits that satisfied their needs, including ease of harvest and edibility (Hua et al., 2015). A repercussion of such events resulted in a heterogeneous reduction in the level of genetic variation among crops including wheat (Buckler et al., 2001). Genetic diversity has been gradually reduced over time during crop domestication while breeding programs started focusing on new traits for further crop yield improvement (Zhang et al., 2017). A systematic introduction of new genetic diversity is needed to sustain the cycle of crop improvement and in addition improve tolerance to environmental stresses (Dwivedi et al., 2016). Wild species are exposed to harsh climatic conditions, with some of these species inheriting a significantly stronger ability to tolerate severe biotic (Cruppe et al. 2020) and abiotic stress conditions (Zhang et al., 2017). In

order to increase the genetic diversity to develop wheat varieties that can be productive and at the same time tolerate harsh environmental conditions such as heat stress, wild wheat ancestors must be explored.

Wild Emmer wheat (*Triticum dicoccoides*) is one of the earliest domesticated wheat species and is closely related to the modern wheat (Troccoli and Codianni, 2005). Cultivars derived from wild emmer wheat have shown tolerance to drought and heat stress (Nevo, 2014). In addition, the genetic diversity found in wild Emmer wheat can be incorporated into commercial wheat varieties for abiotic stress resilience (Xie and Nevo, 2008). However, the potential of emmer-based genetic diversity for heat tolerance has not been fully researched. Another resource that has potential with higher abiotic stress tolerance is wild wheat *Aegilops speltoides*. These species are diploid and belong to the genus Aegilops, and native to the Fertile Crescent area in western Turkey and west-central Iran (van Slageren, 1994). Friebe et al (2000) developed a set of *Triticum aestivum Aegilops speltoides* chromosome addition lines, originating the Robertsonian translocation lines (RobT's), covering the complete *Aegilops speltoides* genome. Robertsonian translocations are chromosomal rearrangements that result from the fusion of the entire long arms of two acrocentric chromosomes (Zhao et al., 2015). These lines occur when two non-homologous chromosomes get attached, meaning that two healthy pairs of chromosomes, one of each pair adhere together. A gene function may be altered when the translocation joins two otherwise separated genes (Liu et al., 2016). Studies have shown that *Aegilops speltoides* have considerable variability for heat tolerance, based on Pradhan et al. (2012), where in 52 accessions were screened for heat stress tolerance during anthesis until 16 days post-anthesis. The study indicated a wide range where the most susceptible accession

recorded an 82% yield reduction and heat susceptibility index (HSI) of 1.4 whereas the most tolerant accession only recorded a 13% yield reduction and HSI of 0.23 (Pradhan et al., 2012). Similarly, other studies have researched Aegilops species tolerance to post-anthesis heat stress and found that accession TA2899 was tolerant to heat stress, identifying an important source of genetic tolerance (Green et al., 2018). Robertsonian translocations can be incorporated into wheat adapted varieties for improving stress tolerance.

Heat stress during pre-flowering and flowering

Heat stress during pre-anthesis vegetative phase can stimulate the process of cell division and cell elongation rates (Fan et al., 2018). Temperature has the greatest influence on the rate of emergence of leaf, with an increase in leaf elongation rates observed under higher temperatures (Hatfield & Prueger, 2015). This accelerated development during the pre-anthesis period can reduce spike dry weight and consequently number of kernels per area (Fischer, 1985), which is considered a coarse regulator of wheat yield (Slafer et al., 2014). Furthermore, severe heat stress is known to decrease stem growth, resulting in decreased plant height during vegetative growth stage (Prasad et al., 2006). Based on the magnitude and duration of stress, high temperatures not only induce a quicker transition of one developmental stage to the next but also shorten the duration of the developmental stages (Dafni and Firmage, 2000). Sinclair (1994) reported that the development rate of individual organs, such as leaves, and the progress of the whole plant through different ontogenetic stages depends solely on temperature and the duration of crop growth is reflected in the amount of solar radiation that can be intercepted and used to accumulate crop biomass (Sinclair, 1994). In a study designed to quantify the adverse effects of heat on plant yield and duration of phenological stages, with temperature of 36°C

imposed for a period of 8 hours lasting 5, 10, and 15 days at three developmental stages; booting stage, heading, and 6th day after heading (Balla et al., 2019). The study reported that phenology significantly influenced the thousand-kernel weight and reproductive tiller number. The duration of heat stress was the most significant factor determining seed number and seed weight, as well as the grain yield consequently, explaining 51.6% of its phenotypic variance (Balla et al., 2019). The authors concluded that irrespective of the developmental phase, yield related traits gradually deteriorated over time, and even a 5-day heat stress was sufficient to cause significant reduction.

The ideal average daily temperature for anthesis and grain-filling of wheat ranges between 12 and 22°C, and significant reductions in grain yield can occur at higher temperatures (Tewolde et al., 2006). The maximum temperature that wheat can endure during flowering without a decrease in the number of grains is around 31°C, with the sensitive stage reported to last from approximately 20 days before anthesis to 10 days after anthesis (Porter and Gawith, 1999). Temperatures greater than the optimum advance onset of anthesis, leading to fewer spikelets per spikes (McMaster et al., 1997; Rahman et al., 2009). Furthermore, extreme temperatures around anthesis lead to reduced pollen fertility, sterile grains, abnormal ovary development, slower pollen tube growth, resulting in a reduction in seed set (Saini and Aspinall, 1982; Wollenweber et al., 2003; Lobell et al., 2011).

Wheat flowering stage is most susceptible to heat stress as cell division, pollen viability and pollen tube growth are severely affected (Ferris et al., 1998). Wheat is a self-pollinating crop adapted to a wide range of temperate environments (Shewry, 2009). Heat stress reduces number of spikes and florets per plant, and spikelets per spike (Dawson and Wardlaw, 1989; Prasad et al.,

2006; Balla et al., 2019). Wheat response to heat stress (>32°C) around anthesis induces an irreversible reduction in yield by adversely affecting ovary development, pollen, and floret viability (Pradhan et al., 2012). Viable pollen is essential for plant reproduction, and survival of subsequent generations (Saini and Aspinall, 1982). Pollen viability comprises of different aspects of pollen such as germinability, pollen tube growth, and fertilization (Dafni and Frirmage, 2000). Within a floret, anthers and pollen are more sensitive to heat stress than ovules, and floret sterility at temperatures greater than 30°C has been correlated with reduced anther dehiscence (Saini and Aspinall, 1982; Matsui et al., 2000), production of fewer pollen grains (Prasad et al., 2006), and pollen sterility (Sakata et al., 2000). A single day of heat stress (>35°C) at anthesis, reduced grain number and yield by up to 33 and 35%, respectively (Talukder et al., 2014)

Heat stress induced physiological changes

Reactive Oxygen Species (ROS)

Heat stress results in oxidative stress associated with the production of reactive oxygen species (ROS), ultimately affecting the structure of thylakoid membranes, photosystem II (PSII) activity, and chlorophyll (Sharkey et al., 2005; Wahid et al., 2007). These events lead to a decrease in photosynthesis by disrupting chloroplast structure and function, thereby reducing the chlorophyll content and accelerating the loss of green leaf area, which is negatively associated with grain yield in wheat (Dias et al., 2011). Oxidative stress induces lipid peroxidation leading to protein degradation, membrane rupture, and enzyme inactivation (Sairam et al., 2000; Asseng et al., 2011). ROS are produced under both normal and stressful conditions at various locations in the plant, including chloroplasts, mitochondria, peroxisomes, plasma membranes and cell wall

(Asada and Takahashi, 1987). ROS including superoxide radicals (O^{-}_{2}), singlet oxygen ($^{1}O_{2}$), hydroxyl radicals ($OH^{\cdot}$) and hydrogen peroxide (H_2O_2) are produced naturally in cells, but overproduction of these compounds can be harmful for plant growth and development and are generated as toxic products (Esfandiari et al., 2007). In the presence of light, chloroplasts and peroxisomes are the major sources of ROS production, while the mitochondria is the leading producer of ROS under dark conditions (Choudhury et al., 2013).

Reactive Oxygen Species Scavengers

Many studies have indicated that ROS scavenging plays a significant role in protecting plants from heat stress (Wang et al., 2014). Heat stress triggers the production and accumulation of ROS (Mittler et al., 2002) and the detoxification by antioxidant systems is important for protecting plants against heat stress (Asada et al., 2006). The balance between production and quenching of ROS may be disturbed by numerous adverse environmental factors, rapidly increasing intracellular ROS levels which can induce oxidative damage to lipids, proteins, and nucleic acids (Noctor et al., 2002). In order to avoid the oxidative damage, plants raise the level of endogenous antioxidant defense where various components of the defense system involved in ROS scavenging have been manipulated, overexpressed or downregulated to add to the present knowledge and understanding the role of antioxidant systems (Sharma et al., 2010). Enzymatic antioxidants include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), and glutathione peroxidase (GPX) (Wang et al., 2016). Normally, SOD can promote the conversion of O_2^- to H_2O_2 and O_2, POD can disintegrate H_2O_2 to O_2, and CAT, APX, and GPX can dismutase H_2O_2 into H_2O to create a relatively balanced redox environment (Arora et al., 2002; Wang et al., 2014).

Nonenzymatic components of the antioxidative defense system include the major cellular redox buffers ascorbate (ASA) and glutathione (GSH) as well as tocopherols, carotenoids, and phenolic compounds. These compounds interact with numerous cellular components and in addition to crucial roles in defense as enzyme cofactors, these antioxidants influence plant growth and development by modulating processes from mitosis and cell elongation to senescence and cell death (De Pinto and De Gara, 2004). Tocopherols represent a group of lipophilic antioxidants involved in scavenging of oxygen free radicals, lipid peroxyl radicals, and singlet oxygen. Tocopherols are known to protect lipids and other membrane components by physically quenching and chemically reacting with O_2 in chloroplasts, thus protecting the structure and function of PSII (Ivanov et al., 2003). Carotenoids also belong to the group of lipophilic antioxidants and are able to detoxify various forms of ROS (Young et al., 1991). As an antioxidant, they scavenge singlet oxygen to inhibit oxidative damage and quench triplet sensitizer (3Chl*) and excited chlorophyll (Chl*) molecule to prevent the formation of $^1O_2$, to protect the photosynthetic apparatus (Sieferman-Harms, 1987). Carotenoids also serve as precursors to signaling molecules that influence plant development and biotic/abiotic stress responses (Vallabhaneni et al., 2008). Phenolic compounds are diverse secondary metabolites (flavonoids, tannins, hydroxycinnamate esters and lignin) which possess antioxidant properties and are abundantly found in plant tissues (Grace and Logan, 2000). Polyphenols can chelate transition metal ions, and can directly scavenge molecular species of active oxygen, and can inhibit lipid peroxidation by trapping the lipid alkoxyl radical. They also modify lipid packing order and decrease fluidity of the membranes (Arora et al., 2000).

Membrane Damage

Plasma membrane, which surrounds the entire plant cell, plays an important role in interacting with the changing environmental conditions and provides signals necessary for the continual survival of the cell. Lipids form a major portion of the plasma membrane, however under stressful conditions when the level of ROS rise above the threshold value, lipid peroxidation becomes significantly damaging, which is often considered as a key parameter to gauge lipid damage (Apel and Hirt, 2004). Oxidative stress notably increases membrane peroxidation and decreases membrane thermos-stability in wheat (Djanaguiraman et al., 2018). Hydroxyl radicals react with almost all constituents of cells, with continual heat stress causing accumulation of ROS in cell plasma membrane with depolarization of cell membrane, activation of ROS-producing enzymes and triggering programmed cell death (Mittler et al., 2011). Increase in lipid peroxidation under heat stress is extensive with increased ROS production and malondialdehyde (MDA) is one of the final products of peroxidation of unsaturated fatty acids in phospholipids (Halliwell and Cutteridge, 1989).

Post-flowering heat stress on wheat

Leaf Senescence - is the gradual loss of green leaf area that occurs during the post-flowering phase of the crop growth (Nooden, 1998). One of the impacts of heat stress is the reduction in photosynthesis resulting from decreased leaf area expansion, impaired photosynthetic machinery, premature leaf senescence, and associated reduction in wheat production (Ashraf and Harris, 2013; Mathur et al., 2014). Heat stress during grain fill causes early senescence and reduces seed-filling duration, and enhances assimilate remobilization from the source to the sink (Asseng and van Herwaarden., 2003). Heat stress further triggers carbon metabolic changes in wheat (Al-Khatib and Paulsen, 1984; Paulsen 1994), including carbon

assimilation, transpiration, photosynthesis and respiration (Stone, 2001; Mathur et al., 2014; Feng et al., 2014). Studies have shown heat stress induced alterations in carbon balance (increased night respiration versus photosynthesis) and whole-plant metabolic changes in wheat leaves, stems, and spikes (Impa et al., 2019). The plant reaction sites of heat-induced damage are stroma and thylakoid lamellae of chloroplast where carbon metabolism and photochemical reactions occur (Wahid et al., 2007). Heat stress in wheat causes disruption of thylakoid membranes, leading to inhibition of activities of membrane-related electron carriers and enzymes, which eventually results in a reduced rate of photosynthesis (Ristic et al., 2008). Leaf senescence begins early in response to heat stress, particularly when these stresses occur during post-flowering and grain-filling stages. Maintenance of leaf chlorophyll and photosynthetic ability, known as stay-green, is therefore considered a heat tolerance indicator (Fokar et al., 1998). Plants exhibiting the stay-green phenotype are able to maintain green leaf area for longer after anthesis than senescent lines, allowing maintenance of photosynthesis for longer during the grain-filling period (Thomas and Smart, 1993). Stay-green traits can be either functional or non-functional, where plants retain leaf greenness but there is no yield benefit, therefore only functional stay-green traits are of interest for crop improvement (Thomas and Howarth, 2000).

Grain filling affecting yield and yield components

Heat stress between anthesis to grain maturity, reduces grain yield because of shortened duration of resource capture (Farooq et al., 2011). Although there might be a small increase in seed-filling rate but a large decrease in seed-filling duration during heat stress is not compensated by the increase in seed-filling rate, resulting in smaller seed size and decreased yield (Shipler and Blum, 1986; Tashiro and Wardlaw, 1989). For every 1°C above the optimal

growing temperature of 15-20°C, the grain-filling duration was reduced by 2.8 days (Streck, 2005). Similarly, Sofield et al. (1977) determined that the rate of grain growth increases as temperature increases, but this apparently depends on whether the number of grains per spike is reduced. In spikes where the number of grains are less affected by elevated temperature, the rate of grain growth is reduced. Therefore, an increase in the grain-filling rate does not compensate for reduced grain-filling period with temperatures greater than 30°C (Sofield et al., 1977; Prasad et al., 2008). Yin et al. (2009) described that the grain-filling period could be reduced by 3-12 days as a result of heat stress (>30°C) (Yin et al., 2009). Other studies have indicated that heat treatment can reduce the grain-filling period by 45-60% (Yang et al., 2002; Shah and Paulsen, 2003). Nonetheless, with 35/25^{o}C exposure, substantial amount of damage was induced to the photosynthetic apparatus which resulted in diminishing both source activity and sink capacity, indicating that a combined impact lead to a reduction in productivity (Harding et al., 1990). After characterizing 12 winter wheat varieties, Bergkamp et al. (2018) concluded that post-flowering heat stress induced early senescence, recorded a shorter grain-fill period, leading to significant yield reduction. Bergkamp et al. (2018) reported that percent reduction in yield ranged from 6 to 51% under severe heat stress imposed during grain filling, under controlled environments and 2 to 27% under field conditions.

The rate and amount of dry matter accumulation and the growth of harvestable organs of a crop is determined by assimilate supply of green leaves (source) and the capacity of organs to store assimilates (sink). Alterations in source-sink balance due to heat stress during these processes can affect growth, yield, and yield components (Stratonovich and Semenov, 2015). Heat stress during the grain-fill period decreases leaf chlorophyll content and accelerates

senescence (Yang et al., 2002), leading to a shorter grain-filling duration with an ultimate decrease in individual grain weight and yield (Zhao et al., 2007). In wheat, both grain weight and grain number appear to be sensitive to heat stress, as the number of grains per spike at maturity declined with increased temperature (Ferris et al., 1998). The effects of heat stress on both the number and grain size varies depending on the growth stage encountering the stress. For example, increasing temperatures ranging from 14 to 22^{o}C between spike initiation and anthesis enhanced the rate of development of the spike but reduced spike dry weight (Fischer, 1985). Temperatures above 30°C during floret development may cause complete sterility (no seed-set) in wheat depending on the tolerance of the genotype (Kaur and Behl, 2010). High day or night temperature (31/20°C) is shown to result in reduced wheat grain size due to changes in cell endosperm, resulting in lower yields (Dias et al., 2008).

The yield reduction of wheat under heat stress is associated with lesser grain number per spike and smaller grain size (Gibson and Paulsen, 1999). Stone and Nicolas (1998) reported that a 3-day period of extreme high temperature (40°C) after anthesis reduced grain number and weight, resulting in a large number of deformed grains (Stone and Nicolas, 1998). Even a single day of heat stress can cause serious damage to grain yield and yield components (Rahman et al., 2009). Heat stress in wheat reduces the number of grains per spike, leading to lower harvest index (Lukac et al., 2011). Gibson and Paulsen reported that grain yield of hard red winter wheat cultivar Karl 92 was reduced by 78%, kernel number by 63%, and kernel weight by 29% at 35/20°C from 10 days after anthesis until maturity under controlled environment conditions (Gibson and Paulsen, 1999). Similarly, Hutsch et al. (2019) concluded that long-term heat stress shortened the developmental phases of grain fill and maturity, causing a 19 to 41% decrease in total above

ground biomass, which is usually positively related to grain fill in wheat (de Oliveira Silva et al., 2020). At grain fill and maturity, the reductions in total shoot biomass resulted in grain yield reduction by 77% and 58% as well as kernel number per spike decreased by 83% to 75% during grain fill and at maturity, respectively (Hutsch et al., 2019).

Grain starch and protein dynamics under heat stress

Wheat is one of the main food crops of the world and it is an important source of protein and carbohydrates for millions of people (Asseng et al., 2011). Wheat quality is relevant as it has a large effect on the market value and consumer acceptance (Zhao et al., 2008). High temperatures above a critical threshold have major negative effects on grain yield and grain starch composition (Dias et al., 2008). Heat stress during grain-filling affects starch synthesis and accumulation due to the sensitivity of key starch metabolism enzymes such as ADP glucose pyrophosphorylase and starch synthase in developing wheat kernels (Jenner, 1994) while protein synthesis is less affected (Bhullar and Jenner, 1985; Dupont et al., 2006; Impa et al., 2020). In cereal endosperm including wheat, protein deposition is initiated around 8-10 days post-anthesis and reaches its peak by at about 20 days post-anthesis, whereas starch deposition is initiated around the same time but can take up to 45 days to complete deposition (Emes et al., 2003). Due to a much shorter window of protein accumulation, reduced grain-fill duration under heat stress exposure does not affect protein in wheat grains, compared to a significant reduction in starch (Herzog & Stamp, 1983; Impa et al., 2020). On exposure to heat stress, the yield losses are primarily caused by a reduction in starch content, since over 65% of wheat kernels are composed of starch (Jenner, 1994; Barnabas et al., 2007). Starch accumulation is correlated with the sucrose content of the kernels and activity of sucrose synthase and other starch metabolism enzymes

that play an important role in starch synthesis (Yang et al., 2004). This suggests that low sucrose content and a decline in the enzymatic activity involved in starch synthesis are responsible for the reduction in starch accumulation in wheat exposed to heat stress. According to Labuschagne et al (2009), the lower ratio of starch in the endosperm in response to heat stress was associated with heat inactivation of starch synthase, the key enzyme in the starch biosynthesis pathway (Labuschagne et al., 2009).

Rationale and objectives of the study

Wheat producers in the Great Plains of the United States face limited agronomic options for managing terminal heat stress during the growing season, with the best practice being the selection of appropriate heat tolerant cultivars. Studies indicate that yield potential and heat tolerance are negatively correlated (Tack et al., 2015). The current wheat cultivars including those adapted to the Great Plains region are bred for higher yield, and hence presents the opportunity to improve their resilience to heat stress. A recent study involving seven popular hard red winter wheat varieties grown in Kansas and the Great Plains, demonstrated a wide range in susceptibility with just one variety (SY Monument) having high level of tolerance to terminal heat stress (Bergkamp et al., 2018). Hence, it is highly important and timely to explore and exploit the ability of wild wheat accessions that can endure extreme level of stresses including heat (Zhang et al., 2017), to help develop wheat varieties that can sustain productivity under future warmer climate. Considering the challenges mentioned above and the opportunity presented by the wild wheat genetic resources, two studies were conducted to characterize 28 wild emmer wheat *Triticum diccocoides* and 20 RobT's developed from *Aegilops speltoides*, for their tolerance to heat stress during flowering and grain-fill stages using controlled environment chambers. These studies were

conducted to: 1) Capture genetic diversity for post-flowering heat stress in wild wheat accessions and translocation lines; 2) Determine physiological and yield related responses; and 3) Identify most promising accessions or translocation lines that can be utilized by the wheat breeding teams to develop heat tolerant wheat varieties for the future.

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Chapter 2- Physiological characterization of wild emmer wheat (*Triticum diccocoides*) for heat stress tolerance under controlled environment chambers

Abstract

Heat stress during flowering and grain-filling stages is a key environmental factor negatively affecting winter wheat yields in Kansas. Optimal temperature for wheat anthesis and grain fill ranges between 12 and 22°C and temperatures greater than 30°C can induce heat stress damage. Wild wheat with the ability to thrive under harsh environments is hypothesized to house traits that can induce greater heat stress tolerance. A controlled environment chamber study was carried out to characterize 28 wild wheat accessions along with two adapted wheat checks (Overley and C306) for flowering and post-flowering heat stress response. Plants were grown at 25/15°C day/night temperature until the start of flowering. At first signs of external appearance of anthers on the main tiller, half of the plants per accession were exposed to high day temperature 35/15°C for 20 days. Physiological parameters such as gas exchange and chlorophyll fluorescence were recorded at 5 and 20 days after stress. At maturity, plants were harvested, and yield parameters were recorded. Temperature had a significant effect on net CO_2 assimilation, seed number and seed weight. Heat stress induced on average 28% and 22% reduction in grain weight and grain number per plant, respectively, compared to the control. The eight best performing wild wheat accessions along with Overley were selected and validated for their tolerance to heat stress in a second experiment. Heat stress induced earlier maturity ranging from one to 13 days. Among the accessions, TA1020 was the standout that retained higher seed weight and number, higher transpiration, and photosynthetic rate under heat stress exposure. Overall, the study indicated low to moderate levels of heat tolerance in the tested accessions of *Triticum diccocoides*.

Introduction

Wheat (*Triticum aestivum* L.) is the most important cereal crop in temperate regions and with burgeoning population, demand for wheat is significantly increasing across the world (Shewry et al., 2015). Winter wheat is the third most cultivated field crop in the United States and accounts for 48 million metric tonnes grown on about 19 million hectares of cropland (USDA ERS, 2019). Global mean surface temperature has increased by 0.8°C from 1880 and has nearly doubled at a rate of 0.2°C increase per decade (IPCC, 2014). This increase in temperature poses a significant threat to global crop production including wheat (Zhao et al., 2017). The optimal temperature during wheat anthesis and grain development ranges from 12 to 22°C and temperatures greater than 30°C can induce significant yield loss. Wheat is highly sensitive to heat stress during flowering and grain-filling stages with yields reduced by 6% for every 1°C increase in temperature (Zhao et al., 2017). Heat stress commonly leads to yield reduction and area abandonment in Kansas (Lollato et al., 2020), which leads to significant economic losses for the US, as the state of Kansas accounts for 15% of wheat grown in the United States, with a value of 2.8 billion US dollars (Tack et al., 2015).

Heat stress disrupts seed germination, vegetative growth, tiller production, dry matter partitioning, reproductive organ development, reproductive processes (Prasad et al., 2011; Sehgal et al., 2018), and grain quality (Gooding et al., 2003). Wheat exposed to high-temperature experiences accelerated senescence, increased rate of chlorophyll loss in leaves, lower CO_2 assimilation, reduced quantum yield of photosystem II and increased photorespiration (Farooq et al., 2011, Aiqing et al., 2018). With the predicted increase in frequency and intensity of heat stress episodes under changing climate, the above processes can be further aggravated leading

a significant increase in yield loss. Hence, it is imperative to develop wheat cultivars with higher tolerance to heat stress.

Wild emmer wheat through natural selection has acquired the ability to tolerate harsh environmental conditions which could be exploited to improve post-anthesis heat tolerance in wheat (Merchuk-Ovnat et al., 2016). Wild emmer wheat (*Triticum turgidum* ssp. *diccocoides*) is the allotetraploid (2n = 4x = 29; genome BBAA) progenitor of cultivated wheat (Feldman, 2001). Studies have indicated that the introduction of alien chromosome segments from wild relatives into wheat can increase tolerance to drought (Ehdaie et al., 2003), high temperatures (Pradhan and Prasad, 2015), salinity (Dvorak et al., 1998), and water-logging (McDonald et al., 2001). Similarly, the introduction of alien chromosomal segments from wild relatives into wheat has improved pest resistance and yield (Ehdaie et al., 2003). Emmer wheat, one of the first wheat species domesticated, is considered to house significant diversity for tolerance to abiotic stresses (Pradhan et al., 2015).

Considering the environmental challenges mentioned above and negative effects of heat stress in wheat, this study provides an opportunity to explore the genetic diversity of wild emmer wheat to help boost the tolerance in current popular cultivars and promising lines in the breeding pipeline. We exposed a collection of 28 *Triticum turgidum* ssp. *diccocoides* accessions along with two adapted wheat check lines to heat stress during flowering and grain-filling stages using controlled environment chambers. Two experiments were conducted to phenotype and validate heat stress responses in these lines and the specific objectives were to: 1) Capture the genetic diversity for post-flowering heat tolerance and identify heat-tolerant wild wheat accessions, and

2) Assess the physiological and yield-related traits inducing heat tolerance in wild wheat compared to adapted checks.

Materials and methods

The experiments were carried out in controlled environment growth chambers, located in Department of Agronomy, Kansas State University, Manhattan, Kansas.

Experiment 1

This experiment involved 28 wild wheat *Triticum turgidium* subsp*. dicoccoides* accessions along with two adapted wheat checks Overley and C306, grown under two temperature treatments (control [25 °C] and heat stress [35 °C]) (Table 1). The Overley variety is widely grown in the state of Kansas. Overley is a semi-dwarf variety with medium height, very good straw strength, and excellent yield potential with early maturity. The C306 is a long duration variety which is widely used in India for its high yield performance and baking quality. 50 seeds of each of the 30 wheat accessions were sown in 30.5×61 cm flat seed trays filled with Sunshine Metro-Mix 380 potting mix (Sun Gro Horticulture, Agawam, MA) and placed in growth chambers at 25 °C/15 °C maximum day/night temperature. After most seeds had germinated, the seed trays were transferred to a vernalization chamber maintained at 5 °C for 6 weeks. Following vernalization, two seedlings were transplanted into 1.6 L pots (10×24 cm, MT49 Mini-Treepot) filled with Sunshine Metro-Mix 380 potting mix. Each pot received 5 g of Scotts Osmocote classic (14-4-14 of N-P-K) and 0.5 g of Scotts Micromax Micronutrients (Hummert International, Topeka, KS) at the time of transplanting.. After transplanting, the pots were kept in trays and placed in controlled environment chambers maintained at 25/15 ^{o}C maximum day/minimum night temperatures. Plants were thinned out down to one plant per pot at five leaf stage. The plants

were well-watered throughout the experiment by maintaining about one cm layer of water in the trays holding the pots. Marathon 60 WP with active ingredient Imidacloprid (insecticide, OHP) was applied to the soil after two weeks of transplanting to avoid infestation by sucking pests such as brown wheat mites (*Petrobia latens*). These accessions were obtained from Prof. Gill's team and were maintained until the transplanting stage by following established methods that their team has developed, to ensure high survival percentage of wild wheat accessions.

The main tiller in each plant was tagged on the day anthesis began and half of the plants were transferred to three independent heat stress chambers maintained at 35/15 ^{o}C maximum day/minimum night temperatures. One plant per genotype were placed in each of the three replicate chambers, individually as they reached the target stage. The other half of the plants remained in three control treatment chambers maintained at 25/15 ^{o}C maximum day/minimum night temperatures. Both control and heat stress chambers were maintained at 16/8 h (day/night) photoperiod, with 900 -1000 µmol m^{-2} s^{-1} light intensity at 5 cm above the canopy and 70% relative humidity (RH). Maximum and minimum temperatures were maintained for 8 h in all growth chambers with a transition period of 4 h between maximum day and minimum night temperature (Fig. 1). Temperature and RH at canopy level were recorded every 30 minutes using HOBO UX 100-011 temperature/RH data loggers (Onset Computer Corp., Bourne, Massachusetts) in all growth chambers. As wild wheat plants grow up to or over one meter high and are prone to lodging, a tall bamboo stick was placed in the corner of the pot with a spring craft clip with a galvanized wire to support the plant stand. The spikes of wild wheat plants approaching maturity were bagged with perforated brown natural Kraft bread bags (Mr. Take out Bags, Pittsburg, PA) to avoid the shattering of seeds. Days to physiological maturity was recorded from the first day

of anthesis to physiological maturity. At physiological maturity (Zadoks growth scale 9-ripening [92-Grain hard, not dented by thumbnail]) plants were hand harvested, and spikes, leaves and shoots were separated.

Experiment 2

Based on the performance of the 28 wild wheat accessions in experiment 1, eight wild wheat *Triticum turgidium* subsp*. dicoccoides* accessions and a check line Overley were selected for experiment 2, to validate findings from experiment 1. This study was carried out in the same growth chambers, following the same plant growth conditions, management, and temperature treatments similar to experiment 1.

Measurements

Physiological Traits

Gas Exchange

Gas Exchange measurements were taken at five and 20 days after anthesis or stress imposition using a portable photosynthesis system Li-6400XT (LI-COR Biosciences, Lincoln, Nebraska). Gas exchange measurements were recorded between 1000 and 1100 h. The CO_2 concentration in the leaf chamber of portable photosynthesis system was set to 400 µmol mol^{-1}, with a flow rate of 500 µmol s^{-1} and a light intensity of 1000 µmol m^{-2} s^{-1} of photosynthetically active radiation supplied by red-blue light-emitting diode. The block temperatures in the photosynthesis system were set at 25 and 35°C in control and heat stress treatments,

respectively. The measurements were recorded on flag leaves of the main tiller from three replicate plants that were tagged on the first day of anthesis.

Chlorophyll Fluorescence (Fv/Fm)

Chlorophyll fluorescence was measured at five and twenty days after anthesis or stress imposition using a handheld chlorophyll Fluorometer OS30p+ (OPTI-Sciences Inc., Hudson, New Hampshire). The fluorescence measurements were recorded between 1000 and 1100 h, from 20-minute dark-adapted flag leaves. The maximum photochemical efficiency of photosystem II (Fv/Fm) were recorded from dark adapted flag leaves at a light pulse intensity of 3000 µmol m^{-2} s^{-1} and pulse duration of 1s.

Agronomic traits

Days to first anthesis (Z61) and physiological maturity (Z92) were recorded for each genotype per replication and treatment. At physiological maturity, the plants were hand harvested and total number of tillers per plant, total number of productive spikes per plant, and total number of sterile spikes per plant were recorded. The harvested plants were separated into spikes and tillers and oven dried at 60°C for seven days to obtain the biomass weight. Oven dried spikes were hand threshed to obtain yield-related parameters including total grain weight, and grain number per plant.

Statistical analysis

The experiments were laid out in a split plot randomized complete block design with temperature as the main plot factor and genotype as the sub-plot factor with three independent

chamber replications for control, three and two independent chamber replications for heat stress in experiments 1 and 2, respectively. Two-way analysis of variance (ANOVA) for all the measured parameters was performed using PROC GLM procedure in SAS software (Version 9.4, SAS Institute). Replication, temperature, and genotypes were used as class variables, replication and replication x temperature as random effects, and all other variables as fixed effects. Means were separated using LSD (least significant difference) test at p=0.05. Number of seedlings obtained from Prof. Gill's team for experiment 1 ranged between 1 to 3 for each treatment and hence a comparative Z score was considered appropriate, to capture the variation and to visually represent the spectrum of genetic variation. The standardized *Z* scores for each genotype was estimated for various traits under control and heat stress. The *Z* scores for individual genotype was estimated using the formula

$$\text{Z score} = \frac{\text{Grand mean of all the genotypes} - \text{Mean of the individual genotype}}{\text{Standard deviation of all the genotypes}}$$

The computed *Z* scores were used to classify genotypes based on the hierarchical algorithm using MORPHEUS (https://software.broadinstitute.org/morpheus/index.html).

Results

Experiment 1

Grain yield and related traits

In experiment 1, seed number and seed weight were significantly ($p<0.001$) affected by temperature (T) and genotype (G) but not by their interaction (Table 2). On average, seed number and seed weight across accessions was reduced by 22% and 28%, respectively, under heat stress compared to control (Table 2). Based on the Z score classification, as expected, the

check lines Overley and C306 outperformed the wild wheat accessions in terms of yield parameters (Fig. 2). Among the wild wheat accessions TA1108, TA1020, TA1465, TA90 and TA1005 were among the better performers classified as heat-tolerant while accessions TA1464, TA56, TA1390, TA74 and TA130 were the poor performers that had lowest seed number and weight under heat stress compared to control.

Physiological Traits

Net CO_2 assimilation rate

Net CO_2 assimilation rate was significantly ($p<0.001$) affected by T, G and T x G interaction at both five and twenty days after stress (Table 2). The negative effect of heat stress on net CO_2 assimilation was more significant at 20 DAS, with an average of 42% reduction compared to control. Wild wheat accessions TA1077, TA1390, TA74 and TA1020 maintained better assimilation rate both under heat stress and control treatments (Fig. 3).

Maximum quantum yield of PS II (Fv/Fm)

Maximum quantum yield of PSII was significantly affected by T ($p <0.001$) at five days after stress but not by G and T x G interaction. Whereas, at twenty days after stress maximum quantum yield of PSII was significantly ($p<0.001$) affected by T, G and T x G interaction. Heat stress significantly reduced the efficiency of PSII by 6% and 13% at 5 and 20 DAS, respectively (Table 2). Wild wheat accessions TA1195, TA1000, TA1059, TA130, TA1171, TA1390, TA123 and TA1398 recorded better efficiency of PSII compared to other accessions under both the temperature treatments (Fig. 4).

Experiment 2

Days to physiological maturity

Number of days between anthesis and physiological maturity was significantly affected by temperature and genotype, but not by their interaction (Table 3). When averaged across accessions, heat stress induced significantly earlier maturity (c.a., 7 days) compared to control grown plants (Table 3). Among the genotypes, TA1191 under heat stress matured 13 days earlier than its respective control, followed by TA1411 and TA1020 with 10 days earlier maturity under heat stress compared to their respective controls (Fig. 5). Meanwhile, wild wheat accession TA1153 did not show any variation in maturity between the temperature treatments (Fig. 5).

Grain yield and related traits

Seed weight and seed number were significantly ($p<0.001$) affected by G but not by T or T x G (Table 3). The check Overley showed higher seed weight and number than all the tested wild wheat accessions under both temperature treatments (Fig. 6 A & B). Accessions, TA1108 (39%), TA1153 (24%) and TA1005 (20%) recorded the highest yield reduction (seed weight) under heat stress compared to control (Fig. 6A). The same three wild wheat accessions TA1108 (25%), TA1153 (16%) and TA1005 (11%) also recorded the highest reduction in seed number under heat stress compared to control (Fig. 6B).

Biomass

Plant biomass (shoot + leaf weight) was significantly ($p<0.05$) affected by T, G and T x G interaction (Table 3). Wild wheat accessions TA1108 (28%) and TA1153 (24%) recorded the

highest reduction in biomass under heat stress compared to control, with the latter differing significantly (Fig. 6C).

Net CO_2 assimilation rate

Net CO_2 assimilation rate at 5 and 20 DAS was significantly ($p<0.001$) affected by T, G, and T x G (Table 3). When averaged across genotypes, heat stress induced a 17% reduction in net CO_2 assimilation rate under heat stress compared to control at 5 DAS (Table 3). Among the accessions almost all the wild wheat accessions recorded a significant reduction in net CO_2 assimilation rate compared to control except TA1020, TA1191 and TA90 (Fig. 7A). Meanwhile, no significant reduction was noticed in net CO_2 assimilation measured at 20 DAS under heat stress compared to control (Table 3). Net CO_2 assimilation reduced drastically at 20 DAS compared to 5 DAS. Among the wild wheat accessions TA1153 and TA1411 recoded similar net CO_2 assimilation rate between the temperature treatments at 20 DAS (Fig. 7b). Accessions TA1020, TA1108 and TA1191 under control conditions were highly sensitive to powdery mildew, which prevented recording a reliable net assimilation rate at 20 DAS (Fig. 7b).

Transpiration rate

Transpiration rate was significantly affected by G ($p< 0.001$) and T X G ($p <0.001$) at 5 days after stress (Table 3). Among the wild wheat accessions TA1005, TA1020 and TA1411 recorded similar transpiration between temperature treatments, while TA1465, TA1020 and TA90 showed significantly higher transpiration rate under heat stress compared to control. TA1108, TA1153, TA1191 and Overley recorded a significantly lower transpiration rate under heat stress compared to control (Fig. 8B). At 20 DAS, transpiration rate was significantly ($p<0.01$) affected by T, G and

T x G (Table 3). On average, heat stress significantly reduced transpiration at 20 DAS compared to control (Table 3). While all the wild wheat accessions along with Overley recorded a significantly lower transpiration rate under heat stress compared to control (Fig. 8).

Maximum quantum yield of PS II (Fv/Fm)

Maximum quantum yield was significantly affected by T ($p < 0.001$) but not by G and T x G interaction at 5 DAS (Table 3). All the genotypes showed similar response between the treatments at 5 DAS (Fig. 9A). At 20 DAS, maximum quantum yield was severely reduced (in most cases were undetectable by fluorometer OSP30^{+}) in all the wild wheat accessions exposed to heat stress compared to control except in TA1411 and Overley (Fig. 9B).

Discussion

Exploiting yield components to minimize heat stress damage

Over generations of natural selection, wild wheat acquires tolerance to a range of biotic and abiotic stresses, including heat stress (Zhang et al., 2017). Considering the significant negative impact of heat stress currently documented or predicted to impact future wheat productivity, exploring and exploiting higher heat stress tolerance in wild wheat could be an effective strategy to cope with the increased food, feed, and fiber needs of a growing population. Tolerance in wild wheat accessions is primarily aimed at survival and not productivity and hence a direct comparison of grain yield between wild accessions and check varieties is not feasible. However, the relative change between heat and control conditions is of interest. Accessions such as TA1020 was tolerant to post-flowering heat stress (Fig. 2), and recorded higher seed weight

and seed number, and maintained the shoot biomass under heat stress (Fig. 6). Until recently, achieving higher seed number and weight in the same genetic background was considered physiologically challenging due to compensatory mechanisms (Sadras et al., 2020). However, Bheemanahalli et al. (2018) demonstrated that this is achievable by extensively testing a diverse set of spring wheat genotypes exposed to heat stress during flowering and grain-filling stages. Similarly, the scenario presented by TA1020 with increased seed weight and numbers without much change in shoot biomass indicates that the phenomenon observed in spring wheat could also be achieved in winter wheat using some of the wild wheat accessions, particularly under stress conditions.

Another interesting aspect with TA1020 is the increase in seed number even with heat stress imposed from start of flowering for 20 days, which would encompass key physiological processes such as pollen germination, pollen tube growth and fertilization. These series of processes leading to fertilization and seed-set are known to be highly sensitive to heat stress in wheat (Aiqing et al., 2018; Bheemanahalli et al., 2019; Jagadish, 2020). TA1020 can be a potential resource for further detailed characterization of heat stress resilience mainly on the sensitive reproductive organ viability that can help minimize heat stress impact on reduced seed numbers, with heat stress coinciding during flowering. Identifying germplasm that have higher tolerance to these processes separately in different genetic backgrounds will make it extremely challenging to pyramid these traits through breeding approaches. Having, tolerance to the entire series of these highly sensitive parameters in the same genetic background will make it considerably easier for breeding programs to develop varieties that can maintain seed numbers under current and future warming scenarios.

Enhance source efficiency to support productivity under heat stress

Heat stress is known to significantly advance senescence and enhance the rate of loss of chlorophyll, affecting the steady supply of assimilates required to meet the sink demand during grain filling (Kumar et al., 2010; Jagadish et al., 2015; Hutsch et al., 2018). Functional stay green requires additional exploration to ensure the current photosynthesis complements the carbohydrates supplied through the stem reserves under heat stress conditions (Macduff et al., 2002; Gregersen, 2011; Thomas and Ougham, 2014). Four wild wheat accessions (Fig. 3) were highly tolerant and maintained the assimilation rate even under extended heat stress conditions, compared to drought tolerant improved checks. One among that was TA1020 which retained photosynthetic rate even in the validation experiment wherein the check Overley recorded a significant reduction (Fig. 7). Albeit non-significant, an increase in transpiration in TA1020 (Fig. 8), could have helped the carbon flow through gas exchange and maintained a more favorable microclimate through transpiration mediated cooling under heat stress conditions helping extend the photosynthesis for a longer duration (Julia and Dingkuhn, 2013). Accessions TA1108 and TA1153 with the highest transpiration (5 DAS; Fig. 8) also translated to similar increase in photosynthesis (Fig. 7), presenting a scenario wherein these traits from these accessions could be effectively utilized to enhance productivity under water sufficient conditions.

In summary, the tested wild wheat accessions did not lead to a striking improvement in yield components under heat stress compared to the checks, indicating that the level of tolerance to be moderate in tested accessions. Among the accessions TA1020 was the standout accession that retained higher seed weight and number, higher transpiration, and photosynthetic rate under heat stress exposure. Additionally, the potential of TA1020 to minimize heat stress effect

and retain grain numbers under heat stress, makes it a promising candidate to be included into wheat breeding programs. However, to obtain a significantly higher heat tolerance, additional sources of diversity among wild wheat relatives need to be explored.

Table 1. List of wild wheat accessions and checks grown in controlled environment chambers in experiment 1 and 2. The accessions that were used in both the experiments are in bold.

Genotype	**Species**	**Country of origin**
C306	*Triticum aestivum* L.	India
Overley	*Triticum aestivum* L.	USA
TA1000	*Triticum turgidum* subsp. d*icoccoides*	England
TA1005	*Triticum turgidum* subsp. d*icoccoides*	England
TA1020	*Triticum turgidum* subsp. d*icoccoides*	Turkey
TA1059	*Triticum turgidum* subsp. d*icoccoides*	Syria
TA1077	*Triticum turgidum* subsp. d*icoccoides*	Turkey
TA1108	*Triticum turgidum* subsp. d*icoccoides*	Turkey
TA1121	*Triticum turgidum* subsp. d*icoccoides*	Turkey
TA1153	*Triticum turgidum* subsp. d*icoccoides*	Lebanon
TA1171	*Triticum turgidum* subsp. d*icoccoides*	Israel
TA1187	*Triticum turgidum* subsp. d*icoccoides*	Jordan
TA1191	*Triticum turgidum* subsp. d*icoccoides*	Jordan
TA1195	*Triticum turgidum* subsp. d*icoccoides*	Jordan
TA120	*Triticum turgidum* subsp. d*icoccoides*	Syria
TA123	*Triticum turgidum* subsp. d*icoccoides*	Syria
TA130	*Triticum turgidum* subsp. d*icoccoides*	Israel
TA1390	*Triticum turgidum* subsp. d*icoccoides*	Israel
TA1398	*Triticum turgidum* subsp. d*icoccoides*	Israel
TA1411	*Triticum turgidum* subsp. d*icoccoides*	Israel
TA1415	*Triticum turgidum* subsp. d*icoccoides*	Israel
TA1434	*Triticum turgidum* subsp. d*icoccoides*	Jordan
TA1459	*Triticum turgidum* subsp. d*icoccoides*	Syria
TA1464	*Triticum turgidum* subsp. d*icoccoides*	Israel
TA1465	*Triticum turgidum* subsp. d*icoccoides*	Israel
TA1471	*Triticum turgidum* subsp. d*icoccoides*	Israel
TA54	*Triticum turgidum* subsp. d*icoccoides*	Israel
TA56	*Triticum turgidum* subsp. d*icoccoides*	Israel
TA74	*Triticum turgidum* subsp. d*icoccoides*	Lebanon
TA90	*Triticum turgidum* subsp. d*icoccoides*	Turkey

Figure 1. Average air temperature inside the controlled environment chambers under control and heat stress treatments in experiment 1. Air temperature at the canopy level was recorded every 30 min using HOBO UX 100-011 data loggers placed at 5 cm above the canopy.

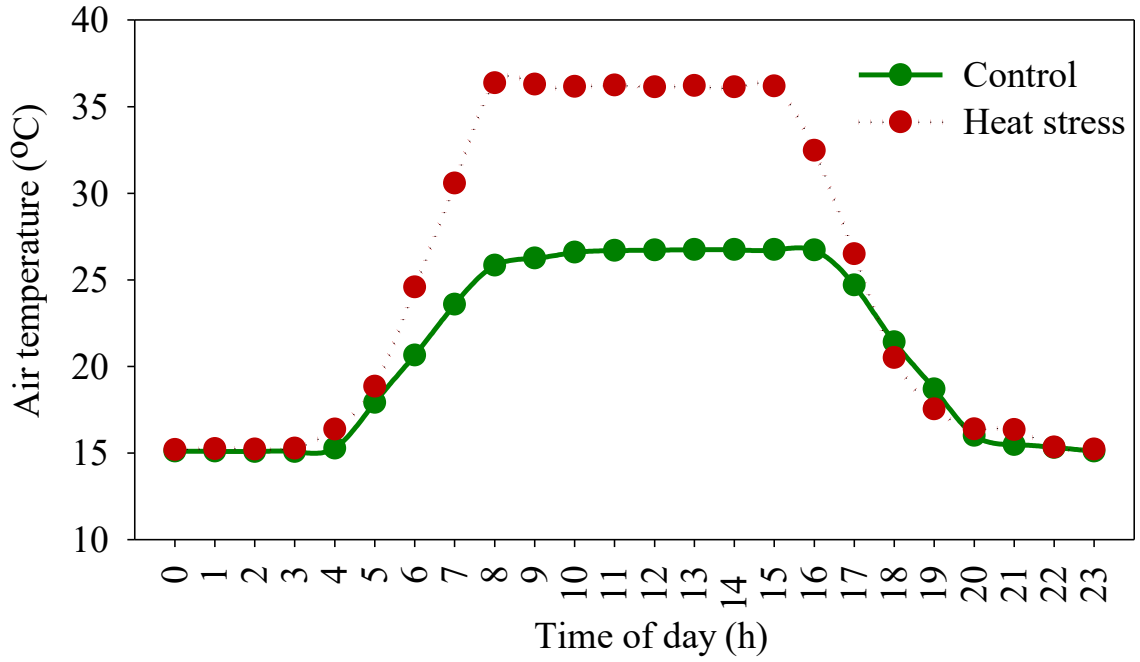


Table 2. Probability of effects of treatment (T), genotype (G), and T x G interactions on physiological traits and yield parameters in experiment 1. Values are averages across 28 wild wheat accessions and two drought-tolerant wheat check varieties. Probability values highlighted in red are significant. Separation of means was carried out using the LSD test ($P < 0.05$). Means with different letters are significantly different at 5% LSD.

Trait	**Treatment (T)**	**Genotype (G)**	**(T x G)**	**Control**	**Heat Stress**
Seed number (plant^{-1})	<0.001	<0.001	0.2194	266.4a	207.2b
Seed weight (g plant^{-1})	<0.001	<0.001	0.3036	7.4a	5.3a
Assimilation rate (µmol m^{-2} s^{-1}) at 5 DAS	<0.001	<0.001	<0.001	21a	18a
Assimilation rate (µmol m^{-2} s^{-1}) at 20 DAS	<0.001	<0.001	<0.001	14.2a	8.3b
Maximum quantum yield of PSII (Fv/Fm) at 5 DAS	<0.001	0.8446	0.9660	0.81a	0.76b
Maximum quantum yield of PSII (Fv/Fm) at 20 DAS	<0.001	0.0040	0.0028	0.77a	0.67b

Figure 2: Z score-based classification of genotypes for seed number and seed weight in response to heat stress in experiment 1. Z score for each genotype was estimated for each trait under control and heat stress and calculated by dividing the difference between the grand mean of all the genotypes and individual genotype mean by standard deviation of all the genotypes.

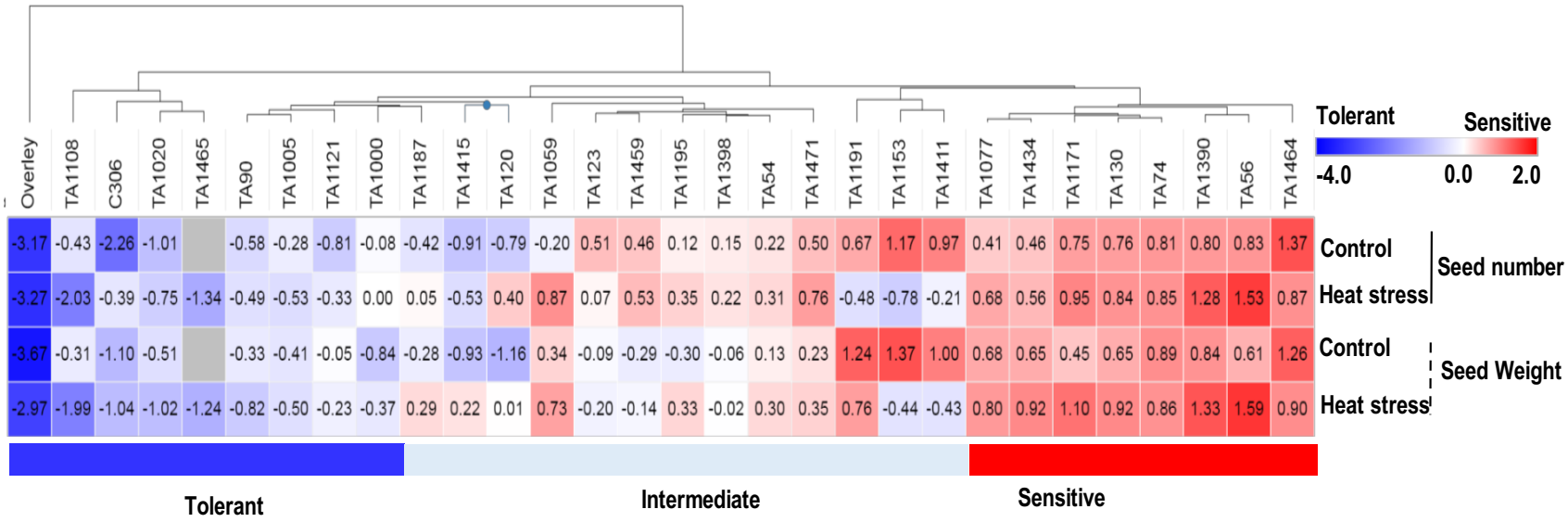


Figure 3: Z score-based classification of genotypes for Net CO_2 assimilation rate at 5 and 20 days in response to heat stress in experiment 1. Z score for each genotype was estimated for each trait under control and heat stress and calculated by dividing the difference between the grand mean of all the genotypes and individual genotype mean by standard deviation of all the genotypes.

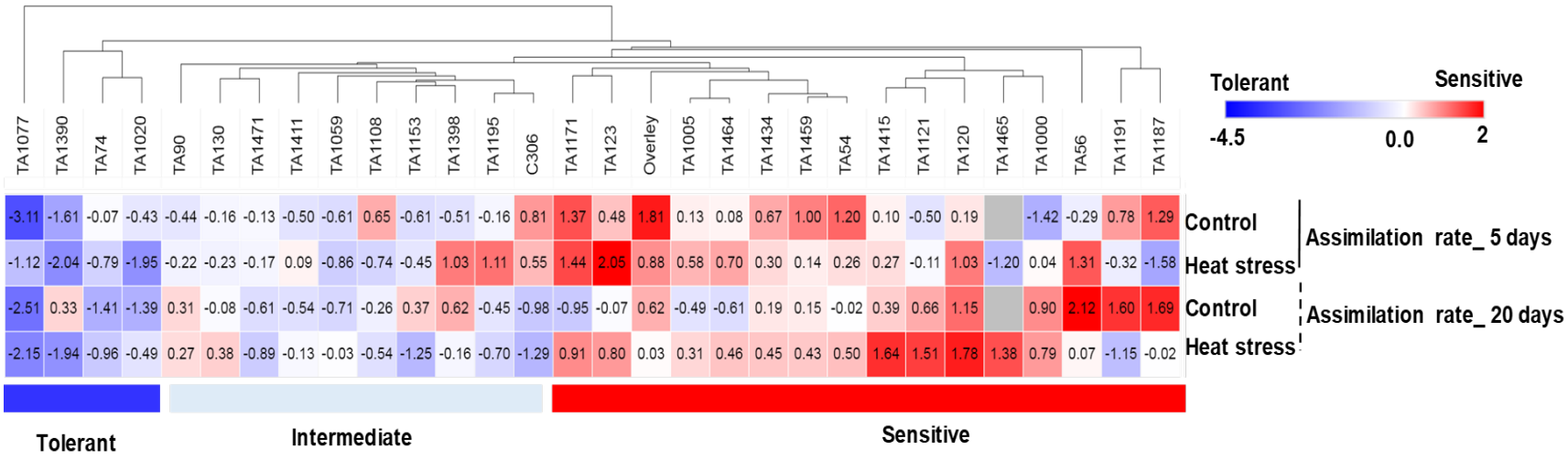


Figure 4: Z score-based classification of genotypes for maximum quantum yield (Fv/Fm) 5 and 20 days in response to heat stress in experiment 1. Z score for each genotype was estimated for each trait under control and heat stress and calculated by dividing the difference between the grand mean of all the genotypes and individual genotype mean by standard deviation of all the genotypes.

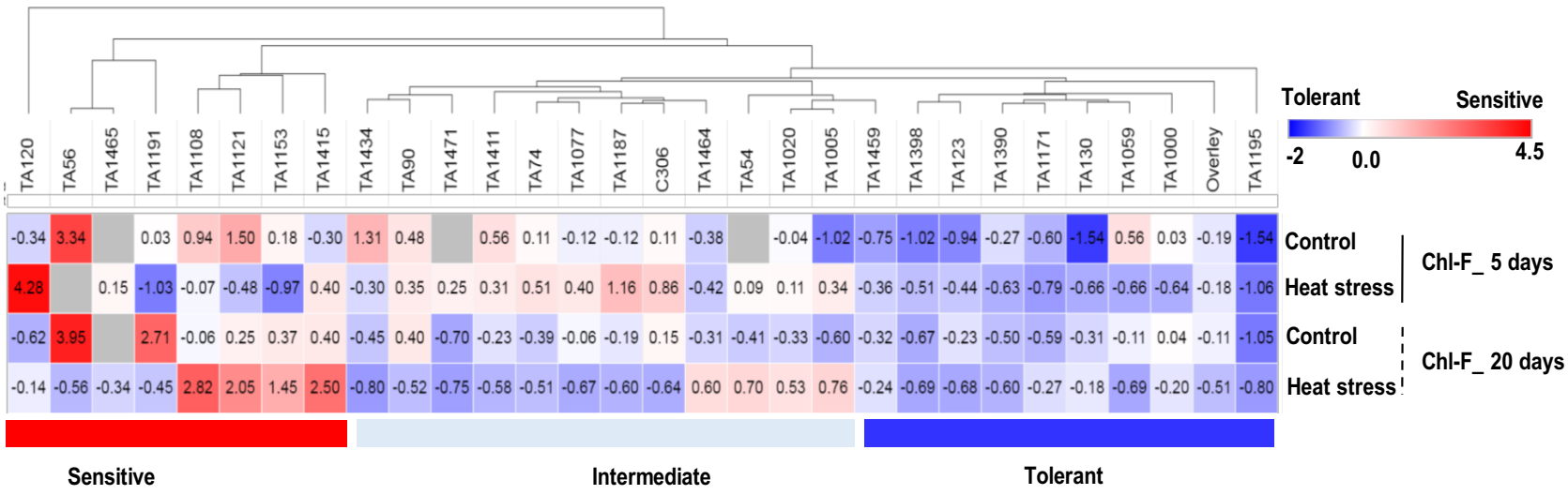


Table 3. Probability of effects of treatment (T), genotype (G), and T x G interactions on physiological and yield parameters in controlled environment experiment 2. Values are averages across eight wild wheat accessions and one drought-tolerant wheat check variety for yield parameters and physiological traits. Probability values highlighted in red are significant. Means with different letters are significantly different between temperature treatments at 5% LSD.

Trait	**Treatment (T)**	**Genotype (G)**	**(T x G)**	**Control**	**Heat Stress**
Physiological maturity (days)	<0.001	0.0157	0.4130	38a	31b
Seed Weight	0.1662	<0.001	0.2783	3.7a	3.4a
Seed Number (g plant^{-1})	0.6612	<0.001	0.0971	125a	123a
Plant biomass (g plant^{-1})	0.0142	<0.001	0.0284	7.3a	6.8a
Net CO_2 assimilation rate (µmol m^{-2} s^{-1}) at 5 DAS	<0.001	<0.001	<0.001	19.9a	16.6a
Net CO_2 assimilation rate (µmol m^{-2} s^{-1}) at 20 DAS	<0.001	<0.001	<0.001	5.0a	6.2a
Transpiration rate (mol m^{-2} s^{-1}) at 5 DAS	0.092	<0.001	<0.001	7.9a	7.6a
Transpiration rate (mol m^{-2} s^{-1}) at 20 DAS	<0.001	<0.001	0.002	3.6a	0.5b
Maximum quantum yield of PSII (Fv/Fm) at 5 DAS	0.010	0.113	0.263	0.792a	0.776b

Figure 5: Days to physiological maturity of eight wild wheat accessions along with a drought tolerant check (Overley) in experiment 2. Days to physiological maturity were calculated as the number of days from the first day of flowering to physiological maturity.

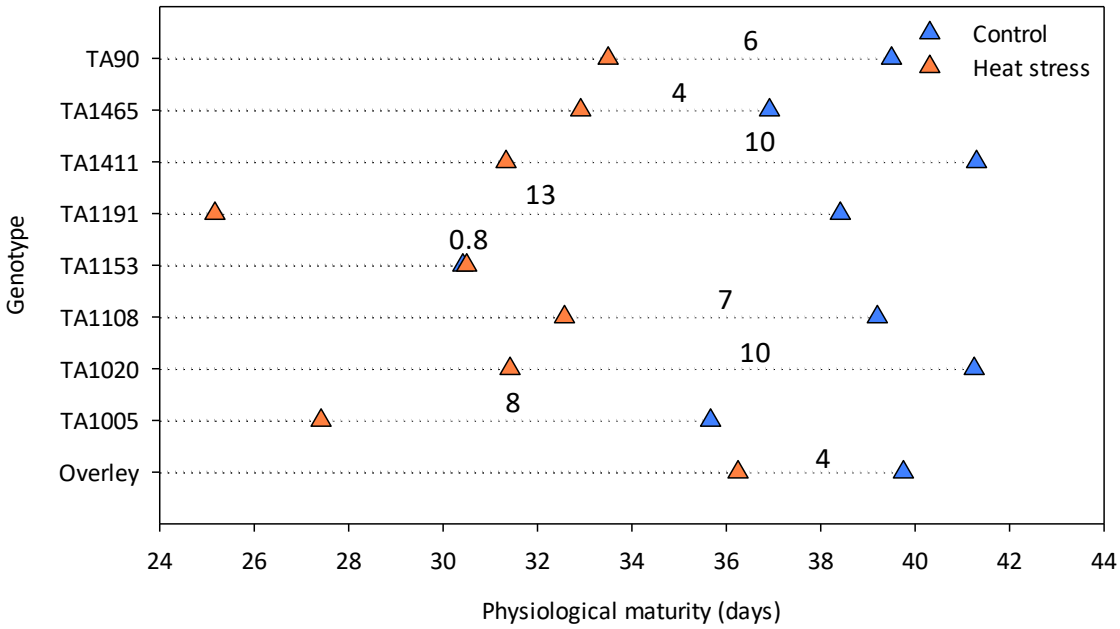


Figure 6: Seed weight (g plant^{-1}), seed number (plant^{-1}) and dry weight plant biomass (g plant^{-1}) of the selected eight wild wheat accessions and a drought tolerant check under control and heat stress in experiment 2. Error bars indicate ± standard error (n=3).

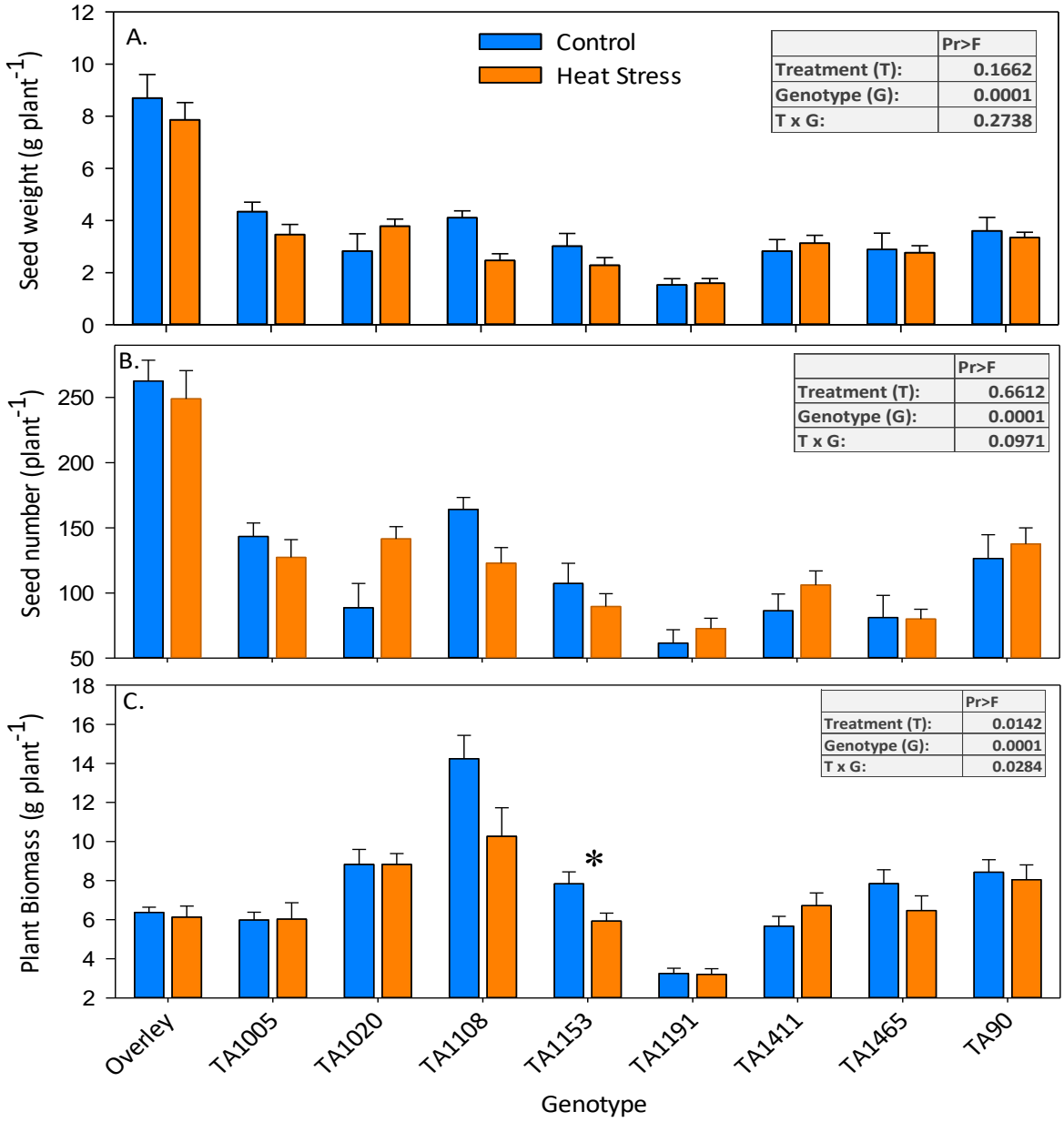


Figure 7: Flag leaf Net CO_2 assimilation rate (µmol m^{-2} s^{-1}) of eight wild wheat accessions and a drought tolerant check (Overley) measured at 5 (A) and 20 (B) days after first flowering/stress imposition under control and heat stress treatments in experiment 2. Different letters above the bars indicate significant difference between treatments in a genotype at 5% LSD. Error bars indicate ± standard error (n=3). DAS-Days after stress.

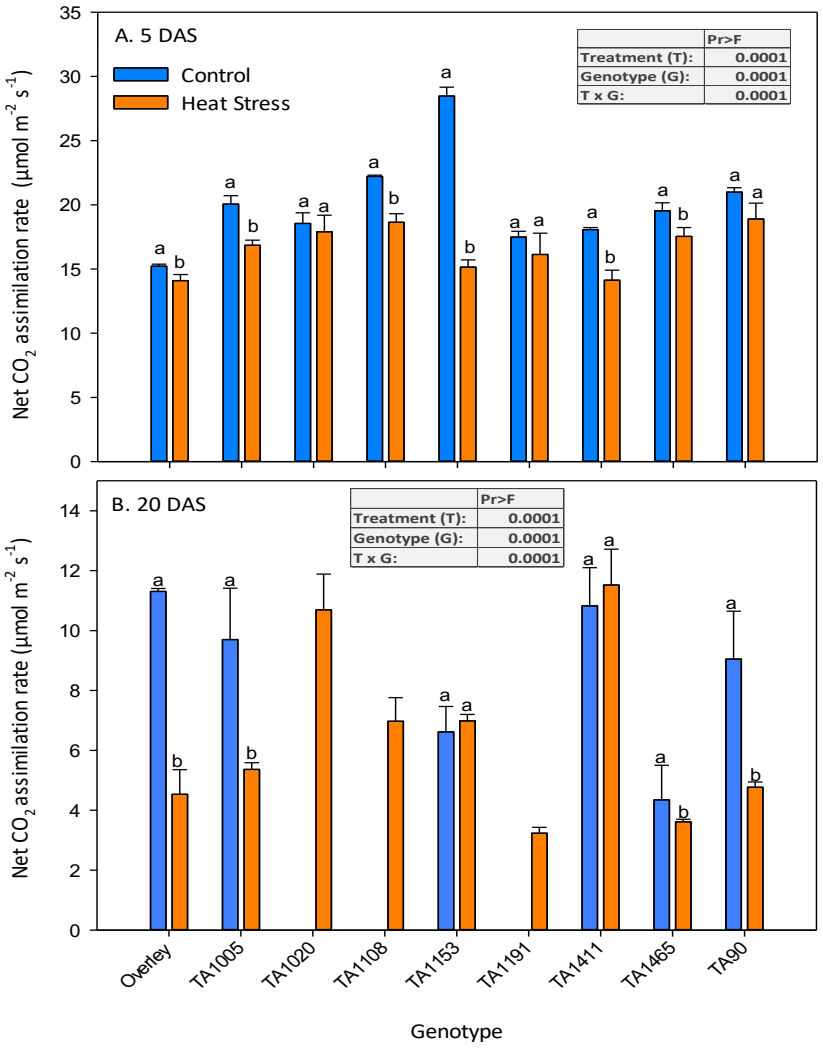


Figure 8: Flag leaf transpiration rate (mol m^{-2} s^{-1}) of eight wild wheat accessions and a drought tolerant check (Overley) measured at 5 (A) and 20 (B) days after first flowering under control and heat stress treatments in experiment 2. Different letters above the bars indicate significant difference between treatments in a genotype at 5% LSD. Error bars indicate ± standard error (n=3). DAS- Days after stress.

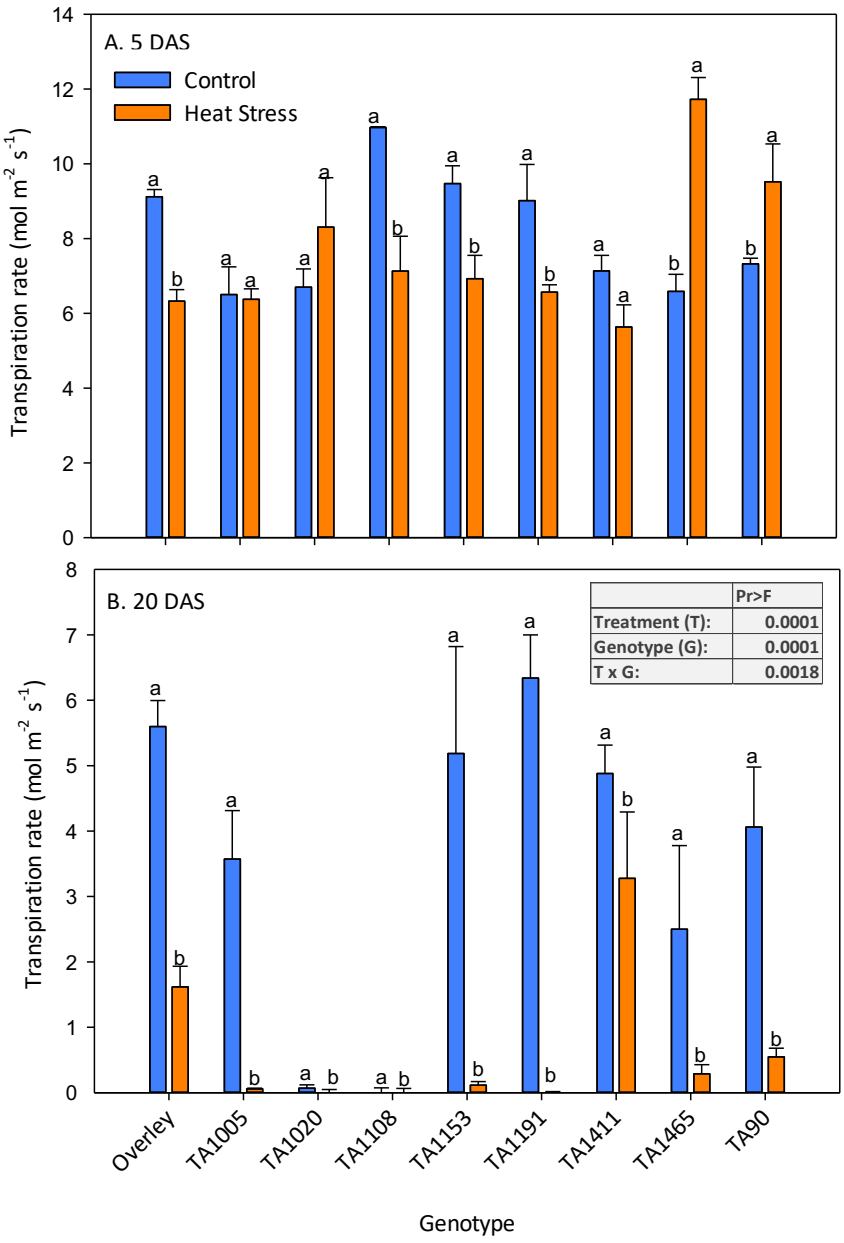
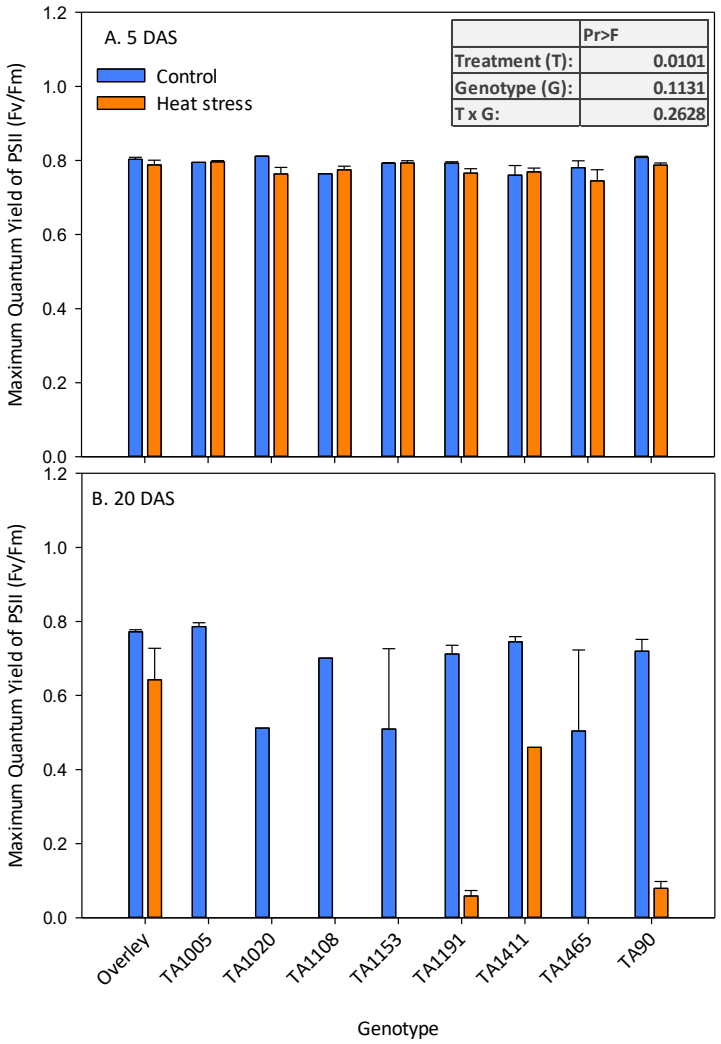


Figure 9: Maximum quantum yield of PSII (Fv/Fm) of eight wild wheat accessions and a drought tolerant check (Overley) measured at 5 (A) and 20 (B) days after first flowering under control and heat stress treatments in experiment 2. Error bars indicate ± standard error (n=3). DAS – Days after stress.



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Chapter 3- Quantifying flowering and post-flowering heat tolerance in *Triticum aestivum-Aegilops speltoides* Robertsonian translocation lines

Abstract

Wheat growing regions often experience temperatures higher than 30°C during flowering and grain-filling stages, resulting in significant reductions in yield. Wild wheat species such as *Aegilops speltoides* are known to possess increased tolerance to abiotic stresses including heat. Chromosomal segments from *A. speltoides* were incorporated into adapted wheat varieties Bob Dole, Joe, and Zenda, leading to the creation of novel Robertsonian translocation lines (RobT's). The hypothesis was that the wild wheat translocations would help enhance terminal heat stress in the adapted backgrounds. A controlled environment chamber experiment helped identify a promising heat tolerant RobT (TA5088) in both Joe and Zenda background. Among the 20 *Triticum aestivum-Aegilops speltoides* RobT's exposed to flowering and post-flowering stages to control (25/15 °C) heat stress (35/15 ^{o}C), TA5088 maintained seed number and weight across two different adapted background. An independent validation experiment involving TA5088 in both backgrounds exposed to four temperatures (25 [control] and 35, 38 and 40 ^{o}C [heat stress]) confirmed the potential of this respective translocation to retain significantly higher seed number and weight even under severe heat stress conditions. Among the two genetic backgrounds, TA5088 translocation was more effective in the Zenda recording a lower heat susceptibility index. Physiological parameters including chlorophyll index and effective quantum yield of photosystem II even under severe heat stress conditions confirmed the higher tolerance of TA5088 in Zenda background. The novel translocation TA5088 provides a unique opportunity for further genetic and molecular studies aimed towards enhancing terminal heat stress in wheat.

Introduction

Wheat (*Triticum aestivum* L.) is a staple cereal for millions of people and is among the highly produced cereal crops in the world (Kropff and Morell, 2019). As wheat is grown under rainfed conditions over a wide range of geographical locations, it is exposed to harsh environmental conditions during different growth and developmental stages throughput its lifecycle. Among the climatic factors, high temperature exposure induces significant reduction in wheat yield both under controlled environments (Aiqing et al., 2018; Bheemanahalli et al., 2019) and field studies (Savin et al., 1999; Bergkamp et al., 2018; Sebela et al., 2020). In addition, the predicted increase in temperature ranging between 0.3^{o}C to 4.8^{o}C by the end of the 21st century (IPCC, 2014), presents a stiff challenge to sustain wheat productivity under future warmer climate.

Even under current conditions, high temperatures commonly impact many wheat-growing regions of the world reducing crop yield and grain quality (Lollato et al., 2017; Teixeira et al., 2013; Zhao et al., 2017). Among the different stages, heat stress limits wheat yield potential more significantly when coincided with flowering or grain-filling period, compared to the vegetative stage (Ugarte et al., 2007; Savin et al., 1999; Farooq et al., 2011; Barkley et al., 2013). Yield losses due to heat stress during flowering are attributed to lower pollen number of reduced pollen viability and the rate of pollen tube growth leading to reduced grain number (Yang et al., 2013), while post-flowering stages are due to abortion of grains and decreased grain weight (Lobell et al., 2012). Post-anthesis heat stress decreases grain size due to the shorter grain-fill duration, even though heat stress increases the grain-filling rate (Prasad et al., 2008). Heat stress disrupts physiological processes such as damage to cellular structure and metabolic pathways

including membrane thermostability, photosynthesis, and starch synthesis (Liu et al., 2000; Ristic et al., 2007; Bheemanahalli et al., 2020). These physiological processes are more significantly affected with post flowering heat stress due to accelerated senescence and altered carbon balance leading to lower yield and poor quality grain (Impa et al., 2020)

One way to mitigate the effect of heat stress on wheat is to develop heat-tolerant varieties (Wahid et al., 2007). An effective approach proposed is to incorporate genetic diversity from wild species to help develop tolerance to different abiotic and biotic stresses (Reynolds et al., 2007; van Ginkel and Ogbonnaya, 2007; Zhang et al., 2017; Cruppe et al., 2020). Aegilops species have been considered a genetic resource for enhancing biotic and abiotic stresses in cultivated wheat (Pradhan et al., 2012; Green et al., 2018). Aegilops species are close relatives of bread wheat, wherein the latter acquired its D genome from *A. tauschii* Coss. (Kihara, 1944; McFadden and Sears, 1946). Genetic diversity housed among the wild wheat relatives has facilitated improving wheat cultivars with enhanced tolerance to heat stress (Nevo, 2014), drought (Nevo and Chen, 2010), salinity (Munns et al., 2003), and leaf rust (Narang et al., 2000). However, the effectiveness of *Aegilops-speltoides* based genetic diversity in addressing heat stress induced damage in wheat has not yet been fully explored (Ullah et al., 2018).

Although wild wheat accessions have been phenotyped for heat stress response (Yang et al., 2002; Pradhan et al., 2012), having translocation lines in adapted background presents a unique opportunity to identify the genetic fragment that induces heat tolerance. Identifying specific fragments that induce tolerance across different adapted background is extremely valuable for breeding new varieties as tolerance then can be incorporated into different varieties are grown across a wide range of environments. To explore the potential of *Aegilops-speltoides*

for enhancing heat tolerance and also to determine the effectiveness of the chromosomal arms across different adapted backgrounds, 20 Robertsonian translocation lines (RobT's) were developed in three popular backgrounds i.e., Zenda, Bob Dole and Joe (referred to as parental lines). Robertsonian translocation lines are chromosomal rearrangements from the fusion of the entire long arms of two acrocentric chromosomes. These lines occur when two non-homologous chromosomes get attached, meaning that two healthy pairs of chromosomes, one of each pair adhere together (Liu et al., 2016). These are the very first set of RobT's that have been developed in adapted varieties that are popularly grown in the US Great Plains, providing a unique opportunity to identify the *A. speltoides* arm that induces highest post-flowering heat tolerance in wheat. Two independent experiments were conducted under controlled environment walk-in chambers using these RobT's and the parental lines to: 1) identify the most promising Robertsonian translocation line/s possessing highest potential for minimizing flowering and post-flowering heat stress induced reduction in yield; and 2) assess the extent of heat stress stability provided by the most promising RobT line/s in terms of physiological responses, and yield and related parameters.

Materials and methods

Two experiments were conducted in controlled environment growth chambers at the Department of Agronomy, Kansas State University, Manhattan, Kansas.

Experiment 1

Experiment 1 was carried out in the spring of 2019 and consisted of 20 Robertsonian Translocation lines better known as RobT's and their respective parent lines, forming a total set

of 23 genotypes (Table 1). 50 seeds were sown in 30.5×61 cm flat seed trays filled with Sunshine Metro-Mix 380 potting mix (Sun Gro Horticulture, Agawam, MA) and placed in controlled environment growth chambers at 25 °C/15 °C maximum day/night temperature. After the majority of the seeds had germinated, the seed trays were transferred to a vernalization chamber maintained at 5°C for 6 weeks. Following vernalization, two seedlings each were transplanted into 1.6L pots (10×24 cm, MT49 Mini-Treepot) filled with farm soil. Once the transplanted seedlings were well established, they were thinned down to one seedling per pot. Each pot received 5g of Scotts Osmocote classic (14-4-14 of N-P-K) and 0.5g of Scotts Micromax Micronutrients (Hummert International, Topeka, KS). The pots were kept in trays and placed in controlled environment chambers maintained at 25/15^{o}C maximum day/minimum night temperatures. The plants were well-watered throughout the experiment by maintaining about one cm layer of water in the trays holding the pots. The main tiller in each plant was tagged on the day anthesis began and three replicate plants were independently transferred to two separate heat stress chambers maintained at 35/15^{o}C maximum day/minimum night temperatures. The other half of the plants remained in three separate control treatment chambers maintained at 25/15^{o}C maximum day/minimum night temperatures. Both control and heat stress chambers were maintained at 16/8 h (day/night) photoperiod, with 900 -1000 µmol m^{-2} s^{-1} light intensity at 5 cm above the canopy and 70% relative humidity (RH). Maximum (25 and 35^{o}C with control and heat stress treatment, respectively) and minimum temperatures were maintained for 8 h in all growth chambers with a transition period of 4 h between maximum day and minimum night temperatures (Fig. 1). Temperature and RH at canopy level were recorded

every 30 minutes using HOBO UX 100-011 temperature/RH data loggers (Onset Computer Corp., Bourne, Massachusetts) in all growth chambers.

Experiment 2

The validation experiment was carried out during in the spring of 2020, to validate the promising RobT's. Experiment 2 consisted of two RobT's TA5088 X Joe and TA5088 X Zenda and their respective introgressed background forming a total of four genotypes. Plant growth conditions and management were similar to experiment 1. Unlike experiment 1, three different levels of heat stress were imposed; 35 °C/15 °C (maximum day/minimum night temperatures), 38°C/15 °C, and 40 °C/15 °C while maintaining control treatment at 25 °C/15 °C. A total of 8 replications per genotype per treatment were used where 3 plants were used for recording physiological parameters and five plants for yield related parameters.

Measurements

Gas exchange was measured 10 days after stress, while chlorophyll index and effective quantum yield of Photosystem II (QY) were recorded at 7, 14, and 21 days in stress and control conditions in experiment 1. All measurements in experiment 2 were similar to those in experiment one, except for gas exchange, which was not recorded. At maturity, yield and yield related parameters including seed number and seed weight were recorded in both the experiments.

Physiological Traits

Gas exchange

Gas exchange measurements were taken at 10 days after anthesis or stress imposition using a portable photosynthesis system Li-6400XT (LI-COR Biosciences, Lincoln, Nebraska). Gas exchange measurements were recorded between 1000 and 1100 h. The CO_2 concentration in the leaf chamber of the portable photosynthesis system was set to 400 µmol mol^{-1}, with a flow rate of 500 µmol s^{-1} and a light intensity of 1000 µmol m^{-2} s^{-1} of photosynthetically active radiation supplied by red-blue light-emitting diode. The block temperature in the photosynthesis system were set at 25℃ and 35℃ in control and heat stress treatments, respectively. The measurements were recorded on flag leaves of the main tiller.

Chlorophyll Fluorescence

Chlorophyll fluorescence was measured on seven, 14 and 21 days after anthesis or stress imposition using a handheld chlorophyll Fluorometer FluorPen FP 100 (Photon Systems Instruments, Drazov, Czech Republic). The fluorescence measurements were recorded between 1000 and 1100 h. The effective quantum yield of photosystem II (QY) was recorded on flag leaves with a light pulse intensity of 3000 µmol m^{-2} s^{-1} and pulse duration of 1s, following Sebela et al. (2015).

Chlorophyll Index was measured on seven, 14 and 21 days after anthesis or stress imposition using SPAD 502 plus Chlorophyll Meter (Konica Minolta, Inc. Tokyo, Japan). The fluorescence measurements were recorded between 1000 and 1100 h on the flag leaf.

Agronomic Traits

The days to first anthesis and physiological maturity (Zadoks growth scale 9-ripening [92-Grain hard, not dented by thumbnail]) were recorded on at least three replicate plants for each

genotype and treatment. At physiological maturity plants were hand harvested and total number of tillers per plant, total number of productive spikes per plant, and total number of sterile spikes per plant were recorded. The harvested plants were separated into spikes and tillers and oven dried at 60°C for seven days to obtain the shoot biomass. Oven dried spikes were hand threshed to obtain total grain weight per plant (constant weight was achieved), and grain number per plant.

Heat susceptibility index

Heat Susceptibility Index (HSI) values for two RobT's and their parental lines were calculated based for seed number and seed weight based on Fischer & Maurer, 1978.

HSI = 1 – [YS/YC]/D

Where, YS = mean of the genotype in heat stress treatment; YC = mean of the genotype under control treatment; D = 1 - [mean of all the genotypes under heat stress/mean of all genotypes under control].

Statistical Analysis

The experiment was laid out in a split plot randomized complete block design with temperature as the main plot factor and genotype as a sub-plot factor with three independent chamber replications for control, three and two independent chamber replications for heat stress in experiment 1 and 2, respectively. Two way analysis of variance for all the measured parameters was performed using PROC GLM procedure in SAS software (Version 9.4, SAS Institute). Replication, temperature, and genotypes were used as class variables, replication and replication

x temperature as random effects, and all other variables as fixed effects. Repeated measures ANOVA was performed for chlorophyll index and QY data recorded over three different time points, using PROC MIXED procedure in SAS software (Version 9.4, SAS Institute), using first order autoregressive covariance matrix structure. Means were separated using LSD (least significant difference) test at p=0.05.

Results

Experiment 1

Days to physiological maturity

Days to physiological maturity was significantly ($p<0.001$) affected by temperature (T) and genotype (G) but not by T x G (Table 2). On average heat stress exposed plants matured 12 days earlier than control grown plants (Table 2). Heat stress induced early maturity varied significantly among genotypes ranging from 7 days in TA5681 x Joe to 28 days in TA5687 X Joe compared to control (Fig. 2).

Growth and yield parameters

Plant biomass varied significantly between treatment and genotype (Table 2). Among the RobT's TA5598L2 X Zenda, TA5686 X Zenda, TA5088 X Zenda and TA5683 X Joe showed no significant reduction in plant biomass under heat stress compared to control, with TA5088 X Zenda and TA5683 X Joe recording a significant increase in biomass. Whereas, TA5680L1 X Zenda (58%), TA5687 X Zenda (55%) TA5678 X Zenda (46%) and TA5682 X Zenda (41%) recorded the highest plant biomass reduction under heat stress compared to control (Table 3).

Seed number was significantly affected by T and G but not by T x G (Table 2). When averaged across genotypes seed number under heat stress was reduced by 14 % compared to control (Table 2). Among the RT lines TA5088 X Joe, TA5686 X Joe, TA5683 X Joe and TA5598L2 X Zenda showed no reduction in seed number under heat stress compared to control (Table 3). Highest reductions in seed number under heat stress compared to control were noticed in TA5680L1 X Zenda (54%), TA5686 X Zenda (45%) and TA5682 X Zenda (44%) (Table 3). Seed weight varied significantly among T and G but not T x G (Table 2). On average seed weight was reduced by 25 % under heat stress compared to control (Table 2). Among RT lines TA5680L1 X Zenda (61%), TA5686 x Zenda (47%), TA5689 X Zenda (47%) and TA5682 X Zenda (42%) exhibited highest reduction in seed weight under heat stress compared to control, whereas TA5598L2 x Zenda and TA5088 x Joe showed no reduction in seed weight under heat stress compared to control (Table 3). Across the three parental lines, Bob Dole was the most tolerant wherein the plant biomass, seed number and weight were not reduced under heat stress exposure.

Physiological Traits

Assimilation rate

Net CO_2 assimilation rate was significantly affected by T and G but not by T x G (Table 2). When averaged across genotypes heat stress induced 11 % reduction net CO_2 assimilation rate (Table 2). Among the RobT's TA5678 X Zenda/Bob Dole//Zenda, TA5598L2 X Zenda and TA5687 X Zenda recorded the highest assimilation rate under heat stress, while TA5688 X Zenda and Joe recorded the lowest assimilation rate under heat stress (Table 4). In terms of relative change, the

range in assimilation rate under heat stress exposure was -55 % (TA5688 X Zenda) and 34 % (TA5689 X Joe) (Table 4).

Transpiration rate

Transpiration rate varied significantly between treatments but not between genotypes (Table 2). On average, heat stress increased transpiration rate by 23 % compared to control (Table 2). Almost all the RobT's and parents recorded an increase in transpiration under heat stress compared to control except TA5088 X Joe and TA5688 X Zenda (Table 4).

Chlorophyll index

Repeated measures anova indicated that chlorophyll index was significantly ($p<0.001$) affected by T, days after stress (DAS) and T x DAS but not by G and T x G x DAS (Fig. 3). On average, heat stress induced 15.5 % reduction in chlorophyll index compared to control. Heat stress reduced chlorophyll index to a greater extent at 21 DAS, followed by 14 and 7 DAS (Fig. 3). The average chlorophyll index data of all the genotypes, treatments measured at different DAS are given in Appendix 2.

Effective quantum yield of PSII (QY)

Repeated measures anova indicated that effective quantum yield of PSII (QY) was significantly ($p<0.001$) affected by T, days after stress (DAS) and T x DAS but not by G and T x G x DAS (Fig. 4). On average heat stress induced 10 % reduction in QY compared to control. Similar to chlorophyll index, heat stress induced reduction in QY under heat stress was significantly greater at 21 DAS, followed by 14 and 7 DAS (Fig. 4). A drastic reduction in QY was noticed starting

at 14 days after stress in heat stress in all the genotypes (Appendix 1). The average effective quantum yield data of all the genotypes, treatments measured at different DAS are given in Appendix 1.

Experiment 2

Days to physiological maturity

Days to physiological maturity was significantly ($p<0.001$) affected by T, G and T x G interaction (Table 5). Days to physiological maturity decreased significantly with increase in temperature indicating that plants exposed to higher temperatures matured earlier than control plants. On average heat stress induced 5, 11 and 18-days earlier maturity with 35 °C, 38 °C and 40 °C temperature treatments respectively, compared to control (25 °C). Among the genotypes heat stress induced early maturity varied from 2 to 9 days at 35 °C, 10-1 days at 38 °C and 11 to 23 days at 40 °C compared to control (Fig. 5). Under control TA5088 X Joe had delayed maturity compared to its parent Joe, whereas TA5088 X Zenda recorded a significantly earlier maturity compared to its parent Zenda (Fig. 5). Among the genotypes days to physiological maturity was least affected in TA5088 X Zenda with 11 days earlier maturity at 40 °C compared to 25 °C, whereas TA5088 X Joe recorded the highest affect with 23 days earlier maturity at 40 °C compared to 25 °C (Fig. 5).

Biomass and yield parameters

Plant biomass varied significantly ($p<0.001$) only among genotypes but not with temperature treatments or their interaction (Table 5). On average Zenda recorded lower plant biomass than all the other genotypes.

Seed number was significantly affected by T, G and T x G interaction (Table 5). On average, seed number was reduced by 37, 75 and 84 % at 35 °C, 38 °C and 40 °C respectively, compared to control (25 °C). Among the genotypes at 25 °C, TA5088 X Joe maintained significantly higher seed number than other genotypes, whereas TA5088 X Zenda maintained significantly higher seed number than other genotypes at 35 °C and 40 °C (Fig. 6). Highest percent reduction in seed number under heat stress compared to control was recorded in TA5088 X Joe (89%), whereas it was lowest (70%) in TA5088 X Zenda (Fig. 6). However, TA5088 X Joe maintained a higher seed number than Joe at 25℃, 35℃ and 38℃, and TA5088 X Zenda recorded a higher seed number than the Zenda under all three heat stress treatments (Fig. 6).

Seed weight was significantly affected by T, G and T x G interaction (Table 5). On average heat stress induced significant reduction in seed weight compared to control (25℃) and among the high temperature treatments starting with 35 °C, with 38 °C and 40 °C recording significantly similar seed weight. Similar to seed number, TA5088 X Joe recorded the highest seed weight than other genotypes at 25, whereas Zenda and TA5088 X Zenda were the lowest yielders at the control temperature (Fig. 7). Among the three heat stress conditions, TA5088 X Joe recorded significantly higher seed weight per plant only at 38℃. On the other hand, TA5088 X Zenda recorded significantly higher seed weight at all three high temperature treatments (Fig. 7).

Heat Susceptibility Index

Heat susceptibility index (HSI) was calculated for yield parameters including seed number and seed weight for heat stress treatments (35°C, 38°C, and 40°C) compared to control (25°C). Among the genotypes, TA5088 X Zenda recorded lower HSI for both seed number and weight

compared to Zenda and other genotypes across all the three high temperature treatments (Table 6). This indicates that TA5088 X Zenda has relatively higher heat tolerance than other genotypes.

Chlorophyll Index

Repeated measures anova revealed a significant effect of T, G, T x G, DAS, T x DAS and T x G x DAS interaction effect on chlorophyll Index (Table 5). On average chlorophyll index showed a linear reduction with increase in temperature from 25 to 40℃. Chlorophyll index decreased over time in all the temperature treatments with faster rates of reduction observed under heat stress compared to control. Among the genotypes, Zenda exhibited a faster reduction in chlorophyll index over time at 35℃ than other genotypes (Fig. 8). At 38 ℃ both the parents showed a faster reduction in chlorophyll index than the RobT's (Fig. 8) and at 40 ℃ all the genotypes recorded similar reduction in chlorophyll index except TA5088 X Joe at 14 DAS (Fig. 8).

Effective quantum yield of PSII (QY)

QY was significantly affected by T, G, T x G, DAS, T x DAS and G X DAS but not T x G x DAS interaction (Table 5). On average heat stress induced 20, 32 and 48 % reduction in QY under 35℃, 38℃ and 40℃ temperature treatments respectively, compared to control (25℃). QY reduced over time from 7 to 14 DAS across all the temperature treatments (Fig. 9A). At 7 DAS, no reductions were recorded in QY under heat stress treatments compared to control (Fig. 9A). Whereas, at 14 DAS, QY at 38℃ and 40℃ showed significant reductions compared to 25, and at 21 DAS all the heat stress treatments recorded significant reductions in QY compared to control (Fig. 9A). QY reduced over time from 7 to 14 DAS across all the genotypes (Fig. 9B). Zenda recorded significantly lower Q than other genotypes at 7 DAS (Fig. 9B)

Discussion

Exploring RobT's potential to induce terminal heat stress tolerance across genetic backgrounds

Historically, breeding programs have used genetic diversity that has been amenable and effectively introgressed for enhancing tolerance to biotic or abiotic stresses including heat. However, a recent study involving popular hard red winter wheat cultivars currently grown in the US Great Plains, exposed to terminal heat stress under controlled environments and field conditions, resulted in only one cultivar recording high level of tolerance due to higher thousand kernel weight and grain number (Bergkamp et al., 2018). Among the seven cultivars yield was reduced by up to 51 % and 27 % under controlled environments and field conditions, respectively, indicating the need to enhance tolerance to heat stress to maintain wheat productivity even under current climatic conditions. A similar response has been documented in rice, wherein among the 18 popular cultivars assembled from across the world, only one cultivar was found to be tolerant to heat stress (Shi et al., 2015), indicating that currently grown popular cultivars across different field crops to be vulnerable to heat stress. Further, considering the projected increase in temperature (IPCC, 2014), a quantum increase in heat stress tolerance is needed to ensure wheat productivity is sustained under future warmer climate.

Aegilops speltoides based RobT's in Joe and Zenda backgrounds provided a unique opportunity to determine the effectiveness of the *A. speltoides* chromosomal arms in reducing terminal heat stress induced yield reduction. Although a wide range in loss of plant biomass, seed number and seed weight were recorded across the RobT's in both Joe and Zenda backgrounds, two of the introgressions namely TA5088 and TA5598L2 were comparatively more promising compared to the others. Although the relative percentage of change in TA5598L2 was

considerably higher in Zenda background, the absolute values under heat stress were significantly lower compared to TA5088. Hence, based on both relative and absolute heat stress tolerance TA5088 translocation was identified to be the most promising translocation having the highest potential to minimize heat stress induced yield loss.

TA5088 translocation a unique and novel opportunity to enhance terminal heat stress tolerance in wheat

Wild wheat by themselves are tolerant to a wide range of biotic and abiotic stresses due to their historical selection pressure, but do parts of their chromosome induce the same response when moved to a more adapted background? A critical threshold of 35^{o}C has been identified (Tack et al., 2015), beyond which significant reduction in wheat yield is induced under field conditions. A similar response with stress levels of 35^{o}C or beyond was observed, averaged across TA5088 translocation in Joe and Zenda background along with the respective parental lines (Figs 6 and 7). Two key components that determine grain yield under stress conditions are seed number and seed weight (Rahman et al., 2009; Balla et al., 2019). Although, seed weight contributes towards yield, it is usually referred to as a finer regulator of wheat yield while seed number play a more important role as coarse regulators sustaining yield under stress (Gibson and Paulsen, 1999; Hutsch et al., 2019; Slafer et al., 2014). Interesting, with 35^{o}C the TA5088 in Zenda background recorded grain number close to the control conditions, indicating higher level of tolerance to physiological processes including pollen germination and fertilization events, which are considered to be highly sensitive to heat stress (Prasad and Djanaguiraman, 2014; Bheemanahalli et al., 2019). Further, the introgressions having a similar significant higher seed number with even severe heat stress condition (38 and 40^{o}C), compared to their parental lines

provides rationale for further detailed investigation to exploit the level of tolerance observed. An exactly similar response was observed with the seed weight (Fig. 7). Due to plant compensatory mechanisms, achieving higher seed number and seed weight simultaneously is physiologically difficult (Sadras et al., 2020), but with the TA5088 we were able to see this demonstrated in two different genetic backgrounds (Figs. 8 and 9). Hence, utilizing this chromosomal arm from the *A. speltoides* into breeding programs could provide the opportunity to maintain seed number and possibly increase the seed weight to compensate for the loss in seed numbers as documented in susceptible popular wheat varieties (Bergkamp et al., 2018). The biggest challenge in breeding new varieties using novel genes or genomic regions has been the lack of consistency in maintaining the advantage under different genetic backgrounds (Yang et al., 2002; Xie and Nevo, 2008; Zhang et al., 2017). To a certain extent, this is observed in our findings where in the translocation TA5088 is expressed significantly better in the Zenda background (low HIS; Table 6) in terms of retaining seed number and weight compared to Joe. In spite of this variability the impact of this translocation being positive across two different genetic backgrounds rendering the need to explore the impact of this translocation in a wider range of cultivars grown across different parts of the US and world, to explore and exploit the advantage to the fullest.

In conclusion, our findings have demonstrated the usefulness of exploring wild wheat translocation lines in adapted background to help enhance terminal heat stress tolerance in wheat. Progress achieved in extracting candidate genes or chromosomal region in the translocation can be used to potentially enhance heat tolerance across winter and spring wheat varieties grown in the US and elsewhere. Designing systematic approaches to identify other novel wild wheat introgressions such as TA5088, is proposed to help enhance resilience in current

popular cultivars that are susceptible to terminal heat stress to help sustain global wheat productivity under current and future warmer climates.

Table 1. List of *Triticum aestivum-Aegilops speltoides* Robertsonian translocation lines and parental lines grown in controlled environment experiment 1 and validated genotypes in experiment 2 (bold).

Genotype	Species
Bob Dole	*Triticum aestivum* L.
TA5678 X Zenda/Bob Dole//Zenda	*Triticum aestivum-Aegilops speltoides*
Joe	*Triticum aestivum* L.
TA5088 X Joe	*Triticum aestivum-Aegilops speltoides*
TA5598L2 X Joe	*Triticum aestivum-Aegilops speltoides*
TA5681 X Joe	*Triticum aestivum-Aegilops speltoides*
TA5683 X Joe	*Triticum aestivum-Aegilops speltoides*
TA5686 X Joe	*Triticum aestivum-Aegilops speltoides*
TA5687 X Joe	*Triticum aestivum-Aegilops speltoides*
TA5688 X Joe	*Triticum aestivum-Aegilops speltoides*
TA5689 X Joe	*Triticum aestivum-Aegilops speltoides*
Zenda	*Triticum aestivum* L.
TA5088 X Zenda	*Triticum aestivum-Aegilops speltoides*
TA5598 L2 X Zenda	*Triticum aestivum-Aegilops speltoides*
TA5678 X Zenda	*Triticum aestivum-Aegilops speltoides*
TA5680L1 X Zenda	*Triticum aestivum-Aegilops speltoides*
TA5681 X Zenda	*Triticum aestivum-Aegilops speltoides*
TA5682 X Zenda	*Triticum aestivum-Aegilops speltoides*
TA5683 X Zenda	*Triticum aestivum-Aegilops speltoides*
TA5686 X Zenda	*Triticum aestivum-Aegilops speltoides*
TA5687 X Zenda	*Triticum aestivum-Aegilops speltoides*
TA5688 X Zenda	*Triticum aestivum-Aegilops speltoides*
TA5689 X Zenda	*Triticum aestivum-Aegilops speltoides*

Table 2. Probability of effects of treatment (T), genotype (G), and T x G interactions on physiological and yield parameters in experiment 1. Values are averages across 20 Robertsonian translocation lines and their parental lines for yield parameters and physiological traits. Probability values highlighted in red are significant. Separation of means was carried out using the LSD test ($P < 0.05$). Means with different letters are significantly different according to the LSD test at ($P < 0.05$).

Trait	**Treatment (T)**	**Genotype (G)**	**(T x G)**	**Control**	**Heat Stress**
Physiological maturity (days)	<0.001	0.0042	0.1067	44.38a	32.00b
Plant Biomass (g plant^{-1})	0.0033	<0.001	0.7074	1.41a	1.11a
Seed Number (plant^{-1})	0.0045	<0.001	0.0669	82.31a	70.63a
Seed Weight (g plant^{-1})	<0.001	<0.001	0.4736	2.65a	2.00a
Assimilation Rate (µmol m^{-2} s^{-1}) at 10 DAS	<0.001	0.0282	0.0944	18.60a	16.53b
Transpiration Rate (mol m^{-2} s^{-1}) at 10 DAS	<0.001	0.0839	0.1750	7.96a	9.78a
Chlorophyll Index 7 DAS	0.0506	0.0932	0.1375	46.99a	44.81b
Chlorophyll Index 14 DAS	<0.001	0.0624	0.1142	46.03a	41.69b
Chlorophyll Index 21 DAS	<0.001	0.5089	0.1490	42.92a	28.38b
Effective quantum yield of PSII (QY) 7 DAS	0.0055	0.0686	0.7100	0.72a	0.70b
Effective quantum yield of PSII (QY) 14 DAS	0.0053	0.0352	0.5486	0.71a	0.68b
Effective quantum yield of PSII (QY) 21 DAS	<0.001	0.5248	0.2927	0.68a	0.51b

Figure 1. Average air temperature inside the growth chambers during control and heat stress treatments in experiment 1. Air temperature at the canopy level was recorded every 30 min using HOBO UX 100-011 data loggers placed at 5 cm above the canopy level.

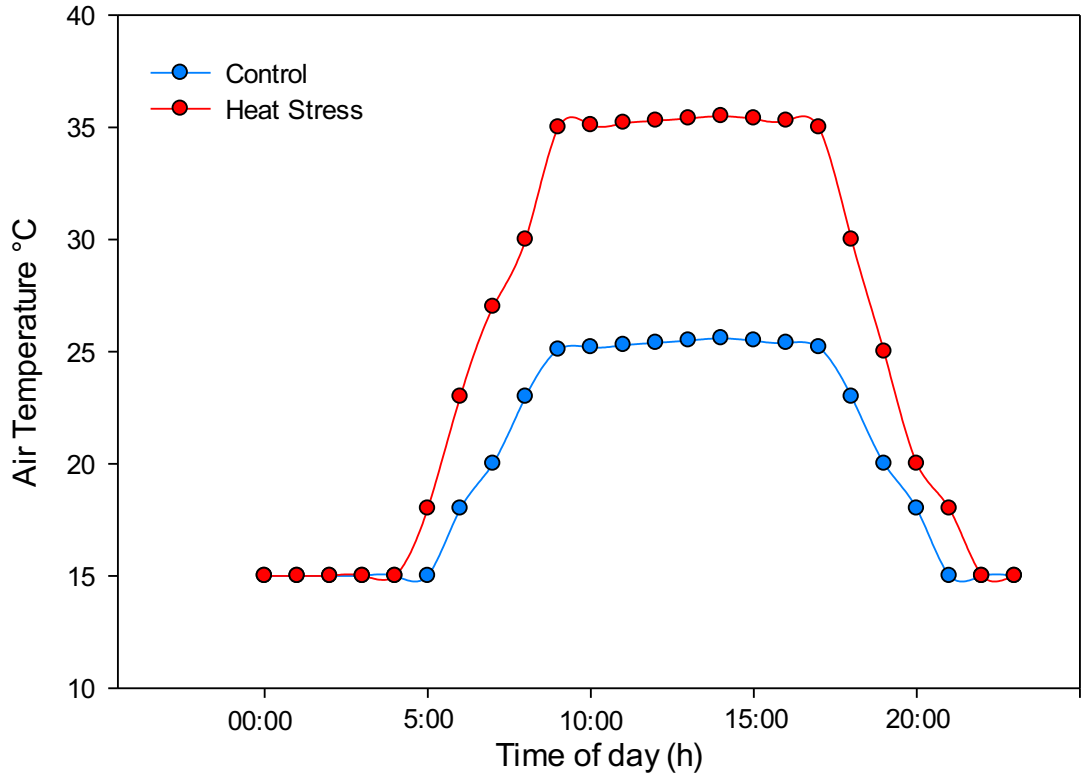


Figure 2. Days to physiological maturity in 20 Robertsonian translocation lines and parental lines in experiment 1. Days to physiological maturity were calculated as the number of days from first day of flowering to physiological maturity. The numbers between the symbols indicates the advancement in maturity (in days) compared to control.

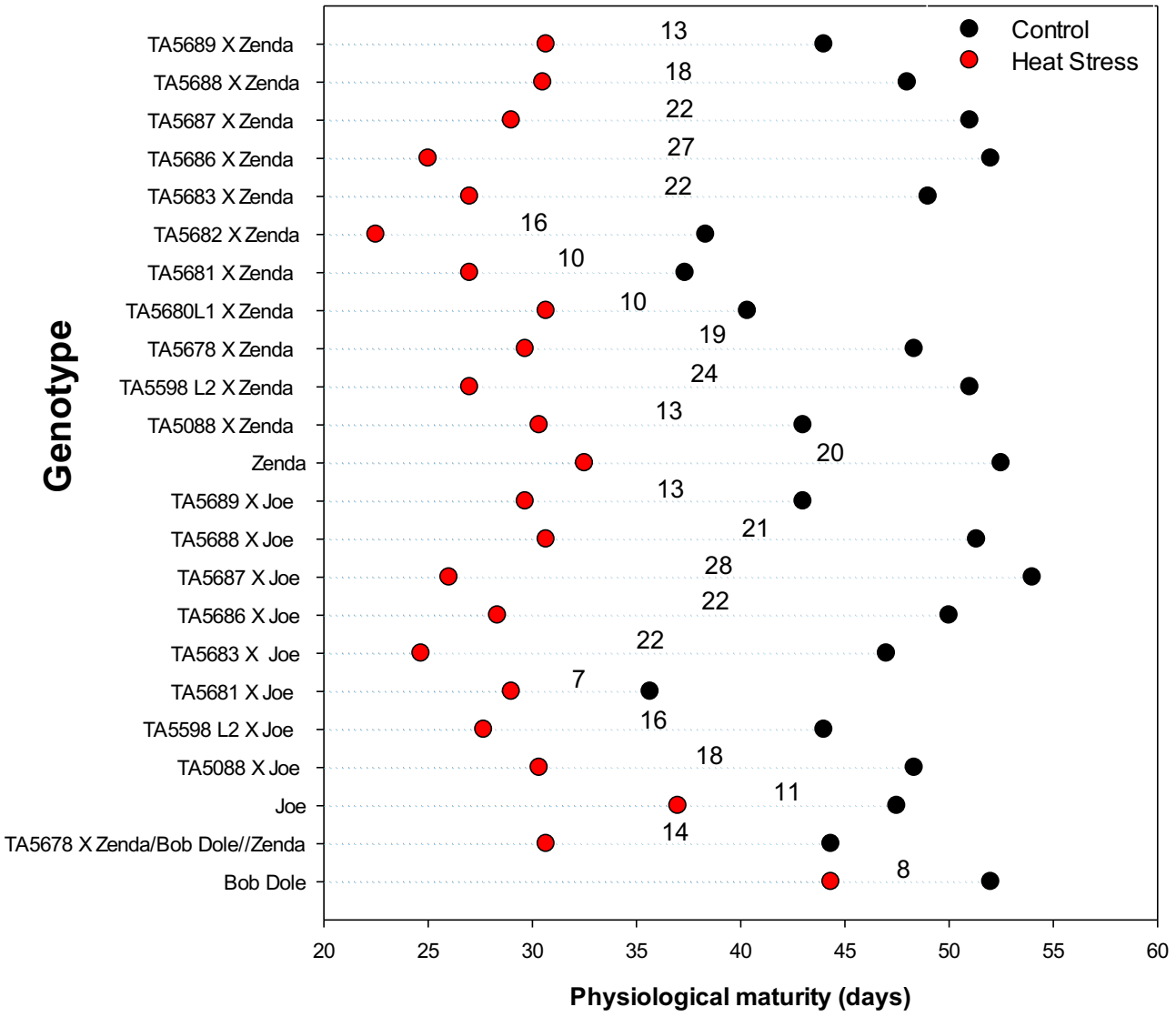


Table 3. Plant biomass (g plant^{-1}), seed number and seed weight (g plant^{-1}) of 20 Robertsonian translocation lines and their parental lines under control and heat stress and % change under heat stress compared to control in experiment 1. % change indicates the change in a trait under heat stress compared to control expressed in percentage. Mean ± standard error (n=3).

	Plant Biomass			**Seed Number**			**Seed weight**		
GENOTYPE	**Control**	**Heat stress**	**% Change**	**Control**	**Heat stress**	**% Change**	**Control**	**Heat stress**	**% Change**
Bob Dole	0.94 ± 0.18	1.10 ± 0.27	17	65 ± 13	101 ± 30	57	1.98 ± 0.45	2.44 ± 0.61	23
TA5678 X Zenda/Bob Dole//Zenda	0.98 ± 0.22	0.69 ± 0.01	-29	73 ± 10	52 ± 7	-28	2.09 ± 0.39	1.48 ± 0.24	-29
Joe	2.48 ± 0.42	2.01 ± 0.14	-19	94 ± 7	105 ± 17	12	3.59 ± 0.39	2.89 ± 0.48	-20
TA5088 X Joe	1.54 ± 0.11	1.35 ± 0.16	-13	69 ± 7	83 ± 5	20	2.30 ± 0.58	2.44 ± 0.33	6
TA5598L2 X Joe	1.53 ± 0.40	1.00 ± 0.16	-34	73 ± 14	75 ± 12	3	2.56 ± 0.55	2.32 ± 0.38	-10
TA5681 X Joe	1.35 ± 0.50	1.35 ± 0.06	0	73 ± 21	66 ± 15	-9	2.47 ± 0.87	2.13 ± 0.35	-13
TA5683 X Joe	1.67 ± 0.30	1.82 ± 0.60	9	87 ± 6	102 ± 23	17	2.62 ± 0.26	2.46 ± 0.31	-6
TA5686 X Joe	1.00 ± 0.03	0.82 ± 0.01	-18	57 ± 10	70 ± 7	23	1.95 ± 0.29	1.91 ± 0.25	-2
TA5687 X Joe	1.26 ± 0.23	1.16 ± 0.29	-8	97 ± 2	76 ± 9	-22	3.08 ± 0.22	2.04 ± 0.22	-34
TA5688 X Joe	0.96 ± 0.43	0.89 ± 0.34	-7	55 ± 10	50 ± 13	-8	1.83 ± 0.49	1.46 ± 0.41	-20
TA5689 X Joe	2.48 ± 0.56	1.60 ± 0.07	-35	97 ± 15	78 ± 3	-19	3.63 ± 0.64	2.49 ± 0.05	-31
Zenda	1.00 ± 0.23	0.72 ± 0.28	-28	83 ± 7	80 ± 19	-4	2.78 ± 0.23	2.11 ± 0.40	-24
TA5088 X Zenda	1.37 ± 0.12	1.40 ± 0.13	2	93 ± 11	87 ± 12	-6	3.08 ± 0.34	2.08 ± 0.33	-33
TA5598L2 X Zenda	0.32 ± 0.00	0.58 ± 0.46	83	35 ± 0	52 ± 26	47	1.03 ± 0	1.41 ± 0.69	37
TA5678 X Zenda	1.81 ± 0.43	0.97 ± 0.05	-46	93 ± 17	77 ± 9	-18	3.01 ± 0.65	2.09 ± 0.19	-31
TA5680L1 X Zenda	1.12 ± 0.21	0.47 ± 0.17	-58	84 ± 8	39 ± 12	-54	2.58 ± 0.27	1.01 ± 0.29	-61
TA5681 X Zenda	1.77 ± 0.10	1.49 ± 0.22	-16	86 ± 3	72 ± 9	-17	2.95 ± 0.13	1.99 ± 0.27	-33
TA5682 X Zenda	1.69 ± 0.45	1.00 ± 0.31	-41	111 ± 13	62 ± 12	-44	3.16 ± 0.32	1.83 ± 0.26	-42
TA5683 X Zenda	0.89 ± 0.11	0.80 ± 0.15	-10	67 ± 3	47 ± 11	-30	2.09 ± 0.06	1.27 ± 0.23	-39
TA5686 X Zenda	0.74 ± 0.00	1.23 ± 0.36	66	91 ± 0	50 ± 34	-45	2.49 ± 0.0	1.31 ± 0.97	-47
TA5687 X Zenda	1.59 ± 0.27	0.72 ± 0.19	-55	82 ± 10	59 ± 15	-27	2.7 ± 0.43	1.67 ± 0.48	-38
TA5688 X Zenda	1.69 ± 0.16	1.07 ± 0.25	-37	116 ± 7	75 ± 10	-35	3.33 ± 0.22	1.98 ± 0.40	-41
TA5689 X Zenda	0.91 ± 0.20	0.79 ± 0.52	-13	84 ± 11	52 ± 8	-38	2.59 ± 0.46	1.38 ± 0.25	-47

Table 4. Flag leaf Net CO_2 assimilation rate (µmol m^{-2} s^{-1}) and transpiration rate (mol m^{-2} s^{-1}) of 20 Robertsonian translocation lines and their parental lines at 10 days after first flowering/stress imposition under control and heat stress treatments in experiment 1. % change indicates the change in a trait under heat stress compared to control expressed in percentage. Mean ± standard error (n=3).

	Net CO_2 assimilation rate			**Transpiration rate**		
Genotype	**Control**	**Heat stress**	**% Change**	**Control**	**Heat stress**	**% Change**
Bob Dole	19.8 ± 1.9	17.9 ± 1.6	-9	7.9 ± 1.0	10.1 ± 1.9	28
TA5678 X Zenda/Bob Dole//Zenda	23.3 ± 0.5	21.6 ± 1.5	-7	9.3 ± 0.3	12.0 ± 0.8	29
Joe	16.5 ± 2.2	12.5 ± 4.2	-24	7.3 ± 1.1	9.1 ± 1.7	24
TA5088 X Joe	18.8 ± 0.7	14.7 ± 2.0	-22	8.8 ± 0.8	7.1 ± 0.6	-19
TA5598L2 X Joe	18.2 ± 0.7	16.9 ± 3.3	-7	7.6 ± 0.8	10.5 ± 1.0	38
TA5681 X Joe	17.6 ± 2.7	16.4 ± 1.8	-7	8.0 ± 1.2	9.7 ± 2.1	21
TA5683 X Joe	16.2 ± 0.7	14.1 ± 2.5	-13	8.2 ± 0.8	9.2 ± 2.5	12
TA5686 X Joe	17.2 ± 1.8	18.3 ± 0.7	6	8.9 ± 0.9	13.0 ± 0.6	45
TA5687 X Joe	18.8 ± 0.7	14.4 ± 0.4	-23	7.7 ± 0.3	9.1 ± 0.7	18
TA5688 X Joe	16.9 ± 0.1	13.2 ± 0.6	-22	7.8 ± 0.0	9.3 ± 1.2	19
TA5689 X Joe	14.3 ± 2.9	19.2 ± 0.7	34	7.2 ± 0.8	11.9 ± 0.3	65
Zenda	18.0 ± 0.2	16.3 ± 1.5	-10	8.4 ± 0.2	9.7 ± 1.3	16
TA5088 X Zenda	16.0 ± 1.0	15.5 ± 0.2	-3	6.1 ± 1.3	9.8 ± 1.5	60
TA5598L2 X Zenda	17.1 ± 0.1	21.5 ± 0.2	26	7.5 ± 0.0	12.1 ± 1.0	62
TA5678 X Zenda	20.0 ± 1.0	14.3 ± 1.4	-28	8.1 ± 0.7	8.5 ± 1.1	5
TA5680L1 X Zenda	17.6 ± 1.9	15.4 ± 1.7	-12	7.4 ± 0.7	9.2 ± 1.3	25
TA5681 X Zenda	20.5 ± 2.3	17.4 ± 1.5	-15	8.3 ± 0.4	9.2 ± 0.6	10
TA5682 X Zenda	18.1 ± 2.4	18.9 ± 1.7	4	7.5 ± 1.2	8.7 ± 0.2	15
TA5683 X Zenda	22.4 ± 0.7	18.4 ± 0.6	-18	8.6 ± 0.8	10.3 ± 2.0	20
TA5686 X Zenda	17.2 ± 0.1	14.5 ± 1.6	-16	6.7 ± 0.0	7.1 ± 0.7	7
TA5687 X Zenda	19.6 ± 2.1	20.2 ± 0.2	3	8.2 ± 0.4	13.4 ± 0.5	65
TA5688 X Zenda	23.2 ± 0.1	10.3 ± 1.5	-55	9.2 ± 0.0	5.1 ± 0.9	-44
TA5689 X Zenda	20.6 ± 0.6	18.2 ± 0.9	-12	8.2 ± 0.2	9.9 ± 0.7	20

Figure 3. Chlorophyll index averaged across genotypes grown under control and heat stress treatments at 7, 14, and 21 days after stress (DAS) in experiment 1. Different letters indicate significance between treatments at each time point based on LSD at 0.05.

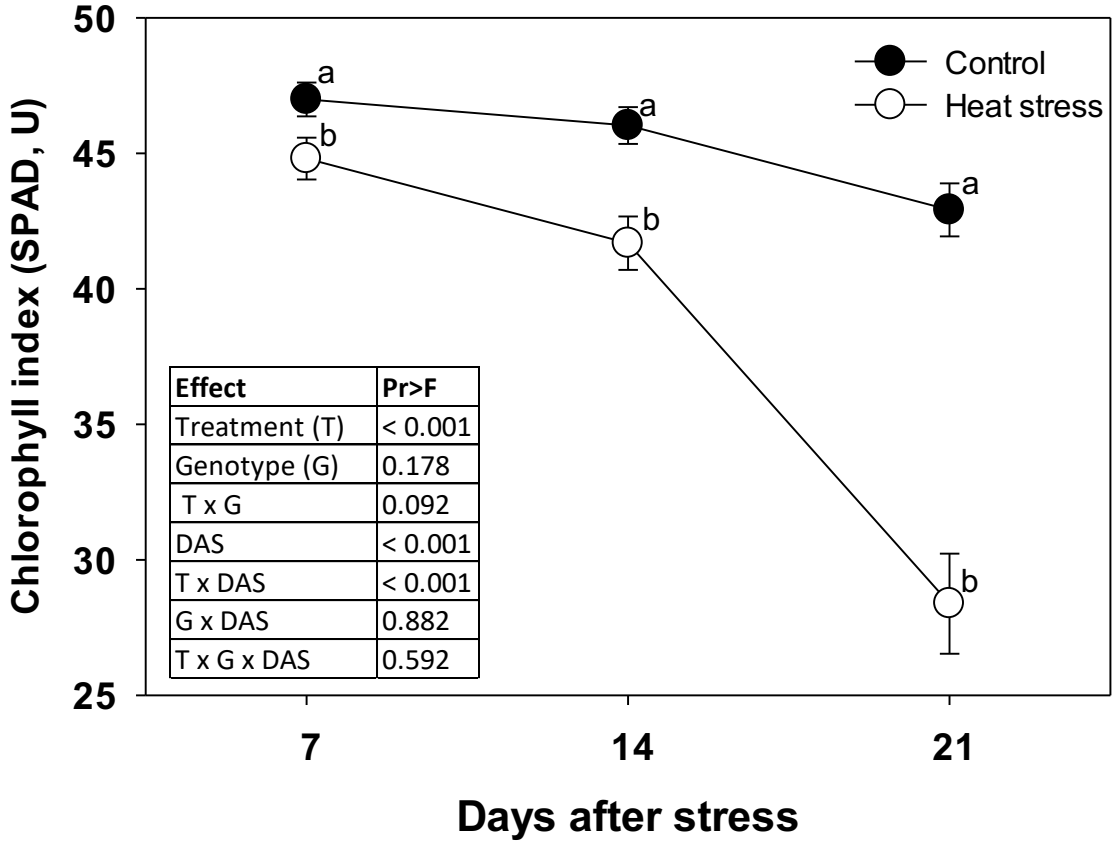


Figure 4. QY averaged across genotypes grown under control and heat stress treatments at 7, 14, and 21 days after stress (DAS) in experiment 1. Different letters indicate significance between treatments at each time point based on LSD at 0.05.

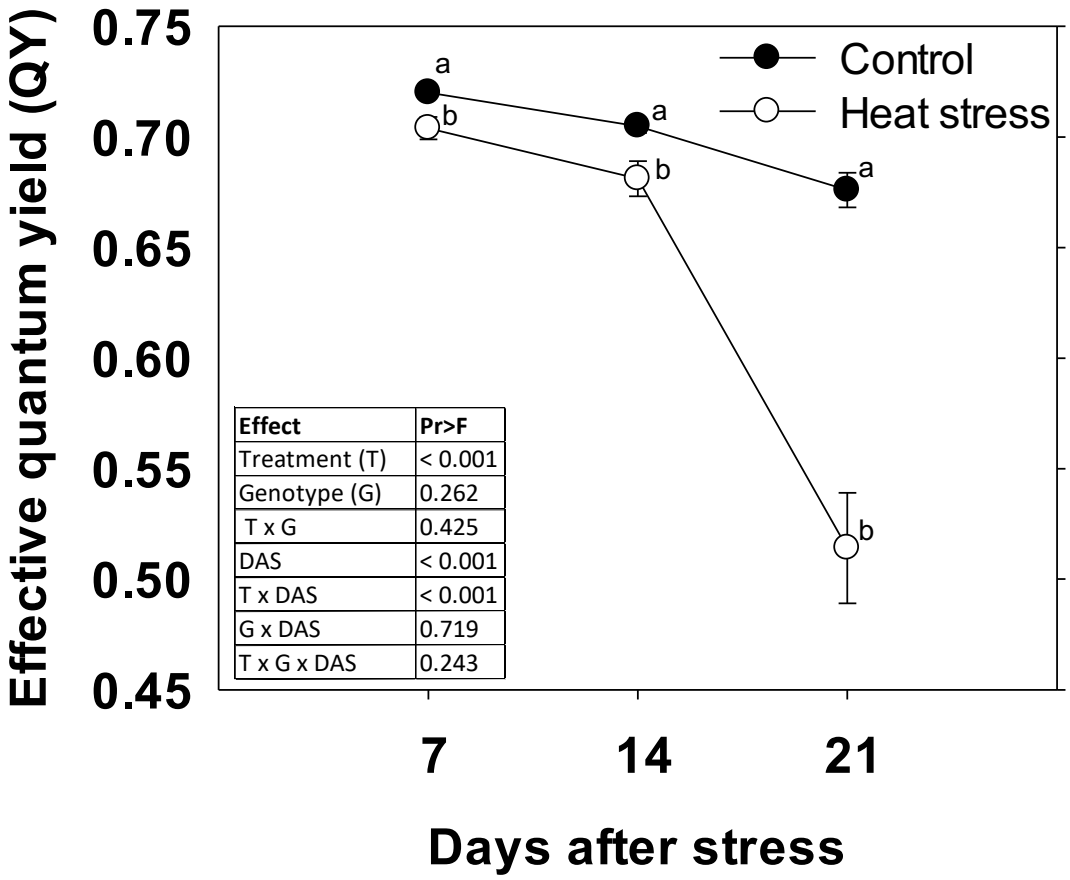


Table 5. Probability of effects of treatment (T), genotype (G), days after stress (DAS) and their interactions on physiological and yield parameters in experiment 2. Probability values highlighted in red are significant.

Trait	**Treatment (T)**	**Genotype (G)**	**T x G**	**DAS**	**T x DAS**	**G x DAS**	**T x G x DAS**
Physiological maturity (d)	<0.001	<0.001	<0.001	-	-	-	-
Plant Biomass (g plant^{-1})	0.838	<0.001	0.550	-	-	-	-
Seed Number (plant^{-1})	<0.001	0.0014	<0.001	-	-	-	-
Seed Weight (g plant^{-1})	<0.001	<0.001	<0.001	-	-	-	-
Chlorophyll Index (SPAD, U)	<0.001	<0.001	0.0044	<0.001	<0.001	0.422	0.017
Effective quantum yield of PSII (QY)	<0.001	0.0007	0.0147	<0.001	<0.001	0.014	0.318

Figure 5: Days to physiological maturity of two Robertsonian translocation lines and their parental lines under different temperature treatments in experiment 2. Days to physiological maturity were calculated as the number of days from first day of flowering to physiological maturity.

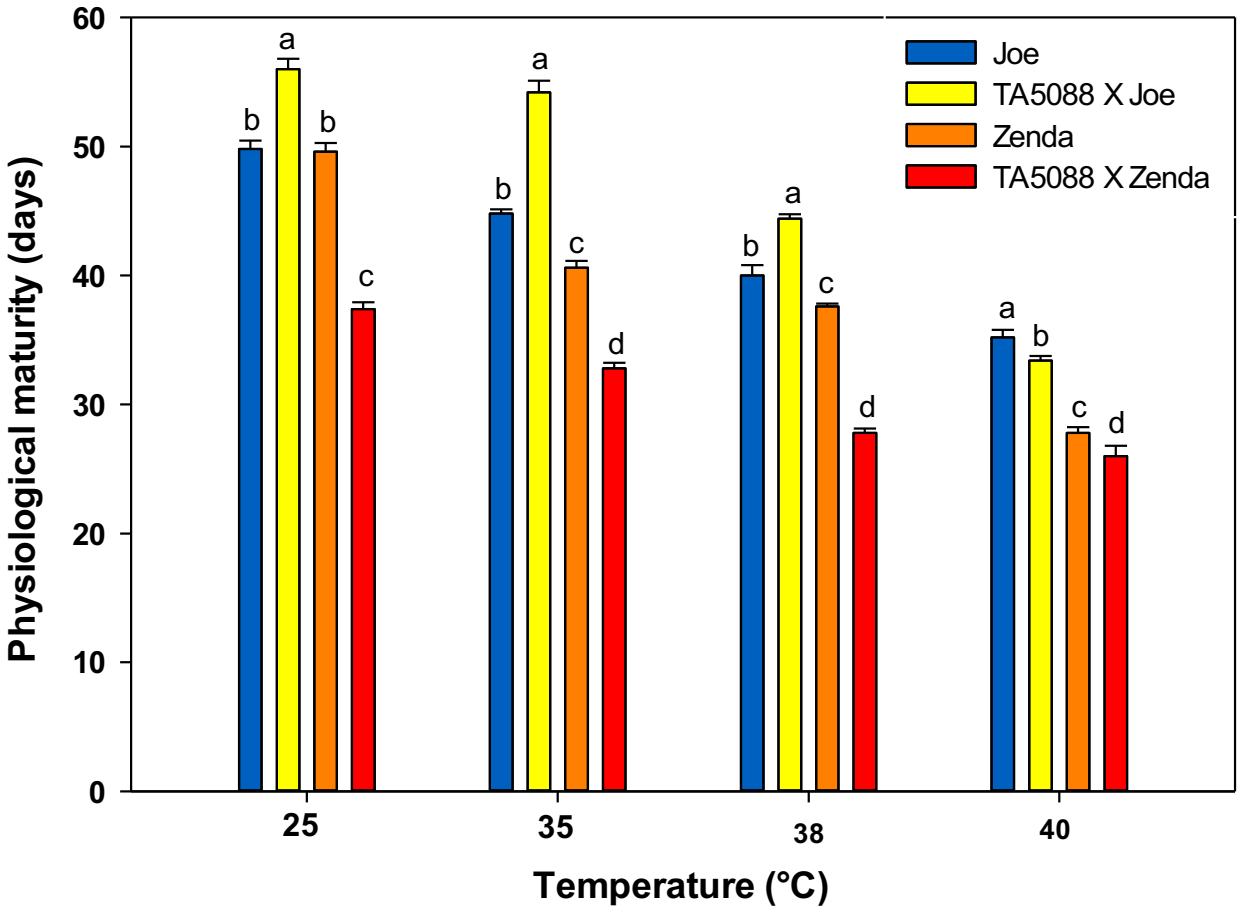


Figure 6. Seed number of the two Robertsonian translocation lines and their parental lines in experiment 2 across four temperature treatments. Error bars indicate ± standard error (n=5).

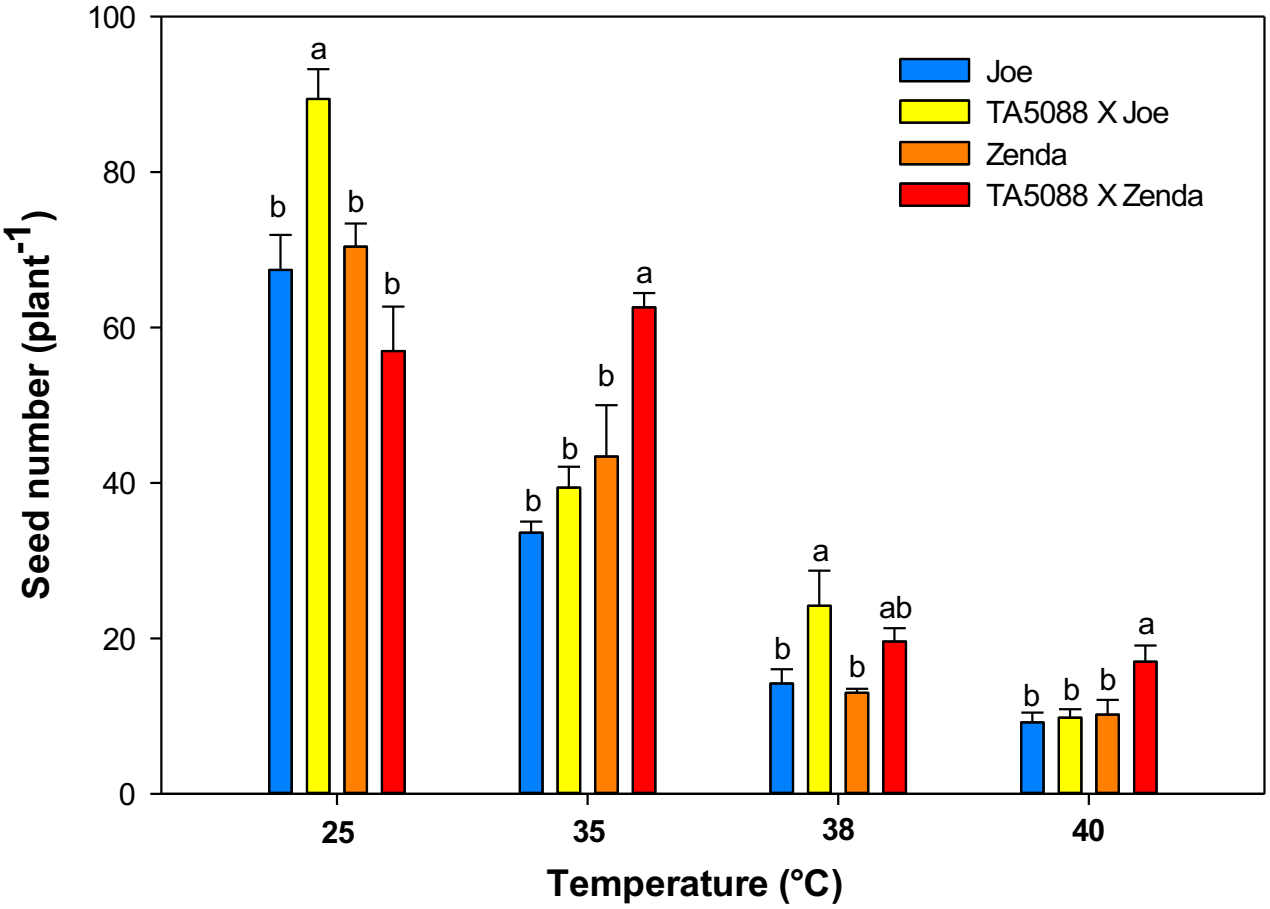


Figure 7. Seed weight in two Robertsonian translocation lines and their parental lines in experiment 2 across four temperature treatments. Error bars indicate ± standard error (n=5).

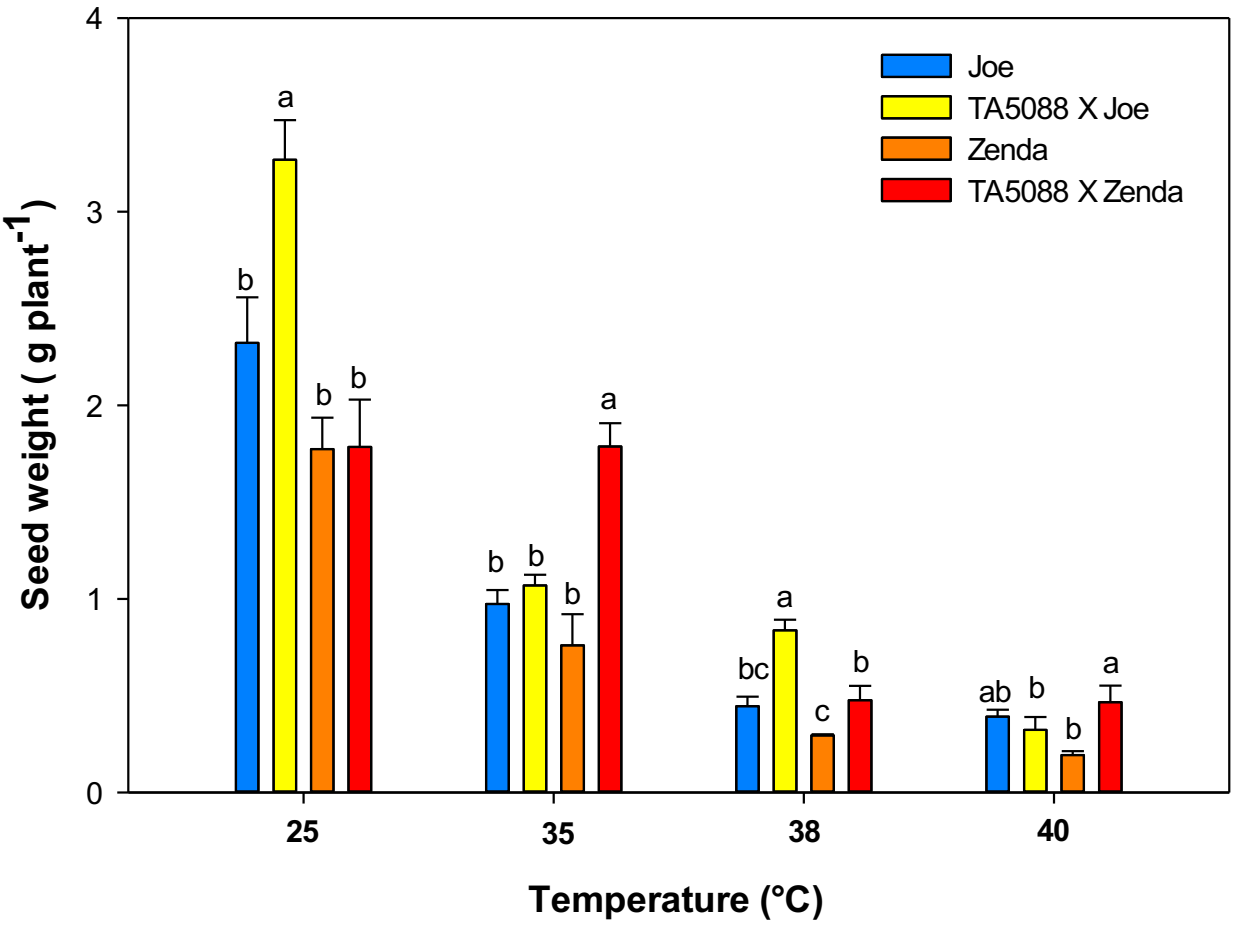


Table 6. Heat susceptibility index (HSI) for seed number and seed weight under heat stress conditions compared to control in experiment 2.

Genotype	Temperature	Seed Number	Seed Weight
Joe	35°C	1.3547	1.1657
Joe	38°C	1.0521	1.0420
Joe	40°C	1.0311	0.9782
TA5088 X Joe	35°C	1.5109	1.3498
TA5088 X Joe	38°C	0.9721	0.9589
TA5088 X Joe	40°C	1.0632	1.0600
Zenda	35°C	1.0360	1.1475
Zenda	38°C	1.0868	1.0755
Zenda	40°C	1.0211	1.0490
TA5088 X Zenda	35°C	-0.2654	-0.0024
TA5088 X Zenda	38°C	0.8746	0.9453
TA5088 X Zenda	40°C	0.8379	0.8696

Figure 8. Chlorophyll Index measured at 7, 14, and 21 days after stress imposition in two Robertsonian translocation lines and their parental lines under control (25°C, A) and heat stress (35°C, B; 38°C, C; and 40°C, D) treatments in experiment 2.

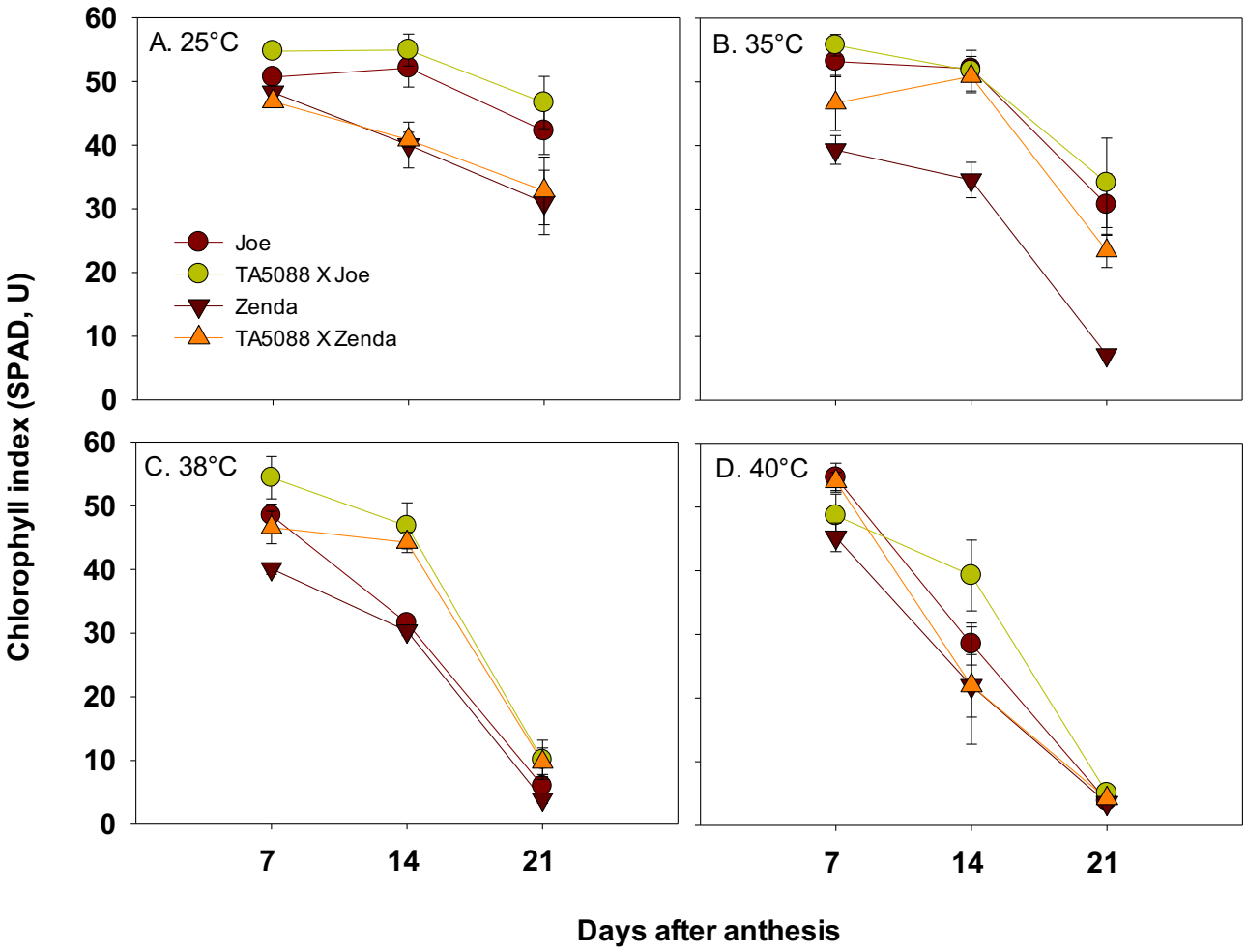
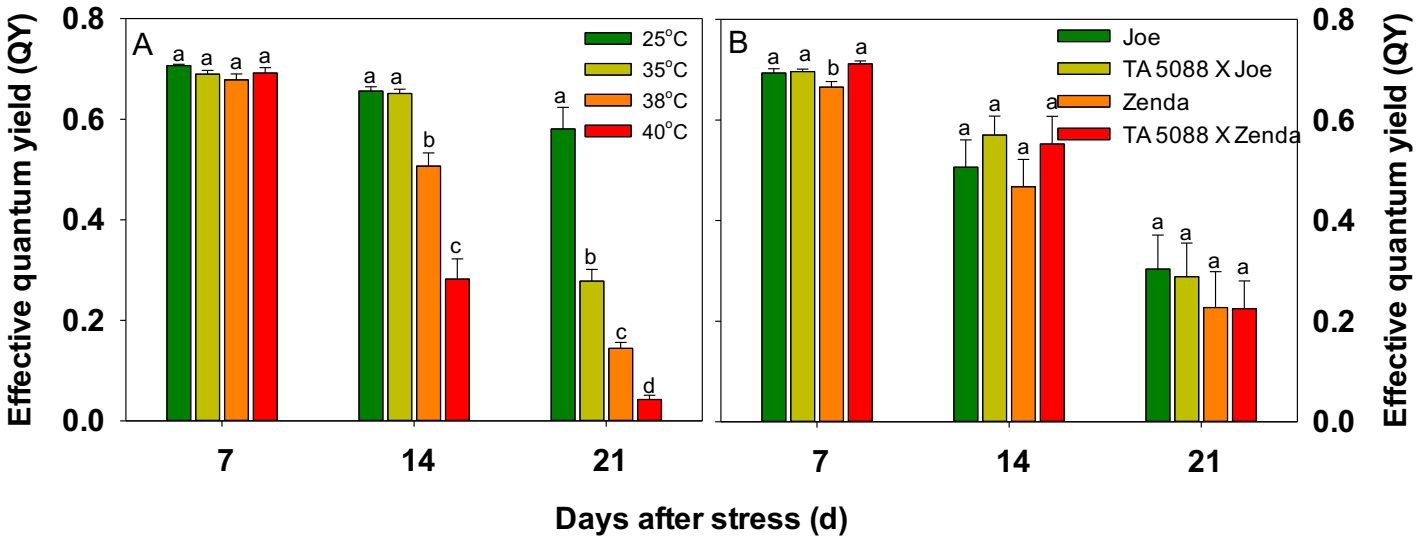


Figure 9. A. QY averaged across genotypes grown under 4 different temperatures at 7, 14, and 21 days after stress (DAS) in experiment 2. B. QY averaged across temperature treatments for 4 different genotypes including 2 Robertsonian translocation lines and their parental lines at 7, 14, and 21 days after stress (DAS) in experiment 2.



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Chapter 4 - General Discussion

Heat stress is known to negatively impact wheat yields and producers do not have a reliable agronomic practice to minimize terminal heat stress damage in wheat. The most feasible, reliable and long term option to address heat stress induced yield reduction is to develop heat-tolerant cultivars. For this reason, and also to ensure wheat productivity is sustained under future warmer climates, identifying alternative genetic resources for heat stress tolerance is necessary. Studies have suggested that using wild emmer wheat *T. diccocoides* or *A. speltoides* can help increase tolerance to different abiotic stresses including heat. Although *T. diccocoides* and *A. speltoides* accessions *per se* have been tested for heat stress response, selected core set of *T. diccocoides* based on molecular markers that captured a large range in diversity and translocations of *A. speltoides* in adapted backgrounds have not been tested for their effectiveness in minimizing heat stress damage. Hence, the above was the major rationale on which both these sets were systematically tested and findings included in chapters 2 and 3.

In chapter 2, among the 28 wild emmer wheat accessions a wide range in tolerance for physiological and yield related parameters was observed. The accession identified to be most prominent TA1020 recorded significantly higher seed number and seed weight under heat stress in the same background, providing evidence for higher level of tolerance. Similarly, in chapter 3, using the *A. speltoides* translocations the TA5088 recorded significantly higher seed number and weight in Zenda background and also to a certain extent in Joe. Interestingly, in both these cases the lines recorded higher seed number and weight in the same accession/translocation indicating the possibility of maintaining both seed numbers and seed weight under heat stress, which are the two key parameters that determine the final yield. Since the stress exposure lasting the entire

flowering stage did allow maintaining final seed numbers gives the opportunity for plant physiologists to explore these lines for genes or mechanism that allows for higher tolerance to pollen viability, pollen tube growth, fertilization and early embryo development. This will help in enhancing our knowledge on reproductive physiology under stress and at the same time allow developing molecular and genetic markers to help enhance resilience of pollen and female reproductive organ to heat stress in wheat breeding programs. Since they also recorded higher grain weight, a similar approach can be taken in these two lines to systematically ascertain the post flowering source-sink relationships, functional stay green, carbon balance (photosynthesis versus respiration), and their ability to maintain starch metabolism enzymes to maintain wheat yield and quality under future warmer climate. In summary, the findings after validation and identification of a narrower genetic region or a gene/s has the potential to help in improving both spring and winter wheat cultivars grown in the US and other parts of the world where wheat is expected to be vulnerable to changing climate

The majority of studies on the impacts of heat stress on wheat have been conducted under controlled environment conditions. Experiments in controlled environment settings provide a consistent temperature and allow to monitor water use and reassure that drought stress does not occur, making heat stress the only abiotic factor. Certain wild wheat species and Robertsonian translocations indicated high level of tolerance to heat stress under controlled environment conditions. However, in order to enhance wheat resistance to heat stress field experiments must be conducted to ensure these lines are adapted to the actual wheat growing environments. The next step would be to test these promising lines by using field-based phenotyping facilities where the infrastructure provides the resources to impose high day-time

heat stress (Bergkamp et al., 2018) or high night-time heat stress (Hein et al., 2019), before they can be included into breeding programs.

Collaborations

Collaborative Output 1

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Plant Methods

METHODOLOGY Open Access

Integrating field-based heat tents and cyber-physical system technology to phenotype high night-time temperature impact on winter wheat

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Abstract

Background: Many agronomic traits have been bred into modern wheat varieties, but wheat (*Triticum aestivum* L.) continues to be vulnerable to heat stress, with high night-time temperature (HNT) stress shown to have large negative impact on yield and quality. Global mean temperature during the day is consistently warming with the minimum night temperature increasing at a much quicker pace. Currently, there is no system or method that allows crop scientists to impose HNT stress at key developmental stages on wheat or crops in general under field conditions, involving diverse genotypes and maintaining a dynamic temperature differential within the tents compared to the outside.

Results: Through implementation of a side roll up and a top ventilation system, heaters, and a custom cyber-physical system using a Raspberry Pi, the heat tents were able to consistently maintain an elevated temperature through the night to differentiate heat stress impact on different genotypes. When the tents were placed in their day-time setting they were able to maintain ambient day-time temperature without having to be removed and replaced on the plots. Data averaged from multiple sensors over three consecutive weeks resulted in a consistent but small temperature difference of 0.25 °C within the tents, indicating even distribution of heat. While targeting a temperature differential of 4 °C, the tents were able to maintain an average differential of 3.2 °C consistently throughout the night-time heat stress period, compared to the outside ambient conditions. The impact of HNT stress was confirmed through a statistically significant yield reduction in eleven of the twelve genotypes tested. The average yield under HNT stress was reduced by 20.3% compared to the controls, with the highest reduction being 41.4% and a lowest reduction of 6.9%. Recommendations for fine-tuning the system are provided.

Conclusion: This methodology is easily accessible and can be widely utilized due to its flexibility and ease of construction. This system can be modified and improved based on some of the recommendations and has the potential to be used across other crops or plants as it is not reliant on access to any hardwired utilities. The method tested will help the crop community to quantify the impact of HNT stress, identify novel donors that induce tolerance to HNT and help the breeders develop crop varieties that are resilient to changing climate.

Keywords: Cyber-physical system, Heat stress, Heat tents, High night-time temperature, Raspberry Pi, Wheat

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My involvement in this research project consisted of assisting with planting the plots of the twelve winter wheat varieties utilized in the study. I was involved in discussions regarding the redesigning, a helping hand in modifying the tents used as the phenotyping infrastructure for the high night temperature stress imposition. I was involved with the wood framing of the heat tents, enclosing the tents with polyethylene film plastic, top vent and side roll vents installation. I contributed to installing the heaters, circulation fans, and wiring for the heating system which helped establish the cyber-physical system to control the temperatures inside the tents. During the growing season I helped maintain the wheat plots which included irrigation and other crop management activities. As the stress period began I was actively involved with the operation of the tents. Further, during the stress period, I helped with igniting the propane tanks and made sure the propane tanks were filled regularly, to ensure the tents had the power to heat throughout the night. Regarding the observations, I played a collaborative role in spike and flag leaf pre-dawn sampling (230 to 430 AM), during the stress period for further molecular and biochemical analysis. As the heat stress period ended and the wheat plots reached physiological maturity I was a part of the team that harvested and threshed for recording yield and yield related parameters.

Collaborative Output 2 (Under review in Scientific Reports)

Title – Improvised cyber-physical system captured post-flowering high night temperature impact on yield and quality of field grown wheat

Running Title – HNT affects field-grown wheat yield & quality

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My contribution to this research project consisted of hand-sowing the 320 winter wheat diversity panel across 6 replicate tents during the fall of 2018. A significant part of my involvement with the project consisted of the establishment of six 48’ by 30’ custom built field-based heat tents that served as the infrastructure for imposing high night-time temperature stress on the diversity panel. I played a key role throughout the entire construction of the tents, which lasted from middle of January well into middle of May of 2019. During the growing season I was involved with crop maintenance, which included regular irrigation and other agronomic interventions. In addition, I was assigned to record flowering phenology and then towards the end the physiological maturity, to be able to calculate the grain-filling duration across the entire panel. Once majority of the population was at 70% flowering heat stress was initiated. During the stress period, which lasted approximately 4-5 weeks I assisted with the daily closing (6:30 pm) and opening (6:00 am) of the tents, to impose or release stress, respectively. Finally, I was a part of a highly collaborative hand-harvesting of wheat plots and threshing to record biomass and grain yield that the team aims to use for further genetic analysis.

Collaborative Output 3 (Review article as a part of AGRON 950 submitted to Plant Physiology Reports)

Plant Physiology Reports

Title - Impacts of heat, drought, and their interaction with nutrients on physiology, grain yield, and quality in field crops

--Manuscript Draft--

Manuscript Number:	INPP-D-20-00147R1
Full Title:	Title - Impacts of heat, drought, and their interaction with nutrients on physiology, grain yield, and quality in field crops
Article Type:	Review Articles
Funding Information:	National Science Foundation, USA (1736192) Dr. Krishna Jagadish
Abstract:	Among different abiotic stresses that negatively affect crop productivity, heat and drought stresses are the most common and their combined stress is highly prevalent under field conditions. Significant research progress has been achieved on heat or drought stress responses in crops, and, more recently, combined heat and drought stress is receiving additional emphasis. Knowledge generated either through controlled environmental chambers or field-based facilities on heat and drought impacts on field crops captures a part of the complex phenomenon. An additional dimension to this interaction and the ability of the plants to respond to and mitigate these stresses depends on their ability to take up and efficiently utilize micro- and macro-nutrients. Improved nutrient use enhances the tolerance potential of the plant through production of essential compounds such as amino acids and carbohydrates. These, in turn, provide energy required to synthesize stress ameliorating compounds such as reactive oxygen species scavengers, including superoxide dismutase and catalase, and balanced hormonal production, which have the potential to reduce or minimize the damage. The review focuses on information generated from controlled environments and field conditions that synthesizes the interactions between heat and nutrients or drought and nutrients in cereals and soybean. The major objective is to highlight if different types of nutrients applied as additional treatment factors either ameliorate or aggravate the impact of these abiotic stress combinations on physiological parameters, grain yield, and quality. In addition, knowledge gaps in addressing these interactions are identified, and directions for future research to help bridge these gaps are given.
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My collaboration in this publication included systematic compilation of relevant scientific papers related to heat stress section of the review. I contributed my part by summarizing the impact of heat stress and nutrient interactions on key physiological traits and yield related parameters. During this collaboration I shared information with co-authors on the effects of drought and nutrient interactions as well as the impacts of heat stress on grain quality and its components. I learnt the approach of extracting data from relevant scientific literature, and to summarize and include in the review to quantify the impacts of heat and drought stresses and their interaction with nutrients. From the data extracted I conducted calculations to determine the percent change of effects of nutrients on heat stresses and were included as supportive information to the submitted review article.

Appendix 1: Mean seed number, seed weight (g plant^{-1}), Net CO_2 assimilation (µmol m^{-2} s^{-1}) and maximum quantum yield of PSII (Fv/Fm) in 28 wild wheat accessions and two check lines under control and heat stress conditions in experiment 1.

Genotype	**Treatment**	**Seed Number**	**Seed Weight**	**Net CO_2 Assimilation**		**Quantum yield of PSII**	
				5 DAS	**20 DAS**	**5 DAS**	**20 DAS**
C306	Control	592 ± 0	11.7 ± 0	19. 1 ± 0.04	18.0 ± 0.3	0.80 ± 0	0.77 ± 0
Overley	Control	723 ± 68	22.03 ± 3	16.99 ± 0.48	12.26 ± 0.33	0.802 ± 0	0.776 ± 0.010
TA1000	Control	279 ± 118	10.67 ± 3.2	23.90 ± 0.21	11.26 ± 1.46	0.799 ± 0.001	0.770 ± 0.002
TA1005	Control	307 ± 11	8.953 ± 0.5	20.58 ± 0.80	19.38 ± 0.36	0.813 ± 0.003	0.798 ± 0
TA1020	Control	412 ± 169	9.346 ± 2.4	21.78 ± 0.80	19.41 ± 0.66	0.8 ± 0.003	0.786 ± 0.016
TA1059	Control	296 ± 30	8.046 ± 0.2	22.17 ± 0.77	17.01 ± 0.93	0.792 ± 0.004	0.776 ± 0.008
TA1077	Control	208 ± 12	4.581 ± 0.2	27.17 ± 2.50	23.39 ± 1.56	0.801 ± 0.009	0.774 ± 0.009
TA1108	Control	329 ± 15	8.550 ± 0.2	19.47 ± 0.22	15.39 ± 1.88	0.787 ± 0	0.7774 ± 0.11
TA1121	Control	383 ± 69	7.504 ± 1.3	21.93 ± 0.23	12.12 ± 0.89	0.779 ± 0.002	0.760 ± 0.023
TA1153	Control	98 ± 0	1.810 ± 0	22.16 ± 0.32	13.16 ± 0.45	0.797 ± 0	0.755 ± 0
TA1171	Control	159 ± 2	5.473 ± 0.2	17.92 ± 0.43	17.84 ± 0.23	0.807 ± 0.001	0.798 ± 0.019
TA1187	Control	327 ± 0	8.427 ± 0	18.11 ± 0.74	8.47 ± 0.20	0.801 ± 0	0.780 ± 0
TA1191	Control	170 ± 40	2.302 ± 0.2	19.19 ± 0.92	8.78 ± 3.47	0.799 ± 0.010	0.651 ± 0.129
TA1195	Control	249 ± 55	8.488 ± 1.6	21.21 ± 0.68	16.07 ± 0.25	0.820 ± 0.008	0.818 ± 0.012
TA120	Control	380 ± 61	11.96 ± 2.6	20.44 ± 0.95	10.38 ± 0.21	0.804 ± 0.009	0.799 ± 0.006
TA123	Control	193 ± 27	7.671 ± 0.8	19.84 ± 0.24	14.74 ± 0.69	0.812 ± 0.012	0.782 ± 0.005
TA130	Control	157 ± 45	4.703 ± 1.5	21.21 ± 0.75	14.77 ± 1.32	0.820 ± 0.002	0.785 ± 0.005
TA1390	Control	152 ± 0	3.904 ± 0	24.30 ± 0.13	13.31 ± 0.28	0.803 ± 0	0.794 ± 0
TA1398	Control	246 ± 10	7.551 ± 0.8	21.95 ± 0.12	12.27 ± 1.04	0.792 ± 0	0.801 ± 0.001
TA1411	Control	127 ± 3	3.267 ± 0.4	21.94 ± 0.13	16.40 ± 1.02	0.792 ± 0.002	0.782 ± 0.004
TA1415	Control	398 ± 92	11.02 ± 2.2	20.64 ± 0.83	13.10 ± 0.21	0.803 ± 0.015	0.754 ± 0.011

TA1434	Control	201 ± 89	4.686 ± 2.1	19.42 ± 0.33	13.80 ± 0.77	0.782 ± 0.002	0.791 ± 0.002
TA1459	Control	201 ± 6	8.476 ± 5.4	18.73 ± 1.40	15.77 ± 0.22	0.809 ± 0.001	0.786 ± 0.003
TA1464	Control	71 ± 54	2.250 ± 1.6	20.68 ± 0.62	16.63 ± 0.65	0.804 ± 0.007	0.785 ± 0.001
TA1465	Control	NA	NA	NA	NA	NA	± 0
TA1471	Control	195 ± 0	6.368 ± 0	21.14 ± 0.18	16.63 ± 0.26	NA	0.803 ± 0
TA54	Control	236 ± 25	6.796 ± 0.5	19.29 ± 0.14	14.55 ± 0.21	NA	0.790 ± 0
TA56	Control	148 ± 41	4.831 ± 1.2	21.49 ± 1.23	6.93 ± 1.77	0.755 ± 0.049	0.595 ±0.087
TA74	Control	151 ± 45	3.740 ± 1.3	21.05 ± 0.26	19.48 ± 0.63	0.798 ± 0.011	0.789 ± 0.002
TA90	Control	350 ± 96	8.627 ± 1.9	21.80 ± 1.20	13.38 ± 0.23	0.793 ± 0.002	0.754 ± 0.014
C306	Heat stress	250 ± 204	8.109 ± 6.5	16.82 ± 0.24	13.68 ± 0.04	0.754 ± 0.22	0.731 ± 0.026
Overley	Heat stress	583 ± 48	13.48 ± 1.2	15.67 ± 0.31	8.46 ± 0.42	0.771 ± 0.22	0.704 ± 0.11
TA1000	Heat stress	205 ± 43	6.222 ± 1.1	18.62 ± 0.67	5.45 ± 0.73	0.778 ± 0.005	0.640 ± 0.44
TA1005	Heat stress	267 ± 57	6.606 ± 1.2	16.71 ± 0.89	7.34 ± 1.14	0.762 ± 0.002	0.436 ± 0.230
TA1020	Heat stress	292 ± 41	8.046 ± 1.1	25.68 ± 1.01	10.52 ± 1.01	0.766 ± 0.003	0.483 ± 0.124
TA1059	Heat stress	105 ± 70	3.164 ± 2.3	21.82 ± 1.83	8.71 ± 0.38	0.779 ± 0	0.743 ± 0.022
TA1077	Heat stress	127 ± 13	2.986 ± 0.1	22.74 ± 0.91	17.08 ± 1.15	0.761 ± 0.003	0.738 ± 0.009
TA1108	Heat stress	440 ± 41	10.75 ± 0.9	21.40 ± 0.96	10.71 ± 0.20	0.769 ± 0.007	±
TA1121	Heat stress	244 ± 18	5.843 ± 0.9	19.17 ± 0.47	2.60 ± 0.38	0.776 ± 0.008	0.162 ± 0.162
TA1153	Heat stress	295 ± 110	6.429 ± 2.7	20.35 ± 1.29	13.50 ± 0.33	0.784 ± 0	0.290 ± 0
TA1171	Heat stress	95 ± 18	2.129 ± 0.4	13.68 ± 0.60	4.97 ± 1.14	0.781 ± 0.004	0.654 ± 0.077
TA1187	Heat stress	200 ± 26	4.403 ± 0.2	24.36 ± 1.65	8.66 ± 0.61	0.749 ± 0	0.723 ± 0.053
TA1191	Heat stress	261 ± 26	3.090 ± 0.4	19.89 ± 0.50	13.12 ± 0.29	0.785 ± 0	0.693 ± 0.010
TA1195	Heat stress	165 ± 36	4.299 ± 1.1	14.85 ± 0.98	11.35 ± 1.34	0.785 ± 0.003	0.765 ± 0.10
TA120	Heat stress	159 ± 67	5.176 ± 2.2	15.11 ± 1.39	1.54 ± 0.22	0.697 ± 0.054	0.627 ± 0.067
TA123	Heat stress	198 ± 13	5.754 ± 0.2	11.53 ± 1.22	5.41 ± 0.52	0.775 ± 0.007	0.741 ± 0.010
TA130	Heat stress	108 ± 19	2.636 ± 0.3	19.57 ± 0.71	7.07 ± 1.34	0.779 ± 0.011	0.635 ± 0.075
TA1390	Heat stress	58 ± 10	1.493 ± 0.1	25.99 ± 1.35	16.23 ± 0.16	0.7785 ± 0.003	0.724 ± 0
TA1398	Heat stress	180 ± 35	5.260 ± 1.1	15.11 ± 0.60	9.22 ± 5.80	0.776 ± 0.002	0.743 ± 0.022

TA1411	Heat stress	229 ± 77	6.406 ± 2.2	18.45 ± 0.92	9.09 ± 5.80	0.763 ± 0.005	0.719 ± 0.013
TA1415	Heat stress	267 ± 10	4.579 ± 0.2	17.81 ± 0.45	2.09 ± 0.67	0.761 ± 0.002	0.068 ± 0.068
TA1434	Heat stress	141 ± 9	2.630 ± 0.1	17.72 ± 0.96	6.80 ± 0.82	0.773 ± 0.005	0.767 ± 0.020
TA1459	Heat stress	144 ± 40	5.596 ± 1.2	18.27 ± 0.76	2.22 ± 0.19	0.774 ± 0.008	0.647 ± 0.065
TA1464	Heat stress	105 ± 37	2.705 ± 0.9	16.29 ± 1.02	6.74 ± 1.26	0.775 ± 0	0.469 ± 0.183
TA1465	Heat stress	360 ± 109	8.556 ± 1.1	23.00 ± 0.29	3.12 ± 0.45	0.765 ± 0.004	0.668 ± 0.037
TA1471	Heat stress	118 ± 0	4.220 ± 0	19.38 ± 0.15	12.10 ± 0.22	0.764 ± 0	0.756 ± 0.010
TA54	Heat stress	170 ± 17	4.366 ± 0.5	17.86 ± 0.42	6.60 ± 1.11	0.766 ± 0.004	0.448 ± 0.183
TA56	Heat stress	29 ± 3	0.784 ± 0.1	14.12 ± 0.10	8.31 ± 0.12	NA	0.715 ± 0.039
TA74	Heat stress	107 ± 14	2.817 ± 0.4	21.57 ± 1.48	12.37 ± 1.84	0.759 ± 0.013	0.704 ± 0.025
TA90	Heat stress	262 ± 61	7.842 ± 2.3	19.56 ± 0.76	7.49 ± 0.81	0.762 ± 0.007	0.706 ± 0.013

Appendix 2: Mean flag leaf chlorophyll index (SPAD, U) and effective quantum yield (QY) of 20 Robertsonian translocation and 3 parental lines under control and heat stress conditions measured at different days after stress in experiment 1. Values are mean ± standard error (n=3).

Genotypes	**Treatment**	**Days after stress**	**Chlorophyll index**	**Effective quantum yield**
Bob Dole	Control	7 DAS	36.4 ± 0	0.715 ± 0.003
TA5678 X Zenda/Bob Dole//Zenda	Control	7 DAS	46.1 ± 2.3	0.721 ± 0.005
Joe	Control	7 DAS	40.25 ± 2.2	0.715 ± 0.008
TA5088 X Joe	Control	7 DAS	43.3 ± 3.0	0.711 ± 0.003
TA5598 L2 X Joe	Control	7 DAS	49.6 ± 0.3	0.718 ± 0.007
TA5681 X Joe	Control	7 DAS	41.43 ± 3.9	0.724 ± 0.004
TA5683 X Joe	Control	7 DAS	43.5 ± 1.0	0.715 ± 0.007
TA5686 X Joe	Control	7 DAS	44.93 ± 1.8	0.716 ± 0.004
TA5687 X Joe	Control	7 DAS	44 ± 0.6	0.725 ± 0.004
TA5688 X Joe	Control	7 DAS	43.1 ± 0.5	0.720 ± 0.002
TA5689 X Joe	Control	7 DAS	43.36 ± 1.0	0.708 ± 0.003
Zenda	Control	7 DAS	42.9 ± 2.0	0.734 ± 0.001
TA5088 X Zenda	Control	7 DAS	47.96 ± 1.5	0.707 ± 0.003
TA5598 L2 X Zenda	Control	7 DAS	40.6 ± 0	0.716 ± 0
TA5678 X Zenda	Control	7 DAS	45.56 ± 2.0	0.734 ± 0
TA5680 L1 X Zenda	Control	7 DAS	45.73 ± 2.6	0.715 ± 0.007
TA5681 X Zenda	Control	7 DAS	39.3 ± 2.3	0.734 ± 0.001
TA5682 X Zenda	Control	7 DAS	41.23 ± 2.8	0.723 ± 0.005
TA5683 X Zenda	Control	7 DAS	45 ± 1.8	0.714 ± 0.003
TA5686 X Zenda	Control	7 DAS	42.5 ± 0	0.703 ± 0
TA5687 X Zenda	Control	7 DAS	42.93 ± 0	0.711 ± 0.004
TA5688 X Zenda	Control	7 DAS	43.77 ± 0.7	0.707 ± 0.004
TA5689 X Zenda	Control	7 DAS	44.33 ± 1.0	0.717 ± 0.007
Bob Dole	Control	14 DAS	37.1 ± 0	0.670 ± 0.024
TA5678 X Zenda/Bob Dole//Zenda	Control	14 DAS	45.13 ± 2.3	0.695 ± 0.006
Joe	Control	14 DAS	33.4 ± 2.1	0.685 ± 0.012
TA5088 X Joe	Control	14 DAS	38.36 ± 4.4	0.691 ± 0.005
TA5598 L2 X Joe	Control	14 DAS	48.03 ± 0.4	0.696 ± 0.009
TA5681 X Joe	Control	14 DAS	39.5 ± 4.1	0.663 ± 0.023
TA5683 X Joe	Control	14 DAS	42.9 ± 1.7	0.661 ± 0.041
TA5686 X Joe	Control	14 DAS	44.36 ± 2.1	0.707 ± 0
TA5687 X Joe	Control	14 DAS	44.9 ± 1.1	0.653 ± 0.016

TA5688 X Joe	Control	14 DAS	43.8 ± 0.5	0.715 ± 0.010
TA5689 X Joe	Control	14 DAS	42.3 ± 0.5	0.703 ± 0.006
Zenda	Control	14 DAS	40.8 ± 0.4	0.718 ± 0.008
TA5088 X Zenda	Control	14 DAS	42.26 ± 5.0	0.693 ± 0.006
TA5598 L2 X Zenda	Control	14 DAS	39.4 ± 0	0.701 ± 0
TA5678 X Zenda	Control	14 DAS	44.56 ± 2.1	0.715 ± 0.006
TA5680 L1 X Zenda	Control	14 DAS	44.86 ± 2.5	0.720 ± 0.001
TA5681 X Zenda	Control	14 DAS	37.63 ± 2.8	0.709 ± 0.005
TA5682 X Zenda	Control	14 DAS	40.26 ± 3.7	0.708 ± 0.014
TA5683 X Zenda	Control	14 DAS	43.96 ± 1.9	0.700 ± 0.007
TA5686 X Zenda	Control	14 DAS	42.8 ± 0	0.713 ± 0
TA5687 X Zenda	Control	14 DAS	43.93 ± 0.4	0.706 ± 0.004
TA5688 X Zenda	Control	14 DAS	43.72 ± 0.4	0.702 ± 0
TA5689 X Zenda	Control	14 DAS	41.96 ± 0.6	0.668 ± 0.025
Bob Dole	Control	21 DAS	37.5 ± 0	0.624 ± 0.061
TA5678 X Zenda/Bob Dole//Zenda	Control	21 DAS	44.4 ± 2.6	0.678 ± 0.025
Joe	Control	21 DAS	37.75 ± 1.2	0.557 ± 0.010
TA5088 X Joe	Control	21 DAS	41.16 ± 2.7	0.641 ± 0.037
TA5598 L2 X Joe	Control	21 DAS	45.3 ± 2.3	0.681 ± 0.004
TA5681 X Joe	Control	21 DAS	35.63 ± 7.0	0.447 ± 0.107
TA5683 X Joe	Control	21 DAS	44.96 ± 5.2	0.6 ± 0.070
TA5686 X Joe	Control	21 DAS	35.76 ± 1.4	0.504 ± 0.128
TA5687 X Joe	Control	21 DAS	41.73 ± 2.3	0.603 ± 0.051
TA5688 X Joe	Control	21 DAS	45.16 ± 0.3	0.523 ± 0.134
TA5689 X Joe	Control	21 DAS	38.83 ± 3.8	0.652 ± 0.042
Zenda	Control	21 DAS	40.75 ± 0.03	0.671 ± 0.034
TA5088 X Zenda	Control	21 DAS	44.76 ± 2.7	0.691 ± 0.017
TA5598 L2 X Zenda	Control	21 DAS	38.3 ± 0	0.686 ± 0
TA5678 X Zenda	Control	21 DAS	41.16 ± 1.8	0.681 ± 0.024
TA5680 L1 X Zenda	Control	21 DAS	44.23 ± 2.7	0.655 ± 0.022
TA5681 X Zenda	Control	21 DAS	37.26 ± 8.1	0.636 ± 0.047
TA5682 X Zenda	Control	21 DAS	40.73 ± 8.6	0.680 ± 0.026
TA5683 X Zenda	Control	21 DAS	39.83 ± 3.7	0.648 ± 0.041
TA5686 X Zenda	Control	21 DAS	44.9 ± 0	0.726 ± 0
TA5687 X Zenda	Control	21 DAS	44.86 ± 0.2	0.651 ± 0.031
TA5688 X Zenda	Control	21 DAS	41.37 ± 3.2	0.628 ± 0.053
TA5689 X Zenda	Control	21 DAS	37.13 ± 2.9	0.494 ± 0.153
Bob Dole	Heat Stress	7 DAS	42.9 ± 1.4	0.729 ± 0.005
TA5678 X Zenda/Bob Dole//Zenda	Heat Stress	7 DAS	45.53 ± 2.2	0.672 ± 0.008
Joe	Heat Stress	7 DAS	39.9 ± 1.7	0.71 ± 0
TA5088 X Joe	Heat Stress	7 DAS	47.8 ± 0	0.682 ± 0.008

TA5598 L2 X Joe	Heat Stress	7 DAS	46.26 ± 1.2	0.677 ± 0.013
TA5681 X Joe	Heat Stress	7 DAS	43.1 ± 3.4	0.725 ± 0.004
TA5683 X Joe	Heat Stress	7 DAS	48.6 ± 1.5	0.698 ± 0.007
TA5686 X Joe	Heat Stress	7 DAS	45.83 ± 2.3	0.713 ± 0.008
TA5687 X Joe	Heat Stress	7 DAS	48 ± 2.1	0.708 ± 0.004
TA5688 X Joe	Heat Stress	7 DAS	36.8 ± 6.6	0.686 ± 0.013
TA5689 X Joe	Heat Stress	7 DAS	38.93 ± 5.5	0.721 ± 0.002
Zenda	Heat Stress	7 DAS	44.95 ± 2.2	0.726 ± 0.004
TA5088 X Zenda	Heat Stress	7 DAS	47.23 ± 0.9	0.715 ± 0.007
TA5598 L2 X Zenda	Heat Stress	7 DAS	44.4 ± 1.1	0.733 ± 0.002
TA5678 X Zenda	Heat Stress	7 DAS	46.46 ± 1.6	0.703 ± 0.003
TA5680 L1 X Zenda	Heat Stress	7 DAS	42.93 ± 2.8	0.719 ± 0.004
TA5681 X Zenda	Heat Stress	7 DAS	46.7 ± 3.4	0.716 ± 0.005
TA5682 X Zenda	Heat Stress	7 DAS	50.65 ± 0.1	0.706 ± 0.007
TA5683 X Zenda	Heat Stress	7 DAS	45.56 ± 2.2	0.718 ± 0.006
TA5686 X Zenda	Heat Stress	7 DAS	47.65 ± 2.5	0.726 ± 0.004
TA5687 X Zenda	Heat Stress	7 DAS	47.03 ± 1.5	0.711 ± 0.004
TA5688 X Zenda	Heat Stress	7 DAS	36.9 ± 8.2	0.689 ± 0.002
TA5689 X Zenda	Heat Stress	7 DAS	44.46 ± 5.0	0.711 ± 0.011
Bob dole	Heat Stress	14 DAS	40.8 ± 0.2	0.698 ± 0.009
TA5678 X Zenda/Bob Dole//Zenda	Heat Stress	14 DAS	46.76 ± 4.6	0.665 ± 0.11
Joe	Heat Stress	14 DAS	40.2 ± 0.3	0.66 ± 0
TA5088 X Joe	Heat Stress	14 DAS	42.16 ± 0	0.673± 0.012
TA5598 L2 X Joe	Heat Stress	14 DAS	36.96 ± 3.1	0.654 ± 0.004
TA5681 X Joe	Heat Stress	14 DAS	39.86 ± 2.8	0.693 ± 0.012
TA5683 X Joe	Heat Stress	14 DAS	45.66 ± 2.5	0.685 ± 0.009
TA5686 X Joe	Heat Stress	14 DAS	44.33 ± 2.2	0.669 ± 0.009
TA5687 X Joe	Heat Stress	14 DAS	39.86 ± 3.4	0.661 ± 0.023
TA5688 X Joe	Heat Stress	14 DAS	35.45 ± 5.9	0.66 ± 0
TA5689 X Joe	Heat Stress	14 DAS	37.4 ± 4.8	0.718 ± 0.009
Zenda	Heat Stress	14 DAS	42.8 ± 2.5	0.69 ± 0.007
TA5088 X Zenda	Heat Stress	14 DAS	40.53 ± 1.9	0.668 ± 0.44
TA5598 L2 X Zenda	Heat Stress	14 DAS	37.75 ± 6.7	0.709 ± 0.002
TA5678 X Zenda	Heat Stress	14 DAS	40.96 ± 4.5	0.707 ± 0.003
TA5680 L1 X Zenda	Heat Stress	14 DAS	42.9 ± 2.7	0.716 ± 0.009
TA5681 X Zenda	Heat Stress	14 DAS	40.23 ± 2.7	0.719 ± 0.008
TA5682 X Zenda	Heat Stress	14 DAS	48.1 ± 0.9	0.702 ± 0
TA5683 X Zenda	Heat Stress	14 DAS	44.4 ± 1.9	0.713 ± 0.005
TA5686 X Zenda	Heat Stress	14 DAS	41.75 ± 5.3	0.653 ± 0.033
TA5687 X Zenda	Heat Stress	14 DAS	38.7 ± 3.6	0.704 ± 0.019
TA5688 X Zenda	Heat Stress	14 DAS	33.95 ± 6.2	0.674 ± 0.006
TA5689 X Zenda	Heat Stress	14 DAS	42.1 4.3	0.688B ± 0.009

Bob dole	Heat Stress	21 DAS	40.75 ± 0	0.627 ± 0.042
TA5678 X Zenda/Bob Dole//Zenda	Heat Stress	21 DAS	40.23 ± 7.0	0.534 ± 0.077
Joe	Heat Stress	21 DAS	40.8 ± 0	0.506 ± 0
TA5088 X Joe	Heat Stress	21 DAS	31.26 ± 0	0.556 ± 0.080
TA5598 L2 X Joe	Heat Stress	21 DAS	22.96 ± 2.3	0.470 ± 0.043
TA5681 X Joe	Heat Stress	21 DAS	30.3 ± 6.0	0.509 ± 0.123
TA5683 X Joe	Heat Stress	21 DAS	23.53 ± 6.1	0.408 ± 0.136
TA5686 X Joe	Heat Stress	21 DAS	31.13 ± 2.8	0.579 ± 0.015
TA5687 X Joe	Heat Stress	21 DAS	21.5 ± 7.8	0.491 ± 0.151
TA5688 X Joe	Heat Stress	21 DAS	31.7 ± 6.9	0.523 ± 0.009
TA5689 X Joe	Heat Stress	21 DAS	13.76 ± 8.6	0.529 ± 0.132
Zenda	Heat Stress	21 DAS	25.7 ± 6.3	0.664 ± 0.034
TA5088 X Zenda	Heat Stress	21 DAS	24.66 ± 4.3	0.591 ± 0.068
TA5598 L2 X Zenda	Heat Stress	21 DAS	33.15 ± 10.2	0.669 ± 0.03288
TA5678 X Zenda	Heat Stress	21 DAS	31.26 ± 6.5	0.562 ± 0.050
TA5680 L1 X Zenda	Heat Stress	21 DAS	34.06 ± 5.3	0.696 ± 0.014
TA5681 X Zenda	Heat Stress	21 DAS	18 ± 8.0	0.533 ± 0.139
TA5682 X Zenda	Heat Stress	21 DAS	22.95 ± 10.0	0.566 ± 0.084
TA5683 X Zenda	Heat Stress	21 DAS	31.46 ± 6.2	0.650 ± 0.018
TA5686 X Zenda	Heat Stress	21 DAS	18.75 ± 12.3	0.39 ± 0.190
TA5687 X Zenda	Heat Stress	21 DAS	24.03 ± 9.2	0.672 ± 0.027
TA5688 X Zenda	Heat Stress	21 DAS	10.7 ± 6.2	0.341 ± 0.187
TA5689 X Zenda	Heat Stress	21 DAS	12.8 ± 4.2	0.388 ± 0.127