Effects of post anthesis heat stress on chlorophyll retention and yield components of 43 winter wheat genotypes.

by

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B.S., Kansas University, 2020

A THESIS

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Agronomy
Collage of Agriculture

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2023

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Abstract

Emmer wheat (*Triticum dicoccum Schrank*) and wild Emmer (*Triticum turgidum ssp. dicoccoides*) are potential sources of novel alleles for a wide range of traits including tolerance to heat (elevated temperature or above optimum temperature) stress. These sources may permit breeding to increase yield and yield plasticity of hexaploid wheat sown in fluctuating seasonal temperature regimes. Mean annual temperatures globally are predicted to rise 0.3 to 4.8° C by the year 2100, constituting higher average temperatures seasonally and a need to shape crop phenotype to changing environments. One strategy to minimize high-temperature damage is to create heat-tolerant cultivars via traditional breeding techniques, which involve identifying resilient lines and integrating their traits into commercial varieties. This research was conducted under controlled environmental conditions where tolerance to heat stress was characterized via chlorophyll index and seed characteristics to identify promising wheat lines with a focus on materials derived from crosses with Emmer or Wild Emmer (WEW). Forty-three lines of wheat were tested; KanMark and KS090387K-20, which were used as parents in crosses with Emmer and Wild Emmer, 26 lines of hexaploid BC1F5:7 lines derived from Emmer or Wild Emmer accessions TA-1000, TA-1077 and TA74, 11 HWWAM (hard winter wheat association mapping) Panel lines and 3 near isogenic (NIL) lines for the wheat streak mosaic virus resistance gene *Wsm1*. Germplasms were phenotyped across time (sowing dates 2/17/2022 and 4/6/2022) and spaces constituting 8-hydrospheres in 7-tropospherically controlled chambers, of which, 5 had diurnal temperature regimes set at 20/32°C (night/day), and 2 at 16/22°C. Chlorophyll index measurements commenced on the 8th day post anthesis and every 4th day thereafter until senescence completion. Plants were harvested at maturity with grain number, grain weight, and thousand kernel weight (TKW) recorded. Results showed that lines U8453_D2 (KanMark*2/TA1000) and U8439_U8554_U8440_B5 (KS090387K-20*2/TA1000) both outperformed parent KanMark in terms of TKW. An emmer derived line having

high chlorophyll retention presented here—line U8454_U8446_A7 (KanMark*2/TA 1077)—had higher percent control TKW and chlorophyll index compared to line X111482-1.8.2.2 (Wsm1-Overley/Fuller). A positive correlation in both experiments was found for the percent control of chlorophyll index on Day 8 with 12 and seed number per primary tiller with seed weight per primary tiller. The heat-tolerant genotypes discovered in this study are likely to prove beneficial for future breeding programs, following further experimentation.

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Acknowledgements

I wish to express my profound gratitude to everyone who has guided and supported me throughout my research. I am deeply indebted to my Major Professor, Dr. Fritz, for his exceptional supervision, patience, and guidance. I am also thankful to my co-professors, Dr. Prasad and Dr. Roozeboom, whose insightful feedback and expertise greatly enriched my research. Their comprehensive reviews and timely recommendations have helped to make my study robust and well-rounded. I would be remiss not to mention the invaluable contribution of Mr. Hoffman. His constant assistance, particularly in the provision of essential research supplies, ensured that my work progressed smoothly and without unnecessary delays. Jim's reliability and helpful nature made him a great pillar of support throughout my research journey.

On a personal note, I would like to express my deepest appreciation to my fiancée—her constant love, understanding, and unwavering support throughout this demanding process have been a source of strength and motivation. I am eternally grateful for her resilience and belief in my abilities. I would further like to extend my gratitude to Dr. Kost. His mentorship and guidance have been invaluable throughout this passage of my life.

Finally, I would like to thank my peers, the faculty of the department, and anyone else who contributed directly or indirectly to this thesis. The journey was arduous, but the shared wisdom and encouragement made the excursion a rewarding experience. I am extremely grateful for the opportunity to gain experience from such esteemed professionals and deeply cherish the support and love from my partner during this academic endeavor—thank you.

Dedication

This paper is dedicated to the town of Zeandale Kansas in thanks for my use of their time and space, which is enveloped by the majestic flora and fauna of the resplendent Flint Hills.

Chapter 1 - Literature Review

The ABD's of Wheat

The quest to understand the evolution, origin, and manipulation of wheat by humans has enamored and perplexed people long before Charles Darwin's grandfather Erasmus wrote, "life is short, & wheat is much adulterated…" (Ramsbottom, 1961). One of society's oldest crops, wheat (*Triticum aestivum* L.) co-evolved with humans (*Homo sapiens*) reaching a stasis of mutualism (Purugganan, 2019) within the Fertile Crescent approximately 10,000 years ago leading to the evolution of a monetary-fixed civilization formed by way of utilizing grain as currency, which dates proximal to ancient Egyptian and Mesopotamian agrarian cultures (Johnston, 1932; Sarkar and Stebbins, 1956). With this capitol fixation, cultures mixed and homogenized over the course of millennia forging a monoculture minded population that centralized space, thought, and life reducing diversity culturally, thus too, ancient landrace varieties homogenized into the current genetically impoverished, unsustainable, and unstable wheat germplasms and models of grain production we have today (Shiva, 2016). Moreover, we account for multiple domestication events that reduced population sizes, resulting in a genomic bottleneck as seen in the 84% nucleotide diversity loss observed in tetraploid durum wheat (*T. turgidum ssp. durum*) (Balla et al., 2022). Common allohexaploid wheat—using a binomial nomenclature classification—is in the Kingdom *Plantae*, Subkingdom *Tracheobionta*, Superdivision *Spermatophyta*, Division *Magnoliophyta*, Class *Liliopsida*, Subclass *Commelinidae*, Order *Cyperales*, Family *Poaceae*, Genus *Triticum* L., of which belongs Specie *T. aestivum* (*USDA,* 2022.).

Modern hexaploid wheat is said to have been domesticated by way of hybridization events involving the ancestors of tetraploid wild emmer wheat (WEW, *Triticum turgidum ssp. dicoccoides*) providing the A and B genomes, and diploid specie goat grass (*Aegilops tauschii*) providing the D

genome—these crossbreeding events coupled with mutation led to the current free-threshing BBAADD cultivars of today (Dvorak et al., 2012). *Aegilops tauschii* chromosomes are shown to structurally evolve at a rate ten-times that of other grass genomes due to heavily repeated transposable elements (TE) representing 84.4% of the presented 4.3Gb genome with 65.9% of that sequence being long terminal repeat transposons (LTRT). These LTRTs induce recombination errors and gene duplications, all of which, fueled the dysploidy reduction that resulted in a twelve to seven chromosome copy change over the course of 350 chromosomal arrangements in the last 35-million years consequentially developing the antecedent *Aegilops tauschii* (DD), which is shown to provide the D genome in hexaploid wheat (Ming Cheng Luo et al., 2017).

Currently, due to the low genetic diversity in common wheat disabling individual and population plasticity in everchanging environments, decades of interspecific hybridization utilizing the Genus *Aegilops* and intraspecific hybridization with emmer wheat have achieved increases of novel genetic diversity to combat abiotic stress by way of trait specific loci insertions to develop novel polyploid cultivars (Mirzaghaderi et al., 2020). The use of diagnostic machinery to advance precision and accuracy when identifying quantitative trait loci (QTLs) in combination with advances in marker assisted selection has revealed emmer wheat as an invaluable ancestral genetic source for improving biotic and abiotic stress tolerance in modern wheat, enhancing productivity, and thus increasing global food security (Merchuk-Ovnat et al., 2016). Presently a radical change from the Green Revolution, agricultural research is being carried out by private companies entrenched in industrialized nations with the public sector taking a backseat in research and development, thus, the research, technologies developed, and distribution of information has reduced accessibility to small farmers globally (Fuck et al., 2008).

Wild Emmer Wheat (WEW) and Domestication

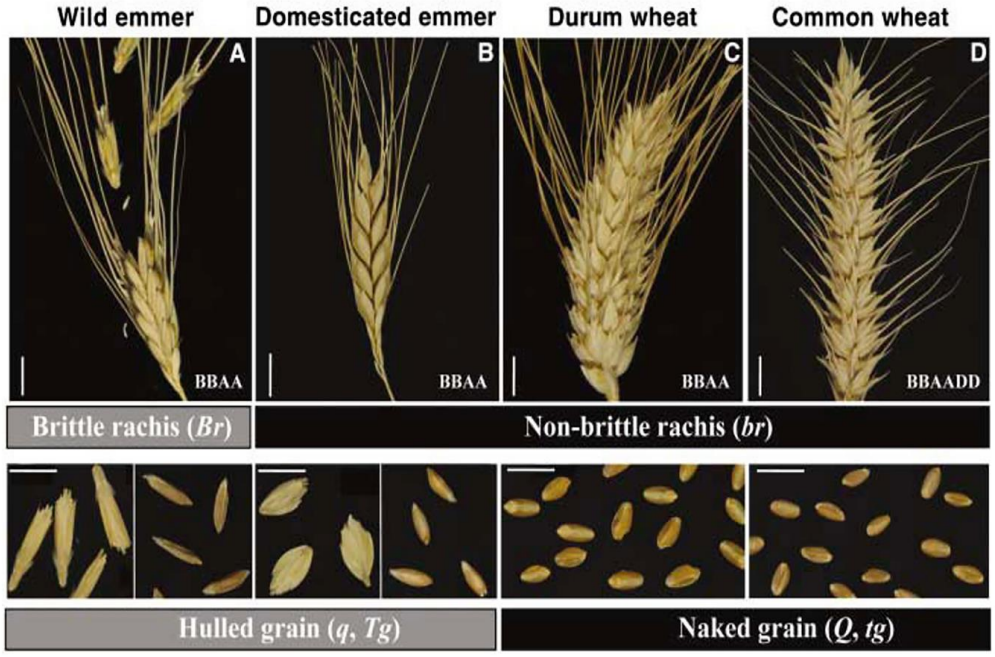
Wild emmer wheat (*Triticum turgidum ssp. dicoccoides*) originated proximal to the Mesopotamian cradle of civilization's Tigris-Euphrates River system of Southwestern Asia in the vicinity of modern-day Southern Turkey and Northern Syria, from where, diffusion of ancient emmer germplasm to Southern Europe, Northern Europe, and Northern Africa began approximately 5500 BC, 3500 BC, and 3000 BC respectively (Fadida-Myers et al., 2022). Emmer wheat *T. turgidum subsp. dicoccon* (2n = 4x = 28; genome BBAA) is the descendant of wild einkorn wheat *T. Urartu*, which is the current accepted progenitor of the emmer A genome, though under discussion, evidence supports that *Ae. speltoides* could be the chloroplast and B genome donor via the S genome found in section *Sitopsis* type specie of *Aegilops* L. (Haider, 2012).

Wild emmer's ancestral populations have been suggested to have genetically bottlenecked over 10-millennia ago following founder events via dispersal and subsequent selection over time in vastly varying microcosmically isolated geographies. Disruptive ecological selection is said to have continued as the driving evolutionary mechanism propelling the independent adaptive sympatric speciation events that have garnered a plethora of our current known accessions (Wang et al., 2020). These naturally evolving accessions contain a myriad of significant traits, such as biomass, earliness, and the complex trait yield; abiotic stress tolerance to heat, drought, and salinity; biotic stress tolerance and resistance to insects, *Fusarium* head blight, powdery mildew, tan spot, stripe rust, stem rust, leaf rust, wheat soil-borne mosaic virus, and *Stagonospora nodorum* leaf blotch (Xie and Nevo, 2008). Further, wild emmer is a prodigious genetic donor for grain quality traits such as raised iron (Fe) and zinc (Zn) content and concentration, which has been shown to combat human health severities such as mental and cognitive development, anemia, morbidity, and mortality caused by micronutrient deficiency affecting approximately 10% of the combined United States and Canadian population and more than three-billion people globally (Cakmak et al., 2004).

Advantageous wild emmer alleles have been mapped for the main components of wheat grain weight, proteins, and carbohydrates, both of which shape the human diet and contribute to the elasticity, viscosity, and extensibility characteristics of dough, and thus, pasta, bread, and an insurmountable number of wheat products (Fatiukha et al., 2020).

Domesticated emmer wheat (*Triticum dicoccum* Schrank) arose from a geographical subdivision of northern and southern wild populations, of which the domesticated population in the southeastern Turkey region of Diyarbakir was later introgressively hybridized by neighboring wild populations in Levant (Luo et al., 2007). Archaeological evidence of humans dispersing wild emmer across geographical barriers combined with nuclear gene sequence analysis suggests domestication of emmer was a reticulated evolution of hybridization events commencing with nomadic Epipaleolithic and early Neolithic societies gathering stands of wild grain and transporting the kernels while migrating for the purposes of hunting (Civáň et al., 2013). The domestication of wild emmer wheat primarily involved trait shifts of seed dormancy, grain development, and spike morphology such as the selection for mutated alleles of genes that control for increased rachis rigidity, which is a key component of the domestication syndrome in modern emmer wheat as can be seen with the phenotypes presented in Figure 1.1 (Avni et al., 2017).

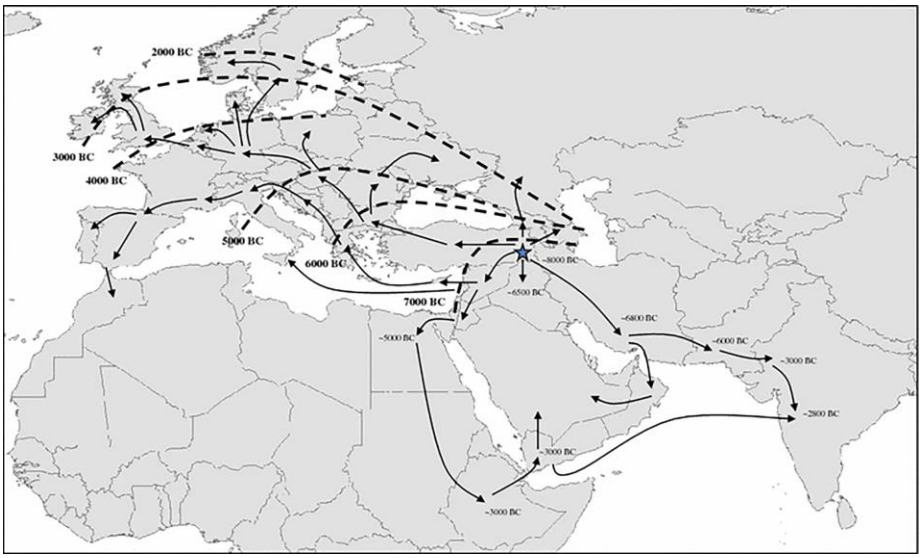
Figure 1.1 Non-brittle rachis syndrome† (Peng et al., 2011)



†Wheat spikes showing (A) brittle rachis, (B to D) non-brittle rachis, (A and B) hulled grain, and (C and D) naked grain. (A) Wild emmer wheat (*T. dicoccoides*), (B) domesticated emmer (*T. dicoccum*), (C) durum (*T. durum*), and (D) common wheat (*T. aestivum*). White scale bars represent 1 cm. Letters at the lower right corner indicate the genome formula of each type of wheat. Gene symbols: Br, brittle rachis; Tg, tenacious glumes; and Q, square head. (adopted from Dubcovsky and Dvorak, 2007)

Archaeological excavations of grain remnants have been utilized by biogeographers to reconstitute the center of origin and geographic diffusion of cultivated emmer wheat showing the rate and distance of diffusion and adoption for emmer wheat was defined by the Caucasian region which geographically parts harsh climates to the West having retarded adoption and moderate climates to the Northeast having accelerated adoption shown in Figure 1.2 (Zaharieva et al., 2010).

Figure 1.2 Map of the diffusion of *Triticum dicoccon* † (Zaharieva et al., 2010)

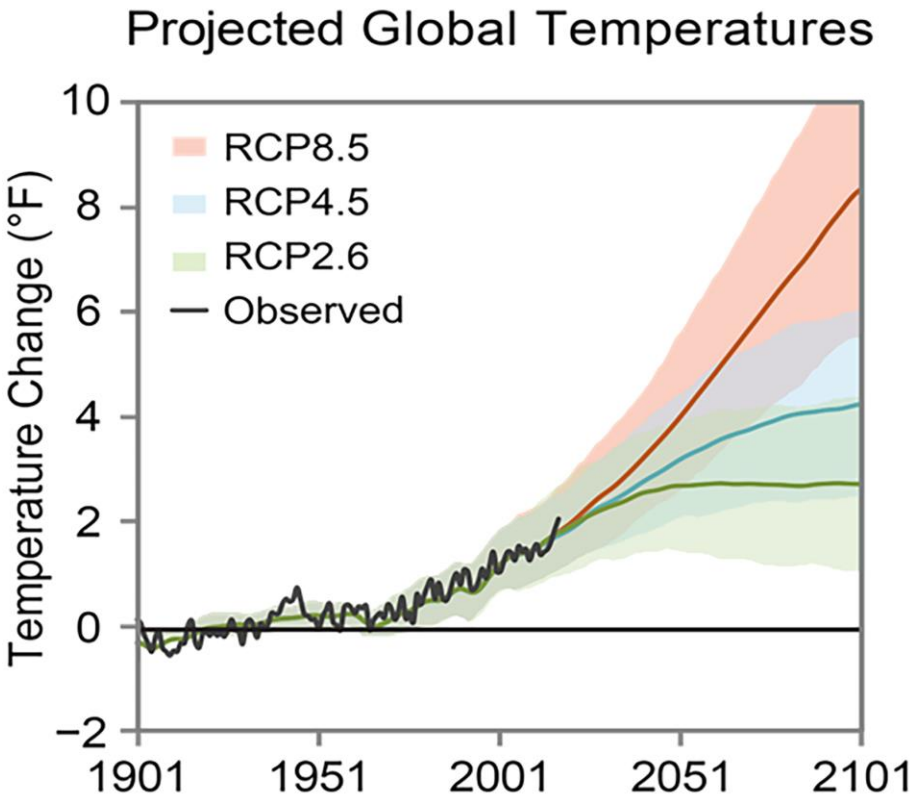


†Established on the basis of presence in archeological sites. The star shows the center of origin of the species and the arrows the presumed ways of diffusion. The bold dotted lines rely sites of the same date and the distance between them suggest the speed of the diffusion to the West and the North

Heat Stress and Wheat

In all mediums, heat stress is commonly defined as an abiotic stress constituting macro to micro time periods of elevated-temperature which permanently cause damage to the development, growth and function of a plant or induces an epigenetic stress-tolerance response of dormant gene transcription used for synthesis of proteins that can buffer physiological performance under stress (Wahid et al., 2007). Mean annual temperatures on planet Earth are predicted to rise 0.3 to 4.8°C by the year 2100 constituting average higher temperatures seasonally over many years of long-term climate warming that will invoke an ecological response to increased frequent high temperatures in the form of severe heat waves and short-term heat-shock impacting the survivability of plants and yield of crops (Jagadish et al., 2021). Climate change projections are based on scenarios that consider human activities and population dynamics, technological changes and implementations, and their respective impact on the environment with some scenarios reflecting continued reliance on fossil fuels and others requiring deliberate emission reduction actions focused on specific end-goals like limiting global temperature increase or achieving net-zero carbon emissions (Wuebbles, D.J., et al. 2017). Some probable outcomes for global mean temperatures presented in Figure 1.3. show that under a lower scenario global mean temperatures are significantly likely to exceed 3.6°F, and if carbon dioxide emissions start declining by 2020 and go to zero by 2100, global temperatures are not expected to reach an increase of 3.6°F .The rate of human population growth indicates production of wheat must increase by 2% per year to meet future global needs. Temperature increases ranging between 3—4°C during grain fill of wheat has shown to result in yield decreases ranging from 15–35%, thus new novel lines of wheat must produce approximately 169—189% yield increase by the year 2100 to meet the obstacles of a changing climate and rising population (Al-Ashkar et al., 2020).

Figure 1.3 The Climate Science Special Report† (CSSR) (Wuebbles, D.J., et al. 2017)



†Historical observed and future temperature change that would result for a range of future scenarios relative to the 1901–1960 average, based on the central estimate (lines) and a range (shaded areas, two standard deviations) as simulated by the full suite of CMIP5 global climate models (right). By 2081–2100, the projected range in global mean temperature change is 1.1°–4.3°F under the even lower scenario (RCP2.6; 0.6°–2.4°C, green), 2.4°–5.9°F under the lower scenario (RCP4.5; 1.3°–3.3°C, blue), 3.0°–6.8°F under the mid-high scenario (RCP6.0; 1.6°–3.8°C, not shown) and 5.0°–10.2°F under the higher scenario (RCP8.5; 2.8°–5.7°C, orange).

The invaluable genetic diversity of emmer wheat that codes for the phenotypic complex trait such as heat stress tolerance has naturally evolved from a dynamically warm, cool, wet, and dry geographical distribution generating novel chromosomal regions that have influenced the increased plasticity of hexaploid wheat sown in fluctuating seasonal temperature regimes (Ullah et al., 2018). All stages of a plant's life—from seed to seed—are subject to and in danger of heat stress: but, the intersecting cycles of seasonality and wheat development dictate latter primary growth stages are of utmost concern regarding accelerated phasic development as a cause of reduced biomass, early and accelerated senescence, reduced photosynthesis, starch synthesis inhibition within emerging kernels, and photosynthate transport reduction during grain fill (Khanna-Chopra and Viswanathan, 1999). Pre-anthesis to anthesis heat stress has been shown to induce complete pollen sterility when temperatures are consistently greater than 30°C (Thomason et al., 2018). When high temperature regimes of 35/20°C (day/night) are imposed 10-days post-anthesis over the grain-filling period on a whole plant basis, yield, kernel number, and kernel weight have been shown to reduce by 78%, 63%, and 29% respectively for the hard red winter wheat cultivar Karl 9 (Thomason et al., 2018). This reduction of crop yield under heat stress is attributed to an increase of radical oxidative species inducing oxidative stress, a decrease of chlorophyll content, accelerated senescence, and reduced rates of photosynthesis when temperatures rise 5°C above the ideal winter wheat tropospheric temperature of 25°C (Thomason et al., 2018). Yield and yield components, canopy temperature, cell membrane stability, and stomatal conductance are common physiological diagnosis systems for the evaluation of wheat crops experiencing elevated temperatures, of which each °C increase has been shown to result in a 4% reduction in grain weight when mean air temperature during grain-filling is 17—24°C (Mohammadi et al., 2004). As a measurement of plant tolerance to heat stress, early emergence and ground cover, leaf rolling, shortness, and stay-green are associated with the heat tolerance of wheat (Mohammadi et al., 2004).

Stay-Green (SGR) and Wheat

The character stay-green was coined circa 1962 proximal the Proefstation voor de Akker-en Weidebouw, Wageningen in the Netherlands by E. Steinbuch, W.S. Poelstra, and T.C. van der Kamp as a phenotype descriptor used to describe legume color-phenology and, with recognition of increased duration of greenness and yield arising from specified concentrated selection, stay-green became quickly determined an exceptional trait for development and marketing (Thomas et al., 2014). Thus, the elucidation of stay-green trait cellular machinery via discovery of genes associated with the pathway of chlorophyll catabolism, gained insight of leaf senescence when plants are heat stressed. Nitrogen and photosynthate remobilization phases, and a finer understanding of the senescence syndrome regarding rate, timing, and systems regulation has revealed many mechanisms of delayed foliar senescence (Thomas and Ougham, 2008). A complex series of interwoven events and many cellular processes, leaf senescence can be visually observed as a reduction of green color caused by a phasic or environmental change, for which a plant favors the up or down-regulation of gene expression regulating the deconstruction of chloroplasts, degradation of chlorophyll binding proteins, and translocation of photosynthates and nutrients from deteriorating tissue to developing roots, leaves, and kernels (Balazadeh, 2014).

The stay-green trait has been binned into five broad categories constituting the functional types A and B when wild genotype compared to mutant has delayed onset of senescence or when the rate of senescence is retarded in stay-green plants respectively; cosmetic stay-green type C exhibiting impaired chlorophyll cessation, pseudo-stay-green type D identified as mortality brought on before and during senescence, and type E signified by a higher accumulation of chlorophyll than wild-type relatives (Kusaba et al., 2013). Functional stay-green mutants have been widely used for the illumination of the biochemical and molecular inner-workings concerning leaf senescence brought on by environmental stress and plant aging (De Simone et al., 2013) . These plants have also been

useful for expounding the evolution of cohesive resistance defense systems shown to buffer levels of reactive oxygen species (ROS) by way of enzymatic antioxidants and non-enzymatic molecules detoxifying free radicals, such as superoxide and hydrogen peroxide, into beneficial oxygen and water molecules (De Simone et al., 2013). Synthesized investigation of multi-environmental, phenotypic, and genomic data for increased wheat breeding efficiency utilizing canopy temperature (CT), normalized difference vegetation index (NDVI), membrane thermostability (MT), and chlorophyll index (SPAD-based) data, has produced statistical prediction models which incorporate spectrum and spectrum by environment interaction, particularly for plants in stressing environments, significantly associate hyperspectral data with wheat grain yield. (Guo et al., 2020).

Analysis of Heat Stress and Green Color

Although influenced by changing growth conditions involving the redistribution of chloroplasts within mesophyll cells, taxa morphology diversity producing variant scattering effects or light reflection, and collection disadvantages of overtly irregular chlorophyl distribution or reticulate appearance of leaves, the concentration of chlorophyll per-leaf-area or a per-fresh-weight basis is frequently quantified to accurately identify chloroplast development, photosynthetic capability, nitrogen content and plant health in agricultural settings (Ling et al., 2011). The Soil Plant Analysis Development (SPAD) chlorophyll meter is a regularly used diagnostic tool to indirectly estimate concentration of chlorophyll rapidly and non-destructively by measuring the difference between the transmittance of a red 650 nm light wave and an infrared 940 nm light wave through leaf tissue and calculate a one-decimal three-digit SPAD value (Antonio Sperotto et al., 2016). Because wheat flag leaf contribution to grain assimilates has been shown to be approximately 30–50%, SPAD readings have been used to ascertain the leaf area under greenness (LAUG), stomatal conductance, and photosynthetic activity as a measure of the stay-green trait in wheat with relation to yield and yield components (Roy et al., 2020).

Elevated temperatures inhibit maintenance of wheat plant photosystem II (PSII) protein complex due to increased activity of reactive oxygen species and photoinhibition, thus, these stress effects of membrane embedded photosynthetic systems are frequently quantified with chlorophyll (Chl) *a* fluorescence kinetics data in an approximate form to the Kautsky curve attained from a time specific intensification of fluorescence via continuous light to a previously dark-adapted foliar tissue (Brestic et al., 2012). Quantum yield (QY)—an indicator of the maximum quantum efficiency of PSII in terms Fv/Fm where Fv = Fm - F0 and (Fm – Fo) / Fm=QYmax when F0 = fluorescence intensity at 50 μs (F50μs), FM = maximal fluorescence, and Fv = Fm - F0 is the maximal variable fluorescence—illustrates a positive interrelationship with wheat grain yield under field and controlled conditions showing light reaction photochemistry impaired, downregulation of photosynthesis, and reduced activation of Rubisco in response to environmental heat stress (Sharma et al., 2015).

Conclusions

Wheat and humans have a long and complex history twinning advancement since the dawn of civilization and crop, of which both have mutualistically endured the assault of abiotic and biotic stress causing agents. To reach the needs of an ever-rising global population, wheat production must increase almost two times in less than a century. Though production must increase, we are currently seeing smaller yields than previous years due to drought and heat brought on by climate change, human behavior, and war. Technologies and information are accumulating at unprecedented rates and, if integrated with cultural understanding and ethnobotanical wisdom, those advancements will propel the regeneration of sustainable levels of diversity for global agriculture. Increasing the diversity of cultivars increases the geographical space on which we can grow crops, and with progressive mechanistic designs that can harvest newly developed forms of

milpas, maslins, and polyculture systems the projected need of yield can be partitioned to reduce the strain of evolving plants to produce twice as much by the year 2100.

Studies that can identify or produce novel wheat genotypes and expand the diversity of germplasm to encompass locally adapted climactic stress tolerant wild emmer loci in domesticated lines to be grown in endemic-similar environments, which are not centers of origin, are essential. Wild emmer wheat has been shown as an invaluable resource for novel genetic material which can be integrated and utilized to develop novel productive lines of wheat. There exists a plethora of diagnostic tools and software to speed the creation and validate phenotypic strength of new genotypes to be released for public use, but there exists much more wild emmer diversity to investigate, understand and implement into novel climate-change buffered wheat lines.

Rational and Objectives of Study

Because wild emmer wheat can readily contribute allelic diversity to modern hexaploid wheat and increase individual and crop plasticity to changing environments, the tools and equipment exist to rapidly quantify and pinpoint best-genotypes. The global population needs crop advancement to stave off future famine, malnutrition, and the average garden-variety whole-number-plus scientist will find immense importance in the undertaking of the study presented here.

The objective of these experiments was to characterize 43 wheat lines for stay-green traits, yield, and yield components. These experiments were conducted to: (1) determine genotype(s) that exhibit stay-green phenotype; (2) determine lines, if any, outperform their parents and/or competitors in yield and/or stay-green phenotype; (3) discover if any lines have consistent yields and/or stay-green phenotypes across environments of elevated temperatures; and (4) determine lines that could be beneficial for further investigation for tolerance to heat stress.

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Chapter 2 - Characterization of wheat yield components and stay-green in response to post-anthesis elevated temperatures.

Abstract

Emmer wheat (*Triticum dicoccum Schrank*) and wild Emmer (*Triticum turgidum ssp. dicoccoides*) are potential sources of novel alleles for a wide range of traits including tolerance to heat stress. The sources may permit breeding to increase yield and yield plasticity of hexaploid wheat (*Triticum aestivum* L.) sown in fluctuating seasonal temperature regimes. Mean annual temperatures globally are predicted to rise 0.3 to 4.8°C by the year 2100, constituting average higher temperatures seasonally and a need to shape crop phenotype to changing environments. One strategy to minimize negative impacts of high-temperature stress is to develop heat-tolerant cultivars via traditional breeding techniques, which involve identifying resilient lines and integrating their traits into commercial varieties. This assessment was conducted to phenotype and identify potential wheat lines, which could serve as genetic sources of post-anthesis heat stress tolerance by way of the stay-green phenotype. Under controlled environmental conditions of optimal temperatures (OT) and high temperatures (HT), chlorophyll index and yield components were measured and evaluated as a percentage of the non-stress control to identify promising wheat lines with a focus on hexaploid materials derived from Emmer or wild Emmer (WEW). Forty-three lines of wheat were tested; KanMark and KS090387K-20, which were used as parents in crosses with Emmer and wild Emmer, 27 lines of hexaploid BC1F5:7 lines derived from Emmer or wild Emmer accessions TA-1000, TA-1077, and TA74; 11 hard winter wheat association mapping (HWWAM) panel lines and 4 near isogenic (NIL) lines for the wheat streak mosaic virus resistance gene Wsm1. Germplasms were phenotyped across time (sowing dates 2/17/2022 and 4/6/2022) and spaces constituting 8-hydrospheres in 7-tropospherically controlled chambers, of which, 5 had diurnal temperature regimes set at 20/32°C (night/day), and 2 at 16/22°C. Chlorophyll index (CI) and

quantum yield (QY) measurements commenced at 8th day post anthesis and every 4th day thereafter until senescence completion. Plants were harvested at maturity with grain number per primary tiller, grain weight per primary tiller, and thousand kernel weight (TKW) recorded. Results show lines U8453_D2 (KanMark*2/TA1000) and line U8439_U8554_U8440_B5 (KS090387K-20*2/TA1000), both of which outperformed parent KanMark in terms of TKW. Further, an emmer derived line having high chlorophyll retention presented here—line U8454_U8446_A7 (KanMark*2/TA 1077)—had higher percent control TKW and chlorophyll index as a percent control compared to line X111482-1.8.2.2 (*Wsm1*-Overley/Fuller). A positive correlation in both experiments was found between the percentage chlorophyll index days 8 and 12, and the percentage of seed number per primary tiller and seed weight per primary tiller. The heat-tolerant genotypes discovered in this study are likely to prove beneficial for future breeding programs, following further experimentation utilizing field based cybernetically controlled temperature heat tents covering true soil conditions.

Introduction

One of the world's primary food sources, hexaploid wheat (*Triticum aestivum* L.) contributes a large proportion of the protein and calories for the global population (Mishra et al., 2021). As the global population grows, production of wheat must increase by 2% per year to meet future global need and to overcome the negative impact of temperature increases ranging between 3 and 4°C during grain fill, which translates to an approximately 169—to 189% yield increase by the year 2100 (Al-Ashkar et al., 2020). Wheat is not only a significant source of nutrition with an estimated 20% of the calories and protein consumed worldwide (FAO, 2020), but also plays a crucial role in the socio-economic resilience of many countries such as China, which—despite having dynamic population and climate change—the per capita production values of wheat have remained comparatively stable, signifying robustness of the crop's production(Sun et al., 2023). Countries' reliance on global

food trade networks implies regionally different climate change impacts on crop yields will be transmitted across borders, affecting how countries engage in cooperative action(Hedlund et al., 2022). A bioregional versatile crop, wheat is cultivated on more than 218 million hectares globally. Comprising the largest land are of a commercial crop (USDA-ERS, 2022), wheat cultivation spans diverse climatic zones, from the harsh winters of Canada and Russia to the hot, dry summers of the Mediterranean and many diverse parts of the U.S. (USDA-ERS, 2022). In terms of production, the top wheat-growing countries include China, India, Russia, the United States, and France (FAO, 2023) with nearly half of the U.S. wheat crop exported in 2021, thus, making the U.S. one of the world's leading wheat exporters (USDA-ERS, 2022). Beyond the punitive realities of a changing climate, geopolitical conflicts disrupt global food security as can be seen in the current conflict between Russia and Ukraine, of which both countries are significant contributors in world food production and trade, thus, these types of conflicts impose an increase in the world's food prices, posturing a danger to global food security—particularly for low-income countries that depend heavily on yearly food import increases to match population growth (Nasir et al., 2022).

The increase of winter wheat yield is seriously challenged by yield reductions ranging from 15–to 35% due to high temperature (heat) stress during grain fill (Al-Ashkar et al., 2020). The domestication of wheat proximal to the Fertile Crescent, which has been a temperate region for millennia, has evolved cultivars that are susceptible to high temperatures (Heun et al., 1997). Heat stress in plants is typically classified as a 10–to 15 °C increase above a species specific threshold for a time long enough to cause irreversible harm to plant growth and development (Wahid et al., 2007). At the molecular level, heat stress induces the production of heat-shock proteins (HSPs), which act as molecular chaperones to protect other proteins from denaturation and maintain cellular homeostasis (Wang et al., 2003). The timing of heat stress is a significant factor in how plants respond to heat stress which can accelerate the development of many plant species including

C3 crops like wheat, thus resulting in earlier maturation, reduction of duration for key development stages such as grain filling in wheat (Porter and Semenov, 2005), and pollen sterility resulting in reduced seed set and yield (Barnabás et al., 2008).

High-temperature regimes of 35/20°C (day/night) imposed 10-days post-anthesis over the grain-filling period reducing yield, kernel number, and kernel weight is attributed to an increase of reactive oxygen species (ROS) inducing oxidative stress, a decrease of chlorophyll content, accelerated senescence, and reduced rates of photosynthesis when temperatures rise 5°C above the ideal winter wheat temperature of 25/15°C (day/night) (Thomason et al., 2018). Molecular responses of wheat varieties to high-temperatures include decreased activities of Rubisco (Sharma et al., 2015), soluble starch synthase, and thus, photosynthesis and starch biosynthesis (Zahedi et al., 2003) while increasing the activities and production of chlorophyllase, ROS, and ethylene, of which all participate in chlorophyll degradation(Wang et al., 2018). The aforementioned phenotypic shifts collectively result in a detrimental effect on yield, yield components, TKW, seed set, and single seed weight, of which, during grain filling periods, can cause a 2.8mg reduction per 1°C increase above optimum temperatures(Fu et al., 2023).

Yield and yield components, canopy temperature, cell membrane stability, and stomatal conductance are common physiological diagnosis systems for the evaluation of wheat crops experiencing elevated temperatures (Mohammadi et al., n.d.). These are phenotypically expressed as reduced green photosynthetic area of the plant, increased rate of senescence, decreased grain filling duration, and increased photorespiration (Jagadish et al., 2016). The green photosynthetic area of plant flag leaves can be evaluated visually or using imaging technology to quantify a representative score through software such as ImageJ (Schneider et al., 2012) or identify increased senescence with chlorophyll index non-invasively using a SPAD meter (Richardson et al., 2002).

Grain filling duration can be tracked by periodic measurement of grain weight during the development stage until maturity (Blum, 1986) and photorespiration, a metabolic pathway that consumes O_2 and releases CO_2, can be quantified indirectly by measuring gas exchange using a photosynthesis system (Busch et al., 2018).

Stay-green variants are broadly used for expounding the evolution of cohesive resistance defense systems—the location and quantification of losses and gains of multiple loci sets which when expressed together operate dynamically to confer a resistance or tolerance to biotic and abiotic stress (De Simone et al., 2015) and naturally evolving wild emmer wheat (*Triticum turgidum ssp. dicoccoides*) accessions contain a myriad of potentially beneficial phenotypic traits including tolerance to heat stress (Xie and Nevo, 2008). Wild emmer wheat has been shown to have inherent resistance to pests such as the Hessian fly (Liu et al., 2005), diseases such as rusts, and readily houses the *Pm13* gene, which was successfully identified as a loci responsible for resistance to powdery mildew and subsequently transferred from wild emmer to bread wheat (Huang et al., 2003). Moreover, wild emmer wheat possesses traits that allow it to tolerate abiotic stresses such as drought, heat, salinity, and traits related to root architecture and grain protein content, all of which could enhance drought tolerance and nutritional quality in modern wheat varieties (Peleg et al., 2005).

Wheat flag leaves—which contribute approximately 30–to 50% of grain assimilates—are commonly analyzed with the Soil Plant Analysis Development (SPAD) chlorophyll meter diagnostic tool as a measure of the stay-green trait of wheat with relation to yield and yield components (Roy et al., 2021). Additionally, synthesized investigation of multi-environmental, phenotypic, and genomic data for increased wheat breeding efficiency utilizing SPAD-based data for plants in stressing environments are significantly associated with wheat grain yield (Guo et al., 2020).

Screening germplasm to identify heat tolerant lines, breeding those tolerance traits into commercial lines, and utilizing novel germplasm pools to increase cultivar diversity, will thus, increase available diversity for future climates. The objectives of this study were to (1) investigate genetic variation and correlations of chlorophyll retention and yield components in a diverse set of winter wheat genotypes derived from crosses with Emmer and wild Emmer, and (2) identify genotypes with high chlorophyll retention and/or thousand kernel weight (TKW) in wheat varieties under heat stress conditions.

Materials and Methods

Plant Materials

Plant materials for this study consisted of 43 varieties of winter wheat sourced from the USDA-ARS Hard Winter Wheat Program (Manhattan, KS). Experiments evaluated the lines KanMark and KS090387K-20, which were used as parents in crosses with Emmer and wild Emmer, 26 lines of hexaploid BC1F5:7 lines derived from Emmer or wild Emmer accessions TA-1000, TA-1077 and TA74, 11 HWWAM Panel lines and 4 near isogenic (NIL) lines for the *wheat streak mosaic virus* resistance gene *Wsm1*. The germplasm origin information can be found within the complete data located in the appendix A.2.4 Link to data.

Plant vegetative growth conditions

Utilizing climate-controlled chambers, 43 wheat genotypes were evaluated in two experiments. For both experiments, all germplasms were sown in four-inch pots filled with ProMix-BX general purpose growing medium (Premier Tech Growers and Consumers, a Business Unit of Premier Tech,

Canada) in well-watered conditions at ~25/15°C Day/night temperatures until germination, and subsequently, vernalized at ~4°C for ~5 weeks. Vernalized seedlings were then transplanted individually to pots having a radius of approximately 10.16 cm and a depth of approximately 30.48 cm pots filled with the same growth medium and placed according to random assignment in a fertigated-water bin. Through the course of both experiments, plants were fertilized by Peters Professional (The Scotts Company, Marysville, OH) containing 10% Nitrogen (N), 20% Phosphorus (P), and 20% Potassium (K) at 4.93 ml per 3.79 L water per block once a week commencing 3-days after transplant. All tubs were filled as needed to maintain fertigated water above the soil interface with a consistent nutrient concentration. Focusing on elevated temperature climate trends that reflect temperature regimes which these plant materials could realistically experience under filed conditions, Viola Kansas was selected as an area of interest and subsequently was used to create climate profiles for these experiments. All chambers were set for 70% relative humidity and a light regime 15hr light/9hr dark (day/night) that mirrored Viola Kansas Mesonet data (Kansas Mesonet, 2022.).

For Exp. 1, transplanted vegetative plants were located within the Kansas State University/USDA greenhouse 108A having daily average temperature regimes 23/19 °C (day/night) reported Appendix data. To control wheat thrips (*Haplothrips tritici*) and reduce copper deficiency syndromes of stunted growth and chlorosis, an adequate amount of Marathon (OHP, Inc., Mainland, PA), 108.43 ml per bin, and a 0.156-gram dose of chelated copper per bin was applied 18 days after transplant to all bins respectively according to USDA protocols. All plants remained in their transplanted positions until 8-days post-anthesis, at which time plants preselected for 1 of 4 chambers having OT and HT settings were moved to their new tropo and hydrosphere environments while continuing in their respective pedosphere environments.

For Exp. 2, during vegetative growth, plants were located within a single Kansas State University/USDA growth chamber having daily average temperature regimes 29.5/15.7°C (day/night). Additionally, to help ensure sturdy stems and reduce copper deficiency syndromes of stunted growth and chlorosis, a single silicon application of Iron Chelate constituting 54.22 ml per bin approximately two-weeks after transplant and a 0.156-gram dose of copper per block two weeks after transplant was applied, respectively. Further, A fungicide treatment for powdery mildew consisting of one 0.5 gram per 7.57 L application of Strike 50 (OHP, Inc., Mainland, PA), and subsequent application of a 14.79 ml:14.79 ml:1 L concoction of Dawn Liquid Soap (Procter & Gamble, Cincinnati, OH) for leaf surface cohesion (surfactant), baking soda (sodium bicarbonate, $NaHCO_3$) to increase the pH levels, and water as the universal solvent respectively, was applied as needed to control powdery mildew infection (Fallik et al., 1997). Upon conclusion of the 8-day period post-anthesis, plants selected for HT treatment were moved to one of 2 chambers set with the elevated temperature regime, and plants selected for the control treatment of OT remained in their respective positions.

Experimental Design

The experimental design was a randomized complete block design (RCBD) constituting two environmental regimes of OT and HT having two replications per chamber such that the lines were each assigned a centrically randomized position per block with each block having 43 genotypes with KanMark placed twice for complete block of 44.

Each chamber in Exp. 1 contained 88 plants placed 44 per replicate and upon conclusion of the 8-day period post-anthesis plants were moved to one of four chambers, of which two treatment chambers had temperatures set by accumulating Kansas Mesonet data which was used to create

seven diurnal profiles based on Viola Kansas temperatures for the years 2016, 2017, 2018, 2019, 2020, 2021, and their average temperatures of the dates 5/22 to 6/3 which dictated the temperature and ramp for 32/19°C (day/night) heat stress (Kansas Mesonet, 2022). Control chambers were set diurnally similar with ideal growth conditions 15/22°C(day/night). Both control and treatment chambers were set for 70% relative humidity and a light regime that mirrored Viola Kansas Mesonet data (Kansas Mesonet, 2022).

Exp. 2 was composed of three chambers of which two were the same treatment chambers described for Exp. 1. The third chamber, set as the ideal growth troposphere, doubled as the vegetative growth setting for the population and a single control chamber, which contained two hydrospheric tubs each containing 88 plants in their own respective pedosphere.

Data Collection

Temperature and relative humidity were recorded every 15min using a HOBO UX data logger and HOBO Marine Pendants (Onset, Bourne, MA). As a measure of the stay green trait, chlorophyll index was recorded using SPAD chlorophyll meter (Model 502 Plus, Konica Minolta, Germany). Measurements were taken on the 8th day post anthesis and every 4th day thereafter as the average of three readings across the primary tiller's flag leaf—distal, middle, and proximal to the junction of the collar and peduncle—until the end of senescence when a zero or negligible reading was obtained. All plants were harvested at maturity and height, grain number, grain weight, and thousand kernel weight were recorded for the primary tiller of each plant. All data collected can be found using the link from appendix A.2.4 Link to data.

Analysis

Analysis was conducted for Exp. 1 and Exp. 2 using the two most differing chambers for each experiment as dictated by the recorded environmental parameters which most closely represented the temperature regimes programmed for each chamber. In scientific experiments, it's common to select the most contrasting conditions to maximize the ability to detect differences or changes (Schober et al., 2018). In this case, choosing the two most differing chambers OT and HT for each experiment likely provided the most significant differences in the responses of the wheat, thereby making any effects of the temperature stress more apparent. In general, degrees of freedom are calculated as the total number of observations minus the number of independent constraints or parameters estimated (Fisher, 1955)—this reduction in chambers did not necessarily reduce the degrees of freedom of these ANOVA analyses as the chambers restricted space dictated a maximum one replication per chamber.

Tolerance of high temperature stress was gauged using the percentage of the control in decimal form (DF) for each genotype to seek phenotypic differences in traits, considering a plant primary tiller as an experimental unit. The chlorophyll retention at various days during grain fill was represented as a percentage in decimal form of the plants grown under HT divided by their respective genotypes grown under OT, a rationale explained in earlier studies elucidating that chlorophyll contents remain relatively constant during the initial 26 days post-anthesis under control conditions for hexaploid common wheat, tetraploid wheat, and maize (Ristic et al., 2008).

The yield traits, and chlorophyll percentages of control, in DF, were computed as: Percent of Control DF = (Y_{HT}/Y_{OT}), where Y_{HT} is the value for each experimental unit under heat stress and Y_{OT} is the average value for the equivalent experimental units under non-stress control conditions (Fu et al., 2023). Greater heat tolerance is indicated by a higher mean percentage decimal of control.

Experiments were analyzed as an RCBD, in linear series (Exp. 1 and Exp. 2). Each experiment was analyzed independently, treating genotype and environment as fixed effects for two-way ANOVAs and genotype as a fixed effect for one-way ANOVAs using SAS PROC GLM (SAS Institute Inc. SAS Studio. 2021). Means were segregated using the Tukey-Kramer multiple comparison test and further trait correlations computed using SAS PROC CORR (SAS Institute Inc. SAS Studio. 2021). Additionally, the chlorophyll retention data of criterion affirming genotypes were plotted using(Microsoft Excel, 2022).

Results

Chamber climates

Distinct temperature settings were established in the chambers, with the high-temperature (HT) and optimum temperature (OT) regimes showcasing significant variations in both maximum (Tmax) and minimum (Tmin) daily averages. Chambers set for high-temperatures and optimum temperatures for Exp. 1 were recorded having daily average Tmax=38.5°C & Tmin=19.2°C HT; Tmax=26.2°C & Tmin=16.0°C OT, and Exp. 2 Tmax=38.5°C & Tmin=19.2°C HT; Tmax=29.5°C & Tmin=15.7°C OT. Sinusoidal daily set ramps and recorded ramps are presented in the appendix Table A.2.1.

Chlorophyll index

The stay-green characteristic was itemized under a comprehensive analysis of chlorophyll indices across different genotypes and environments searching for patterns in chlorophyll index retention, particularly under heat stress conditions, for assessing the heat stress tolerance or susceptibility of selected wheat lines. Experiment one results showed chlorophyll index Day 8 was significantly

different among genotypes, environments, and genotypes by environments**.** Chlorophyll index Day 12 and Day 16 were significantly different among genotypes and environments (Table 2.1)**.** Exp. 2 chlorophyll index Day 16 was significantly different among genotypes and environments (Table 2.2)**.** The percent of control for chlorophyll index at Day 12 of heat treatment among the wheat lines was significantly different in Exp. 2(Table 2.3). The genotype rankings for percent control chlorophyll index decimal form Days 8 and 12 are presented in Figure 2.1 and Figure 2.2 respectively.

Figure 2.1. Percent control in decimal form (DF) chlorophyll index Day 8.

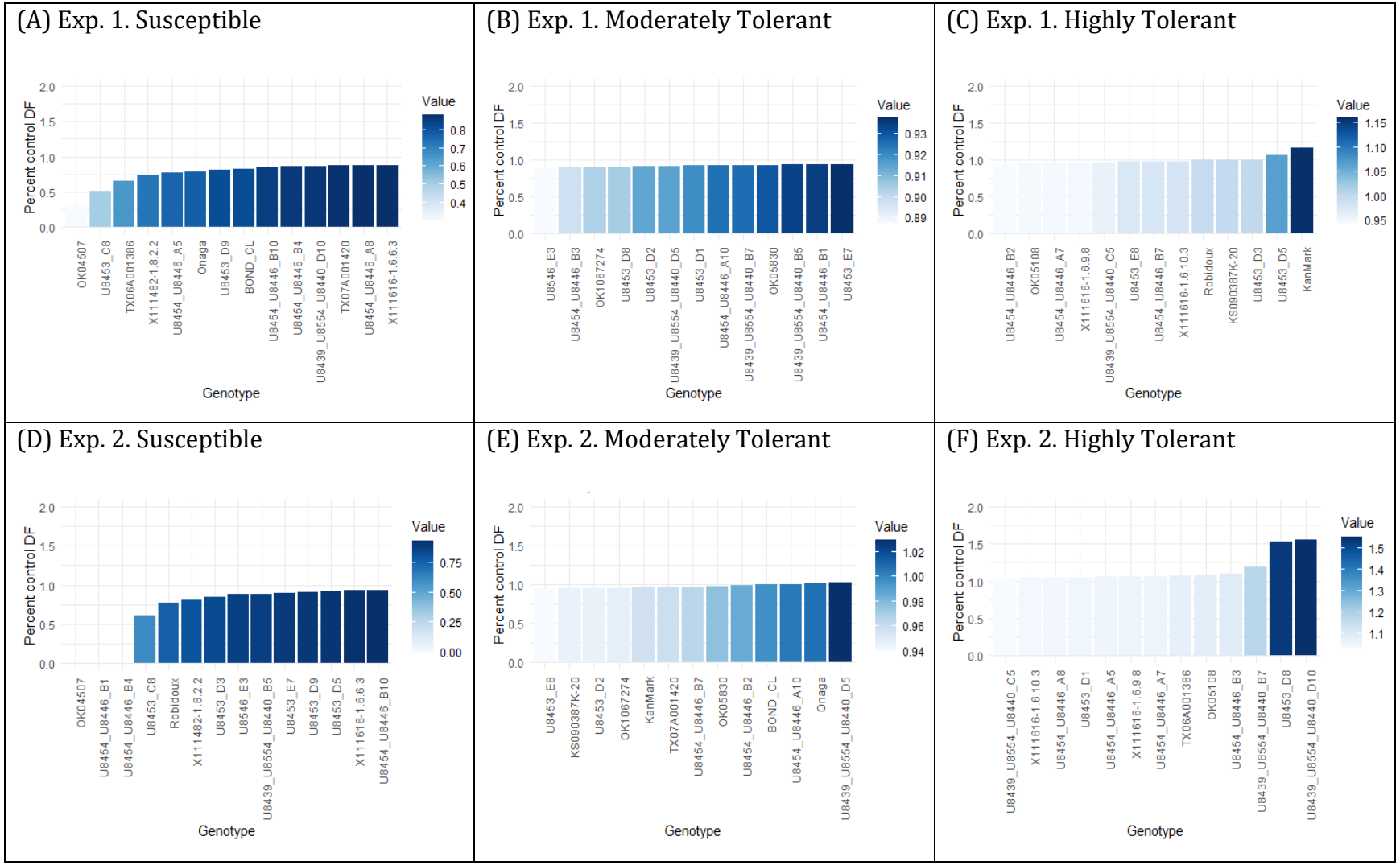


Figure 2.2 Percent control in decimal form (DF) chlorophyll index Day 12.

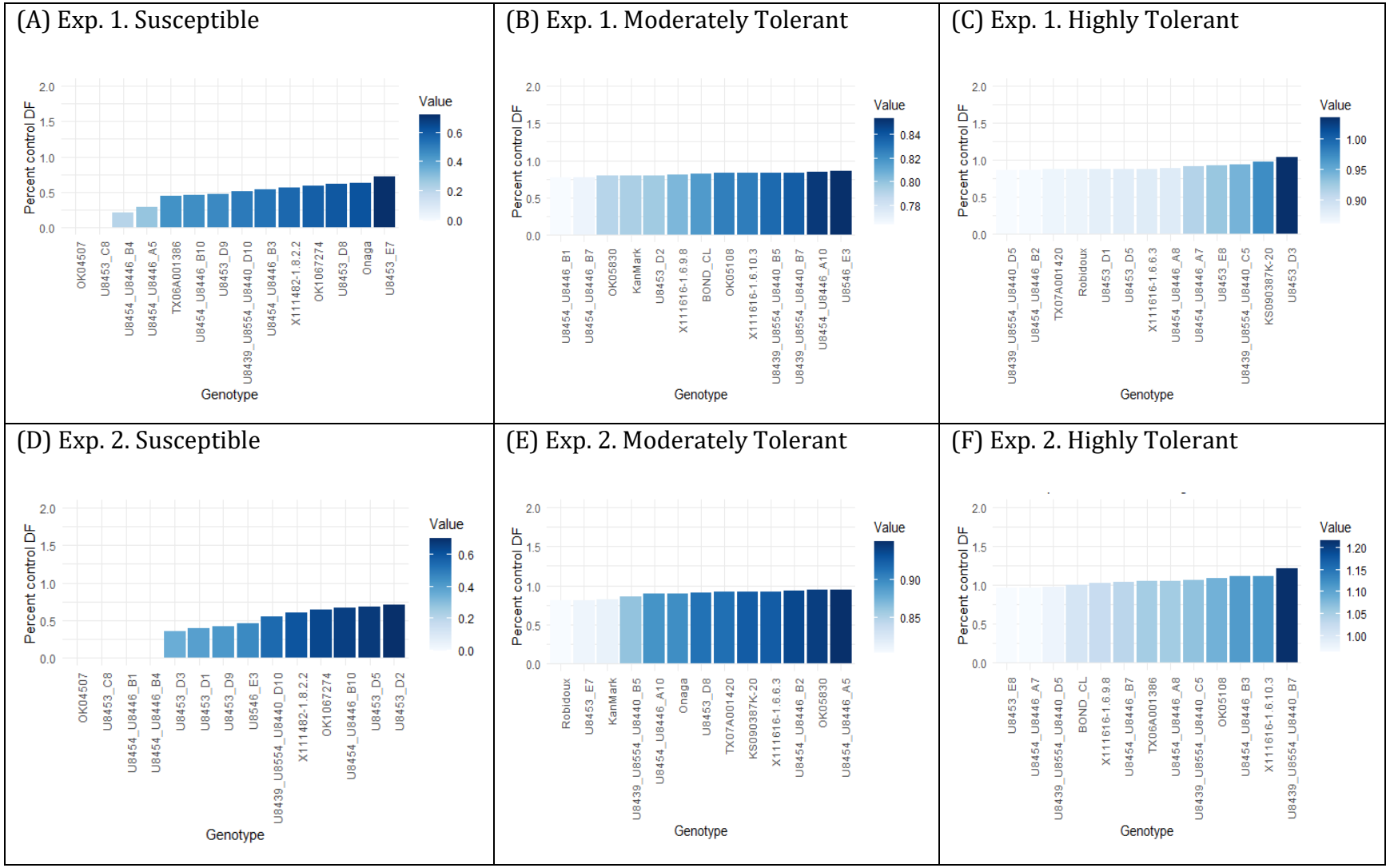


Table 2.1 Exp. 1. Two-way ANOVA SAS PROC GLM for chlorophyll index and yield component traits in winter wheat.

	Model		Genotype		Environment		Genotype by Environment	
Traits	F value†	P > F	F value	P > F	F value†	P > F	F value	P > F
Chlorophyll index Day 4	1.44	0.0503	1.74	0.0172	7.05	0.0095	1.02	0.4653
Chlorophyll index Day 8	2.75	<.0001	2.47	0.0003	51.23	<.0001	1.84	0.0101
Chlorophyll index Day 12	2.47	<.0001	2.20	0.0012	63.75	<.0001	1.14	0.3081
Chlorophyll index Day 16	3.31	<.0001	2.41	0.0004	125.01	<.0001	0.95	0.5566
Seed weight primary tiller	2.59	<.0001	4.14	<.0001	16.05	<.0001	0.66	0.9257
Seed number primary tiller	2.76	<.0001	4.65	<.0001	0.15	0.7020	0.93	0.5955
Thousand kernel weight	5.06	<.0001	5.70	<.0001	100.94	<.0001	1.79	0.0134

†Omnibus test N = 165, model df = 81, Error df = 83; Type III F test Genotype df = 40, Environment df = 1, and Genotype by Environment df = 40.

Table 2.2 Exp. 2. Two-way ANOVA SAS PROC GLM for chlorophyll index and yield component traits in winter wheat.

	Model		Genotype		Environment		Genotype by Environment	
Traits	F value†	P > F	F value	P > F	F value	P > F	F value	P > F
Chlorophyll index Day 4	1.14	0.3056	1.65	0.0481	0.60	0.4408	0.64	0.9080
Chlorophyll index Day 8	0.98	0.5291	1.38	0.1400	0.52	0.4732	0.59	0.9396
Chlorophyll index Day 12	1.05	0.4298	1.30	0.1882	4.35	0.0410	0.67	0.8828
Chlorophyll index Day 16	2.14	0.0015	1.81	0.0244	43.58	<.0001	1.13	0.3381
Seed weight primary tiller	4.20	0.0001	7.54	<.0001	0.00	0.9978	1.00	0.4795
Seed number primary tiller	4.28	<.0001	7.47	<.0001	2.26	0.1373	1.18	0.2847
Thousand kernel weight	2.57	0.0001	4.37	<.0001	0.52	0.4726	0.84	0.6857

†Omnibus test N = 124, model df = 39, Error df = 64; Type III F test Genotype df = 29, Environment df = 1, and Genotype by Environment df = 29.

Table 2.3 One-way ANOVA SAS PROC GLM of genotypic variation of percentage of control DF for stay-green and yield traits in winter wheat.

A† Traits	F value	P > F	N	df
Chlorophyll index Day 4	1.05	0.4372	84	40
Chlorophyll index Day 8	1.66	0.0517	84	40
Chlorophyll index Day 12	1.03	0.4604	84	40
Chlorophyll index Day 16	0.80	0.7562	77	38
Seed weight primary tiller	1.20	0.2759	84	40
Seed number primary tiller	1.16	0.3162	84	40
Thousand Kernel Weight	2.91	0.0004	84	40
B Traits	F value	P > F	N	df
Chlorophyll index Day 4	1.03	0.4610	76	36
Chlorophyll index Day 8	1.28	0.2252	76	36
Chlorophyll index Day 12	1.89	0.0284	74	36
Chlorophyll index Day 16	1.05	0.4459	73	36
Seed weight primary tiller	0.98	0.5288	76	36
Seed number primary tiller	1.45	0.1290	76	36
Thousand kernel weight	0.88	0.6554	76	36

† (A)Exp. 1 (B) Exp. 2.

Seed Characteristics

Seed weight per primary tiller

The experiments demonstrated significant variation in seed weight per primary tiller across different genotypes and environmental conditions. Exp. 1 seed weight per primary tiller was significantly different among genotypes and environments. (Table 2.1). Exp. 2 seed weight per primary tiller, was significantly different among genotypes (Table 2.2).

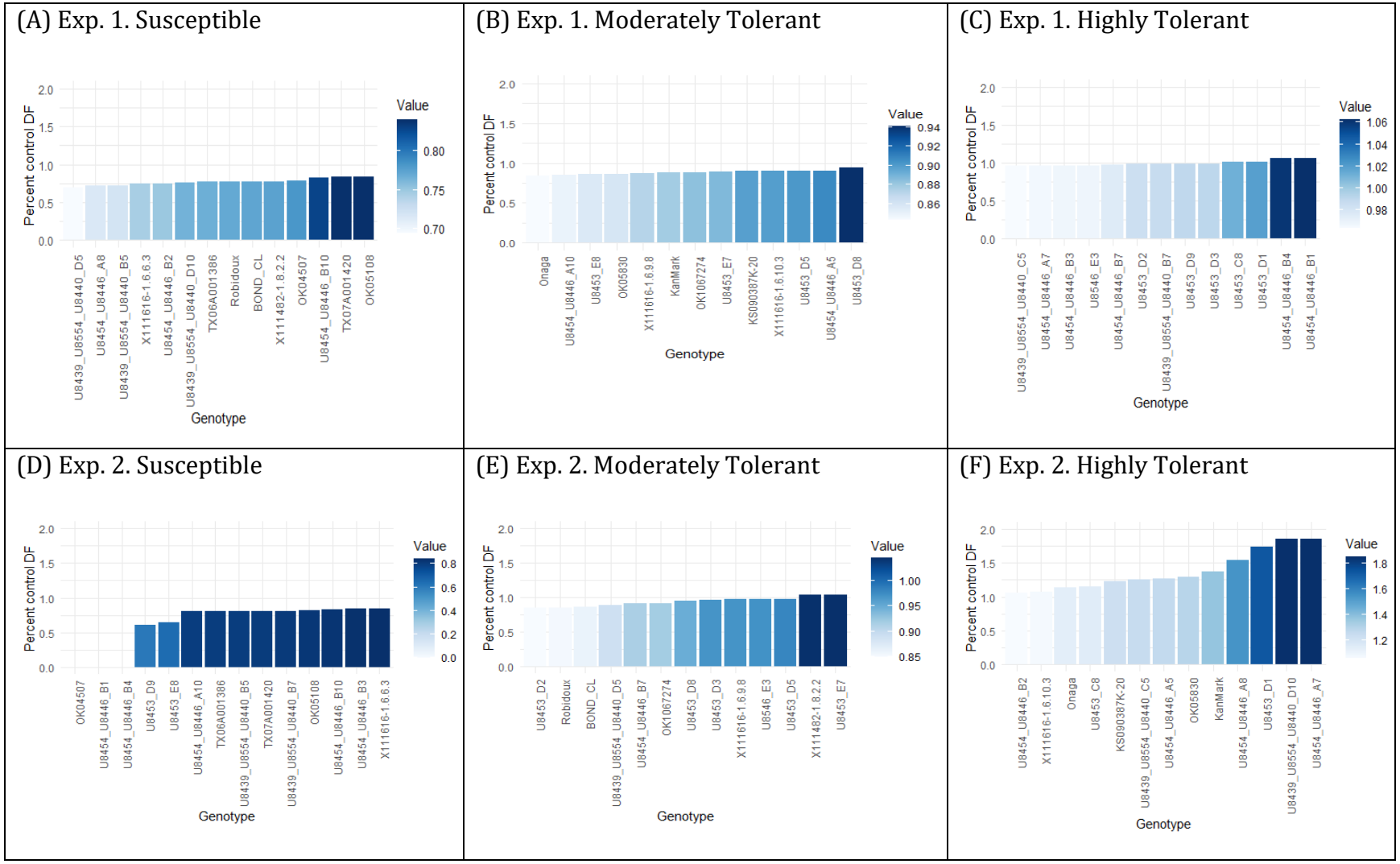
Seed number per primary tiller

Statistical analysis of both experiments revealed significant differences in the seed number per primary tiller among different genotypes, as evidenced by the results of the omnibus test and Type III Model ANOVA. Exp. 1 seed number per primary tiller was significantly different among genotypes (Table 2.1). Exp. 2 seed number per primary tiller, was significantly different among genotypes (Table 2.2). Heat stress post seed set affecting grain fill in relation to stay green characteristics was a primary focal point of these experiments and seed number for environment and genotype by environment was not found significant for Exp. 1 and Exp. 2.

Thousand kernel weight

The experiments revealed significant differences in the Thousand Kernel Weight (TKW) across genotypes, environments, and genotype by environment interaction, along with a noteworthy impact on percent control TKW, as confirmed by the omnibus tests and Type III Model ANOVA results. Exp. 1 TKW, was significantly different among genotypes, environments, and genotype by environment interaction (Table 2.1). Exp. 2 TKW was significantly different among genotypes and environments (Table 2.2). The percent control TKW was significant for Exp. 1 Table 2.3. The percent control decimal form TKW genotypes ranks are shown in Figure 2.3.

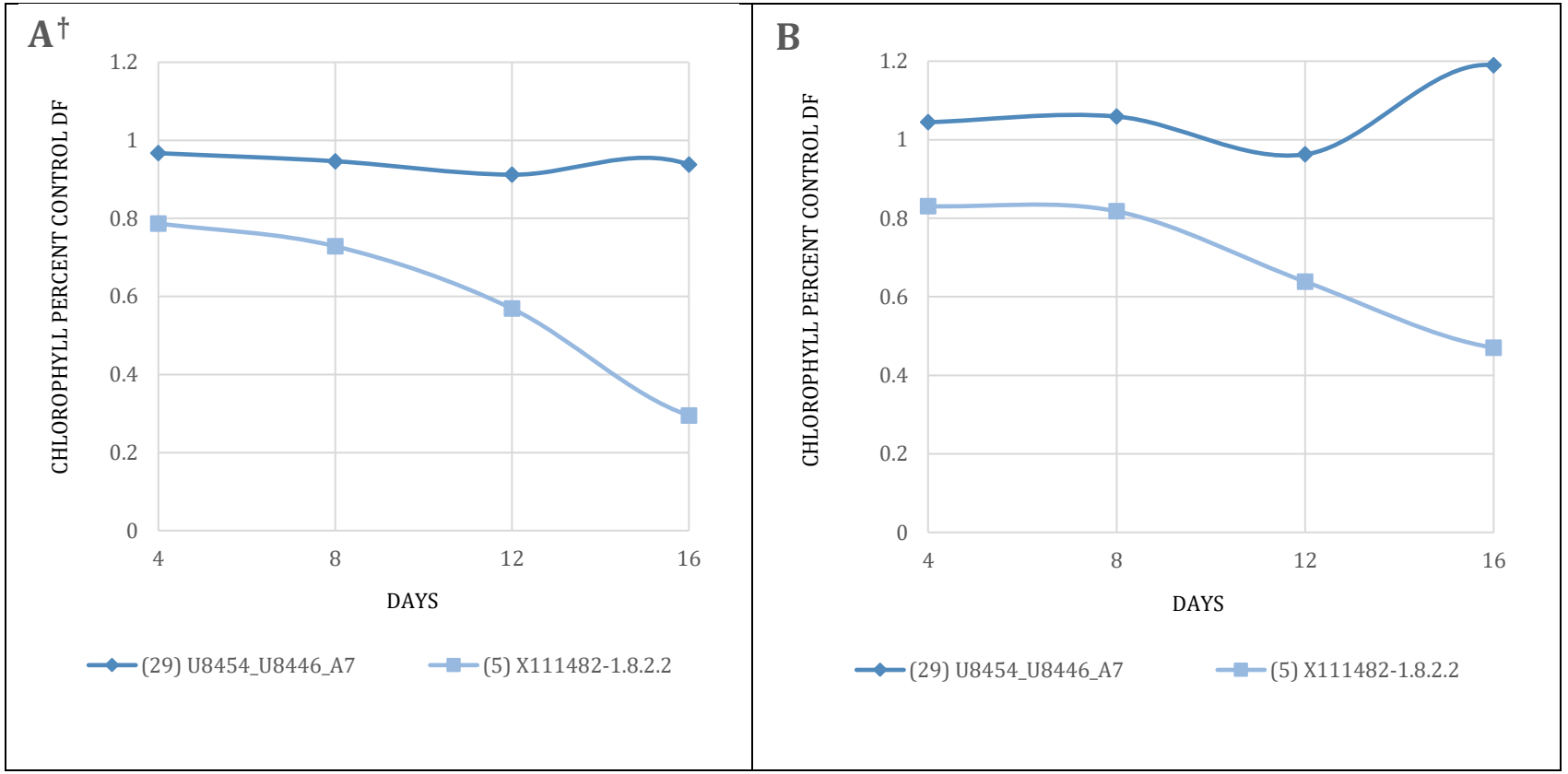
Figure 2.3. Percent control in decimal form (DF) thousand kernel weight.



Chlorophyll retention of dissimilar genotypes

The exploration of the thermal tolerance of different wheat genotypes were analyzed and based on their performance under heat stress conditions, the percent difference TKWs for all surviving genotypes were subjected to multiple comparisons of the least square means using the Tukey-Kramer Grouping for LS-Means to present the top 14 means as highly tolerant, the middle 14 as moderately tolerant and the bottom 14 as susceptible. Figure 2.3. The percent of control for chlorophyll index at Day 12 of heat treatment among the wheat lines was significant for Exp. 1 and 2, and thus subjected to multiple comparisons of the least square means using the Tukey-Kramer Grouping for LS-Means to present the top 14 means as highly tolerant, the middle 14 as moderately tolerant, and the bottom 14 as susceptible. The results used to identify the lines are presented in appendix Table 2.2 Exp. 1 and Table 2.3 Exp. 2 for TKW and Day 12 respectively. One candidate was selected from the highly tolerant categories and one candidate from the susceptible categories—defined in this set from the percent difference TKW and percent control DF Day 12 chlorophyll retention (higher chlorophyll index) — U8454_U8446_A7 (29) (highly tolerant) and X111482-1.8.2.2 (5) (susceptible). An example in terms of percent for chlorophyll retention over time, was shown in Figure 2.4. for Lines (29) and (5) showed 120.9% and 40.42% in Exp. 1 and 93.98% and 31.69% in Exp. 2 after 16 days of heat stress, respectively.

Figure 2.4 Chlorophyll retention rates in two dissimilar genotypes.



† (A) Exp. 1. (B) Exp. 2. Emmer derivative line (29) U8454_U8446_A7 with high chlorophyll retention and Wsm1-Overley/Fuller line (5) X111482-1.8.2.2 having low chlorophyll retention.

Correlations

Significant correlations were observed among the traits of chlorophyll index and yield components in the examined wheat genotypes under heat stress conditions across both experimental setups. The correlations among the studied traits of chlorophyll index and yield components were presented in (Table 2.4)**.** For Exp. 1. correlations of the percent of control for chlorophyll index among Days 4, 8, 12 and 16—excepting Day 16 with Day 4 and Day 8—were significant. For Exp. 2, correlations for chlorophyll Day 12 and Day 8 were found significant. Correlations for the percent of control between seed weight per primary tiller and chlorophyll index at Day 4, 8, 12, and 16 were significant in Exp. 1 and further correlations of the percent of control for chlorophyll index among Days 4, and 8 with seed number per primary tiller were also found. percent of control TKW for Exp. 1 with chlorophyll index Day 8 and 16 was significant and the percent of control seed weight per primary tiller and seed number per primary tiller were significant in both Exp. 1 and Exp. 2. The percent control TKW and percent control seed weight per primary tiller was significant for Exp. 1.

Table 2.4 Correlations among percentage of control for chlorophyll index Days 4, 8, 12, and 16, seed weight per primary tiller, seed number per primary tiller, and TKW.

Traits †	Chlorophyll Day 4	Chlorophyll Day 8	Chlorophyll Day 12	Chlorophyll Day 16	Seed weight / Primary Tiller	Seed number / Primary Tiller	TKW (f) / Primary Tiller
Chlorophyll Day 4	0.11689 0.3146 76	0.03726 0.7365 84	0.02211 0.8417 84	-0.00447 0.9692 77	-0.01626 0.8833 84	-0.06548 0.554 84	0.16367 0.1369 84
Chlorophyll Day 8	0.84464 <.0001 76	0.0931 0.4238 76	0.45881 <.0001 84	0.07133 0.5376 77	0.08802 0.426 84	0.08269 0.4546 84	0.04151 0.7077 84
Chlorophyll Day 12	0.39844 0.0004 74	0.57415 <.0001 74	0.02381 0.8404 74	0.21092 0.0656 77	0.0871 0.4308 84	0.12949 0.2404 84	-0.06537 0.5547 84
Chlorophyll Day 16	0.10515 0.376 73	0.21899 0.0627 73	0.55208 <.0001 73	-0.06245 0.6156 67	-0.03518 0.7613 77	-0.03353 0.7722 77	0.00567 0.9609 77
Seed Weight / Primary Tiller	0.45526 <.0001 76	0.69517 <.0001 76	0.23981 0.0396 74	0.2437 0.0377 73	0.03194 0.7841 76	0.91104 <.0001 84	0.19163 0.0808 84
Seed number / Primary Tiller	0.50548 <.0001 76	0.58705 <.0001 76	0.18801 0.1087 74	0.06486 0.5856 73	0.80353 <.0001 76	0.01895 0.8709 76	-0.20494 0.0615 84
TKW (f) / Primary Tiller	0.21259 0.0652 76	0.43194 <.0001 76	0.17257 0.1415 74	0.4179 0.0002 73	0.56336 <.0001 76	0.20541 0.0751 76	0.18295 0.1137 76

†Exp. 1 (below diagonal) and Exp. 2 (above diagonal), and between Exp. 1 and Exp. 2 (diagonal upper left to lower right). For each cell, upper number is the Pearson correlation coefficient (r), The middle number is the P value for the null hypothesis r = 0, and the lower value=N. TKW is represented as a function (f) of the primary tiller seed weight and count.

Conclusions and Future Directions

In conclusion, this study identified phenotypic expressions which revealed genetic variability among the evaluated wheat lines. Genotypes were classified as having highly tolerant, moderately tolerant, or susceptible phenotypes according to their respective rank of percent control phenotypic trait values. Heat stress effecting grain fill in relation to stay green characteristics was a primary focal point of these experiments and seed number per primary tiller for environment and genotype by environment was not found significant indicating that sed set was not confounded in grain fill. Further, both experiments showed a significant positive correlation for the percent control seed number and seed weight demonstrating similar seed character trends across experiments.

Two genotypes were selected, one commonly residing in the high tolerance category and one commonly residing in the susceptible category, utilizing percent control DF TKW, which was found significant for genotype in Exp. 1, and the percent control chlorophyll Day 12 which was found significant for genotype in Exp. 2. The two genotypes selected, U8454_U8446_A7 (highly tolerant) and X111482-1.8.2.2 (susceptible), showed a phenotypic difference in chlorophyll retention in both Exp. 1 and Exp. 2

The results of this study were from controlled chamber experiments having a single replication. A more robust experiment having increased replication within chambers could allow for better detection and confirmation of heat stress tolerance with respect to the stay green characteristics and grain traits observed in these studied lines. Given substantial chamber-based experimentation to locate the most desirable genotypes, and further extensive field testing, tolerant genotypes

identified in this study may be useful for breeding new global-warming resilient cultivated winter wheat lines.

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Chapter 3 - Discussion

Chambered Experiments

These chamber-based experiments evaluated a limited number of genotypes selected from a diverse set of gene pools for heat tolerance. This could lead to advantages for selecting tolerant genotypes, which might exhibit 'easy to spot' heat tolerance due to a diverse set of responses. Controversially, the limited number of genotypes in this set reduces plausible implications regarding the population of each genotype's respective pool. Because the testing of a diverse collection of genotypes can lead to substantial variation in phenological traits such as days to flowering, chamber-based studies—while lacking the space for examining large genotype sets—allow for the comparison of heat stressed plants at the same developmental stage within near identical environmental conditions. In addition, chamber-based studies minimize the confounding effects of other environmental and biophysical conditions that can exist in field conditions.

Environmentally controlled chambers were identified as advantageous in the early 1950's by Frits Warmolt Went, who happened to demonstrate the existence of auxin in plants (Went and Wissenschaft, 1928), however, 20 years later in the 1970's there was still a lack of concern for unevenness within and between these chambers (Allen Hammer and Langhans, 1972). With between-chamber experiments, the chamber effect, the resolvable or unresolvable discreet differences between chambers, can confound treatments (Porter et al., 2015). To obtain maximum experimental precision, a researcher must know chamber capabilities as well as possible sources of chamber effect pre-experimentation. The researcher frequently loses much of this precision due to spatial distribution and decay of fluorescent light intensity with time; air movement and chamber structure causing temperature oscillations; door, vent, border, and edge effects; differential location

of chambers within a facility, and a difference of model and make of the device (Allen Hammer and Langhans, 1972).

Robust field-based systems having roll up and down side ventilation, top ventilation, heating systems, and cyber-physical intelligent structures are used to effectively impose high temperature stress while algorithmically following the dynamic fluctuations of the outside environment (Hein et al., 2019). Due to the genetic diversity of flowering time, upwards of one month apart, these field-based setups also make it challenging to impose uniform post anthesis heat stress and separate the effects of heat stress from other biotic and abiotic stressors that create terminal effects within a week of stressing a plant. The fluctuating microcosmical nature of stressors in the field would lead to errors in data collection influencing subsequent genetic analysis.

Hindsight-derived erudition of the climates and functionality of chambers in this study, which consisted of seven chambers and two different vegetative growth locations across two sowing dates, propelled a reduced analysis by selecting the most distinctly different two chambers from each experiment with respect to the diurnal temperature regimes that materialized. In this study there was no significant correlation regarding the percent control retention of chlorophyll and thousand kernel weight of the genotypes examined across experiments, indicating that the chamber environments of the two experiments differed.

Grain filling and stay green.

In this study, the data collected approached significance for chlorophyll index Day 4, and Type III tests indicated significant differences between genotypes and between environments with no genotype by environment interaction being significant. Further, thousand kernel weight showed a genotype by environment interaction in Exp. 1 but not Exp. 2 A probable explanation for the

differentiation and lack of correlation between experiments is the early onset of powdery mildew that diseased plants in Exp. 2. Low differentiations within and between experiments could have been brought on by heat stress damaging sink components, genotypes relying mainly on stem reserves under heat stress conditions for grain filling, reduced photosynthate transport to developing grains (Akter and Islam, 2017), and the potential for stay-green to be non-functional or partially functional within lines evaluated (Thomas and Ougham, 2014). During experimentation, having not been quantified, observed genotypes displayed grain filling via relocation of stored soluble sugars from stem reserves, awns, and rapid senescence of flag leaves; continued photosynthesis, transport, and chloroplast maintenance, or increased upregulation of chloroplast maintenance with a reduction in photosynthate transport to kernels (Wang et al., 2018). These mechanisms represent distinct phenotypic strategies utilized by flora, wheat specifically, to survive and reproduce under elevated temperature regimes.

Heat stress that occurs post anthesis can endanger the process of seed filling when stored carbohydrates and nutrients are not available for relocation to the kernel. Carbohydrates can be used immediately for growth or stored and later mobilized during development. The genetic diversity of peduncle starch assimilates in wheat can vary greatly and has been linked to the differential regulation of certain carbohydrate metabolic genes and The Green Revolution dwarfing genes (Hedden, 2003), which are generally related to a decrease in storage capacity due to shorter peduncles (Scofield et al., 2009). Under optimum growing conditions, the peduncle concentration of fructose, sucrose, and other carbohydrates typically peaks 10 to 25 days post anthesis or earlier when stress shortens the grain-filling period (W.M. Blacklow, 1984). Genotypes in this study expressed a wide range of peduncle length, width, and shape with some genotypes having a large flag leaf which retained a considerably high chlorophyll index but had a peduncle completely senesced, and other genotypes which had an early senescent flag leaf with long lasting stay green

peduncles. One must consider the amount of nutrients a crop of green flag leaves will pull from the soil, while not able to translocate nutrients in the form of photosynthates into grain sinks because the dead phloem of a peduncle cannot translocate assimilates—these types of varieties are environmentally nonfunctional stay green.

Senescence is a highly regulated process involving the remobilization of mineral nutrients and carbohydrates from source leaves to sink developing grains (Li et al., 2017). This process is characterized by leaf yellowing due to the prioritized breakdown of chlorophyll (Ougham et al., 2008). A nonfunctional stay-green phenotype, found in various species, denotes genotypes that retain leaf greenness while experiencing a loss of photosynthetic function which is deemed unfavorable as it hampers nutrient movement from leaves to developing seeds: conversely, functional early leaf senescence leads to enhanced grain filling, thus boosting the grain filling rate and grain weight(Thomas and Ougham, 2014). Cytokinin's, known for delaying senescence processes, have been the focus of transgenic approaches aiming to implement the stay-green trait often by increasing the endogenous cytokinin content as demonstrated in tobacco plants with a 52% rise in seed yield (Liu et al., 2012). Unfortunately, the correlation between grain yield and the stay green trait is a more complex matter in cereal crops.

Research Inferences

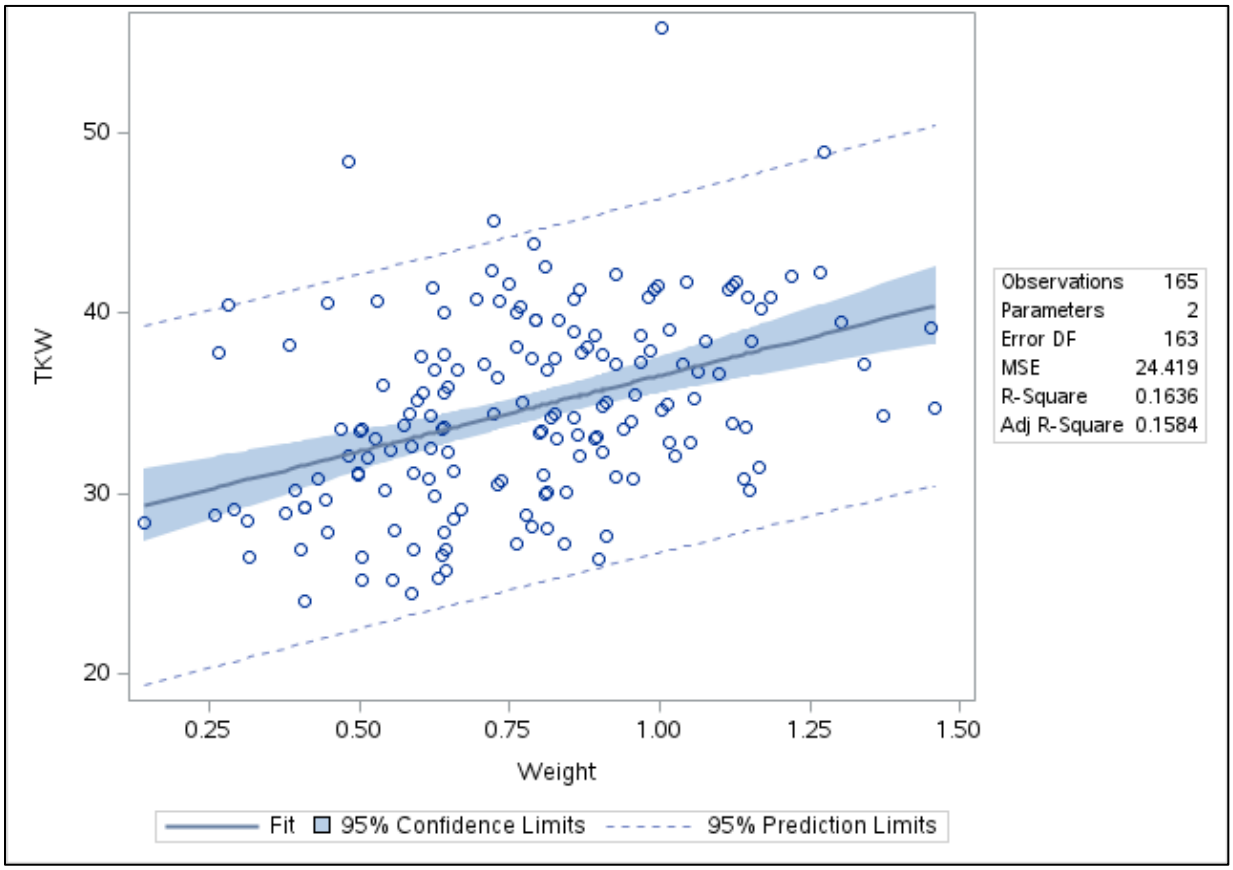
Exploring the intricate dynamics of seed development under heat stress, these experiments illuminate former studies reporting substantial variation in seed characteristics and stay green traits across diverse genotypes of wheat and environmental conditions with a particular emphasis on the impact of post-seed-set heat stress on grain-fill. Previous studies undertook the task of elucidating the impact of domestic emmer, wild emmer, and wheatgrass material introgressed into modern hexaploid cultivars.

These experiments revealed significant differences in the Thousand Kernel Weight (TKW) across genotypes, environments, and genotype by environment interaction for Exp. 1. An analysis of the genotype by environment interaction revealed the lines having a greater TKW in the high-temperature chambers were KanMark*2/TA 1077 emmer derivatives U8454_U8446_B4 and U8454_U8446_B1, and KanMark*2/TA 1000 emmer derivatives U8453_D5, U8453_D1, and U8453_C8. These lines commonly occurred in the tolerant groups for percent control of chlorophyll retention with the presented line U8454_U8446_A7 (KanMark*2/TA 1077) which exhibited high tolerance to high-temperature climates for TKW across experiments. In this study the parent line KanMark was outperformed by emmer introgressed progeny lines across environments and experiments for TKW and related seed components reflecting a recent study which purported the emmer derivative PI 272527 outperforming the line Divide for TKW by 3.64g and 14.41g in the green house and field respectively (Peters et al., 2023). These findings further link emmer wheat as a valuable resource for seed characteristics as opposed to biotic and abiotic stress tolerance traits.

Seed number for environment and genotype by environment was not found significant for these experiments indicating seed set was not impacted by the temperature treatments. Seed number per primary tiller was not significantly different across environments and seed weight per primary tiller was found significant across environments. Regression analysis computed using SAS PROC REG (SAS Institute Inc. SAS Studio. 2021) shows the independent variable weight as highly significant and explaining 15.84% of the variance of the dependent variable TKW shown in Figure 3.1. Further regression analysis shows the independent variable count as not significant and explaining 0.00% of the variance of the dependent variable TKW shown in Figure 3.2. Therefore, the average weight of a seed from the primary tiller can be seen as the driving component of TKW

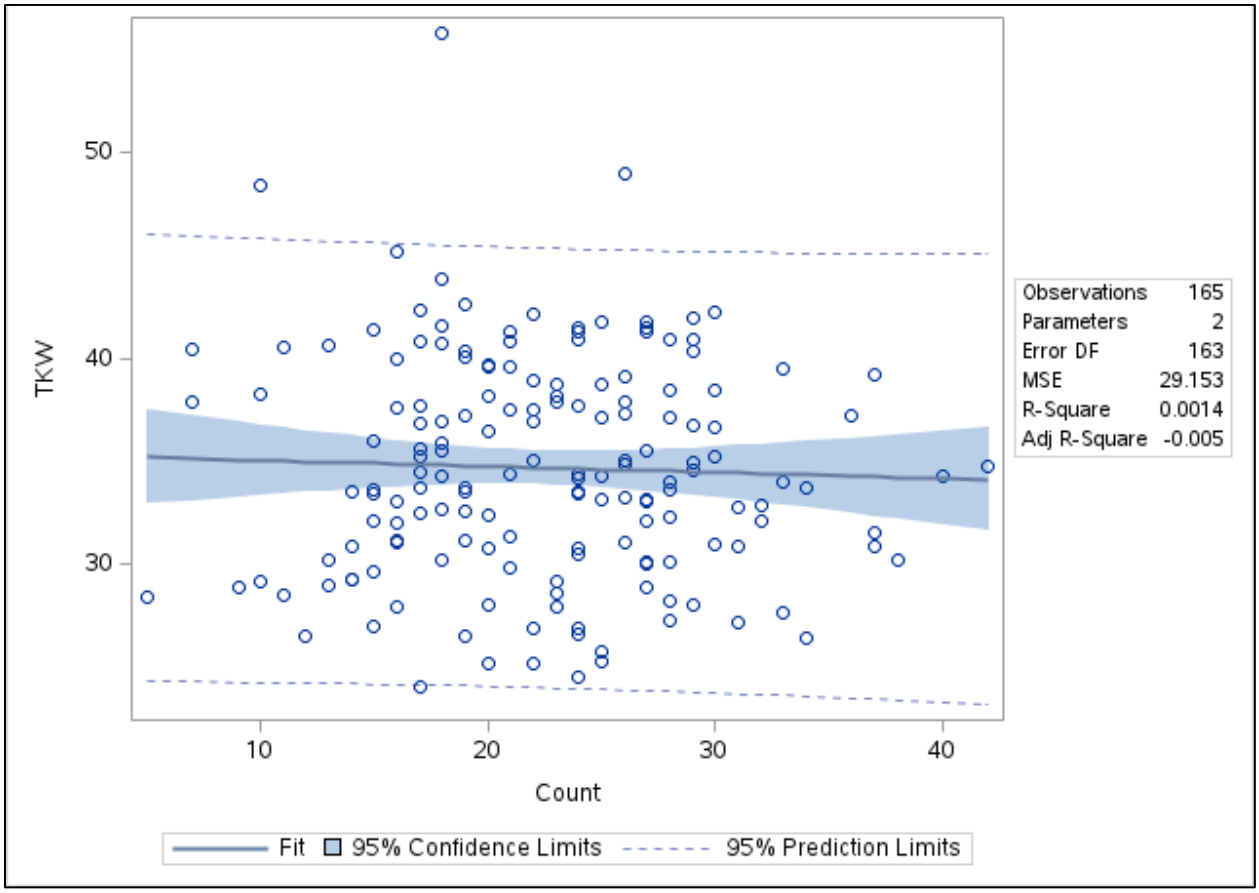
differentiation in these experiments, thus, the significant genotype by environment interaction found for the response variable TKW can be explained as a significant differentiation of grain fill for these genotypes under optimum and elevated temperatures.

Figure 3.1. Simple linear regression of weight per primary tiller as a predictor for TKW†



†Exp. 1. The fitted regression model was Intercept = 28.14 + 8.42 * Weight, the regression was statistically significant ($R^2 = 0.1636$, $F(1, 163) = 31.87$, $p < 0.0001$), and weight significantly predicted the intercept ($\beta = 8.42$, $p < 0.0001$).

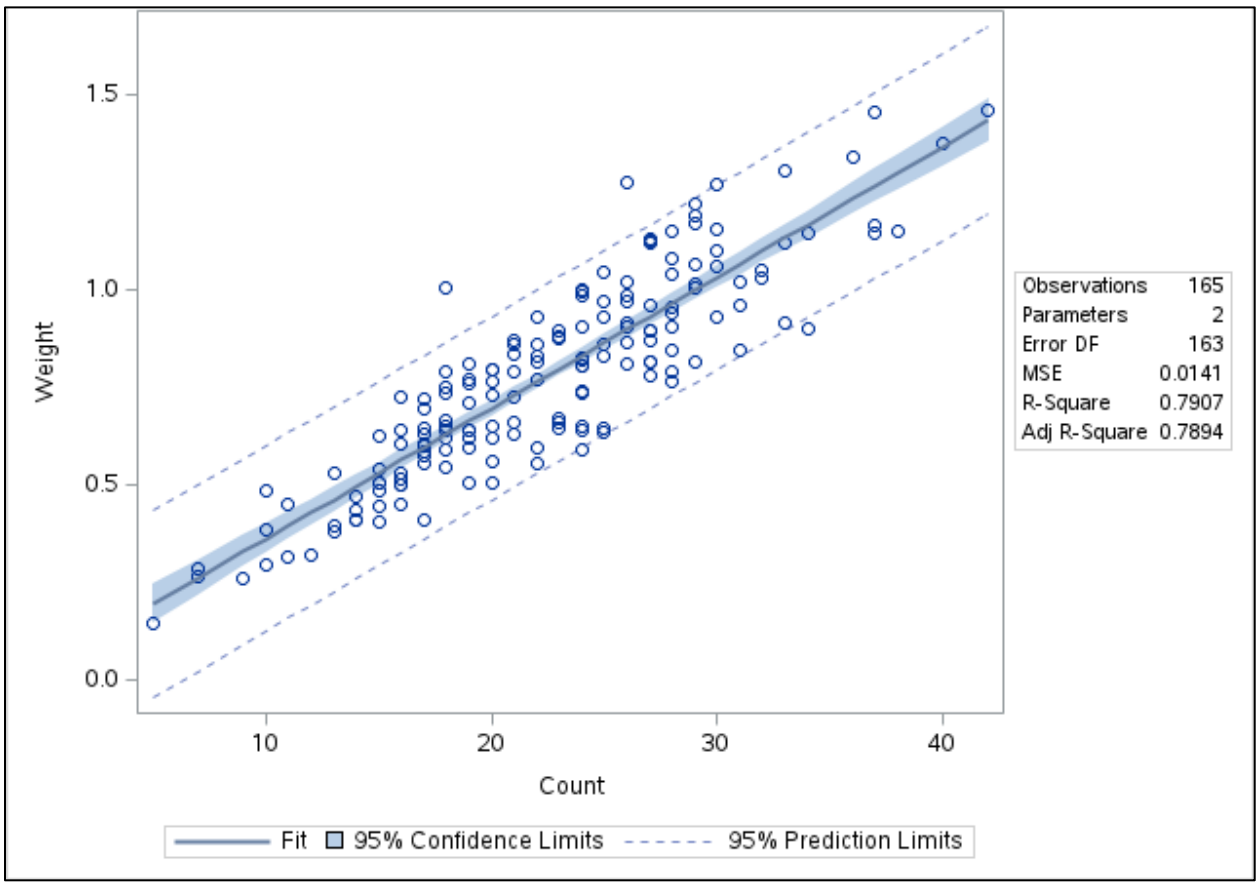
Figure 3.2 Simple linear regression of count (seed number per primary tiller) as a predictor for TKW†



† Exp. 1. The fitted regression model was Intercept = 35.37 + -0.03 * Count, the overall regression was not statistically significant (R^2 = 0.0014, F(1, 163) = 0.23, p = 0.6334).

Correlations were observed among the percent control of the seed characteristics number and weight per primary tiller for the examined wheat genotypes across both experiments. These correlations remained true for mean values within temperature profiles of both experiments. The total weight of a primary tiller is dependent on individual seed weight and the number of seeds for the tiller observed. Conversely, the number of seeds per primary tiller is independent of the variable weight per primary tiller . Therefore, correlations between seed number per primary tiller and seed weight per primary tiller are a construct formed by the collection of data which confounded the variable seed number within seed weight of the primary tiller. A highly significant regression analysis for Exp. 1 shows the independent variable count explaining 78.94% of the variance of the dependent variable weight (Figure 3.3). For common bread wheat, recent studies have reported correlation coefficient of ($r = 0.696$, $P < 0.01$) for TKW and Kernel Weight (KW) suggesting that the average weight of a kernel should be a focal trait for increased grain weight (Su et al., 2018).

Figure 3.3 Simple linear regression of count (seed number per primary tiller) as a predictor for weight per primary tiller†



† Exp. 1. The fitted regression model was Intercept = 0.02509 + 0.03350 * Count, the overall regression was statistically significant ($R^2 = 0.7907$, $F(1, 163) = 615.66$, $p < 0.0001$), and Count significantly predicted the intercept ($\beta = 0.03350$, $p < 0.0001$).

The presented dissimilar line X111482-1.8.2.2 and the line X111616-1.6.6.3, which both appeared commonly in the susceptible groups for investigated traits, are Overley/Fuller derived near isogenic lines having been introgressed with an intermediate wheatgrass locus containing *Wsm-1* gene, which, confers resistance to *wheat streak mosaic virus*, *triticum mosaic virus*, and gene(s) effective against Ug99 races of stem rust (*Puccinia graminis tritici*) (Byamukama et al., 2014). The Overly/Fuller near isogenic lines X111616-1.6.9.8 and X111616-1.6.10.3, which both contain the *Wsm-1* gene but not adjacent intermediate wheatgrass loci, commonly resided in the tolerant groups of investigated traits. The novelty of the *Wsm-1* gene resistance to *wheat streak mosaic virus* compared to the resistance of the line CO960293-2 was originally noted as *Wsm-1* introgressed lines having effective resistance at higher temperatures reaching 24°C than that of the unknown-origin based low-temperature resistance of line CO960293-2 losing resistance at temperatures reaching 18°C with both origins of resistance having shown poor and good rheological properties depending on the genetic background (Friebe et al., 2009; Graybosch et al., 2009). Reasons for the difference in tolerance between these studied *Wsm-1* introgressed lines could be wheat grass loci having linkage drag of unfavorable genes for elevated temperature tolerance, unknow linkage disequilibrium of genes within the genome of parents, and/or epistatic interaction of genes that could have positive or negative consequences given the genetic background of these introgressed lines (Hao et al., 2011). The lack of seed set differentiation across temperature regimes in combination with the significant variation in seed weight per primary tiller across different genotypes and temperature profiles in these experiments shows the detrimental yield outcomes caused by heat stress during grain fill for parents and non-adapted progeny in this study while highlighting the resiliency of these stay green emmer derivatives.

Conclusions and Future Directions

The stay-green phenotype is sometimes viewed as unfavorably delayed because the benefit gained from the prolonged photosynthesis period is overshadowed by the loss from incomplete remobilization of pre-stored carbohydrates, thus, the stay green trait can disrupt the wheat reproductive-strategy, which relies on rapid senescence and metabolite remobilization from senescing organs to developing grains shortly after anthesis (Li et al., 2022). Further, phenotypes that stay green due to increased nitrogen use, high lodging resistance expression, and exhibiting delayed whole-plant senescence are reported as having poor grain filling (Yang and Zhang, 2006). Here, the classification and selection of wild Emmer stay green traits to be adopted by hexaploid genotypes is crucial for identifying novel loci to create a functionally green crop. These experiments suggested that functional stay-green genotypes could aid in developing heat-tolerant varieties that outperform currently grown parents in both optimal and heat stressing environments. Though results from Exp. 1 and Exp. 2 were found to not significantly correlate, progeny that outperformed parents and significant genetic variation for chlorophyll retention and grain yield-related traits under heat stress conditions were identified. Further validation of the stay-green mechanisms utilized by these genotypes could potentially extrapolate which lines could be a useful genetic resource for increased wheat grain yield under post-anthesis heat stress conditions.

The intertwined relationship between wheat and *Homo sapiens* traces back to the origins of civilization, a relationship marked by shared resilience to various abiotic and biotic stressors (Dani et al., 1996). As the global population continues to grow, there is a pressing need to nearly double wheat production within the next century (Al-Ashkar et al., 2020). However, challenges such as climate change-induced drought and heat (Sehgal et al., 2018), human behavior, and conflicts are causing a decline in yields (Nasir et al., 2022). By integrating these cultivar developments with cultural understanding and ethnobotanical knowledge, we have the potential to restore sustainable

diversity in global agriculture, increase the diversity of accessions, and expand the geographical regions for which a crop could grow and produce in elevated temperature climates. The untapped genetic diversity of wild emmer presents an opportunity to explore, comprehend, develop, and implement winter wheat lines resilient to climate change.

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Appendix A - Supplemental Material

Table A.2.1 Average daily chamber temperatures

Optimal temperature (OT) chambers and elevated temperature (HT) chambers for Exp.1 and Exp. 2.

Time	OTExp2	HTExp2	HTExp2	OTExp1	OTExp1	HT-set	OT-set
0	18.04136	22.8881	21.84498	17.58172	18.26863	21.1	16.6
1	17.22455	21.46051	21.12937	17.26328	17.2365	20.1	16.2
2	16.68637	21.34869	20.62358	16.92713	17.15634	19.6	15.9
3	16.51442	20.49513	19.98986	16.58222	16.78128	19.2	15.6
4	16.18563	20.00111	19.52669	16.28434	16.472	18.8	15.4
5	15.91655	19.60409	19.11117	16.05787	16.168	18.4	15.2
6	15.71753	19.19642	19.49721	19.07662	15.95091	18.4	15.0
7	17.2711	20.46125	20.86735	21.57247	16.98084	19.6	15.8
8	20.31457	23.47527	21.95188	23.84128	18.25358	21.9	16.8
9	23.35658	24.77003	24.52013	28.14144	18.65778	24.4	17.9
10	24.81996	29.27865	27.62793	29.76256	21.40169	26.2	18.8
11	25.85832	31.49182	29.55801	30.68028	22.49506	27.6	19.0
12	26.62182	33.05419	31.00538	31.08058	23.41531	28.9	19.6
13	27.04208	34.36455	32.22158	31.40873	23.65973	29.9	20.3
14	27.62358	35.81922	33.39222	32.05448	24.23461	30.8	20.9
15	28.34699	36.71491	34.30961	32.92409	24.79961	31.5	21.3
16	28.93103	37.61097	34.99427	33.45573	25.41318	31.7	21.5

	29.2740	38.2829	35.5546	33.7659	25.7902		
17	1	1	2	4	4	31.3	21.7
	29.4713	38.5115	35.5996	34.0190	26.0145		
18	4	2	8	9	5	30.5	21.5
	29.5085	38.2013	34.1537	30.8820	26.2273		
19	2	7	3	9	3	29.0	21.0
	29.2195	35.1518	32.1320	28.2062	24.0969		
20	6	1	8	7	7	26.0	19.8
	26.8366	33.6037	29.4348	24.9849	24.1204		
21	4	7	1	7	2	23.6	18.5
	23.7654	27.8366	25.7459	20.1762	21.5252		
22	5	7	2	7	7	22.5	17.7
		24.0036	23.1486	18.6478	19.0222		
23	18.9645	6	3	5	4	21.7	17.0

Table A.2.2 Thousand Kernel Weight Percent Difference

	Exp1			Exp2		
Tolerance	Genotype	LS-Means	Significance (*)	Genotype	LS-Means	Significance (*)
High	U8454_U8446_B1	1.062135	*	U8454_U8446_A703	1.851	
High	U8454_U8446_B471	1.059	*	U8439_U8554_U8440_D10	1.849 73	
High	U8453_D1	1.019175		U8453_D1	1.731 77	
High	U8453_C8	1.01188		U8454_U8446_A811	1.546	
High	U8453_D3	0.99111		KanMark	1.373 44	
High	U8453_D9	0.990075		OK05830	1.290 765	
High	U8439_U8554_U8440_B7	0.987575		U8454_U8446_A5085	1.264	
High	U8453_D2	0.98515		U8439_U8554_U8440_C536	1.251	
High	U8454_U8446_B7	0.975885		KS090387K-20	1.228 46	
High	U8546_E3	0.96789		U8453_C8	1.148 405	
High	U8454_U8446_B3	0.967575		Onaga	1.138 335	

		0.965		1.068
High	U8454_U8446_A7	84	X111616-1.6.10.3	86
	U8439_U8554_U8	0.963		1.058
High	440_C5	535	U8454_U8446_B2	09
Mediu		0.941		1.044
m	U8453_D8	255	U8453_E7	19
Mediu		0.907		1.043
m	U8454_U8446_A5	74	X111482-1.8.2.2	795
Mediu		0.902		0.979
m	U8453_D5	06	U8453_D5	96
Mediu		0.899		0.970
m	X111616-1.6.10.3	59	U8546_E3	715
Mediu		0.898		0.969
m	KS090387K-20	88	X111616-1.6.9.8	315
Mediu		0.887		0.968
m	U8453_E7	775	U8453_D3	93
Mediu		0.884		0.947
m	OK1067274	265	U8453_D8	01
Mediu		0.878		0.918
m	KanMark	76	OK1067274	47
Mediu		0.872		0.914
m	X111616-1.6.9.8	935	U8454_U8446_B7	31
Mediu		0.859	U8439_U8554_U8	0.889
m	OK05830	79	440_D5	835
Mediu		0.857		0.861
m	U8453_E8	24	BOND_CL	765
Mediu	U8454_U8446_A1	0.850		0.852
m	0	2	Robidoux	645
Mediu		0.843		0.848
m	Onaga	75	U8453_D2	845
		0.839		0.845
Low	OK05108	92	X111616-1.6.6.3	1
		0.839		0.839
Low	TX07A001420	625	U8454_U8446_B3	62
	U8454_U8446_B1	0.828	U8454_U8446_B1	0.827
Low	0	57	0	72
		0.782		0.821
Low	OK04507	415	OK05108	08
		0.775	U8439_U8554_U8	0.812
Low	X111482-1.8.2.2	22	440_B7	145
		0.773		0.807
Low	BOND_CL	1	TX07A001420	035
		0.770	U8439_U8554_U8	0.802
Low	Robidoux	795	440_B5	74
		0.766		0.802
Low	TX06A001386	835	TX06A001386	675
	U8439_U8554_U8	0.764	U8454_U8446_A1	0.801
Low	440_D10	89	0	88
		0.750		0.651
Low	U8454_U8446_B2	54	U8453_E8	115

		0.745			0.609	
Low	X111616-1.6.6.3	045		U8453_D9	89	
		0.739				
Low	U8453_D6	785				
	U8439_U8554_U8	0.721				
Low	440_B5	32				
		0.716				
Low	U8454_U8446_A8	07				
	U8439_U8554_U8	0.694				
Low	440_D5	97	*			

Table A.2.3 Percent difference DF chlorophyl index Day 12 of treatment

	Exp1			Exp2		
Tolera nce	Genotype	LS-Means	Significanc e (*)	Genotype	LS-Means	Significanc e (*)
		2.9338		U8439_U8554_U84	1.2159	
High	U8453_D6	25	*	40_B7	6	*
					1.1143	
High	U8453_D3	1.0346		X111616-1.6.10.3	85	*
		0.9726			1.1105	
High	KS090387K-20	6		U8454_U8446_B3	4	*
	U8439_U8554_U84	0.9435		U8439_U8554_U84	1.0944	
High	40_C5	15		40_D10	2	
		0.9284			1.0851	
High	U8453_E8	65		OK05108	9	
		0.9142		U8439_U8554_U84	1.0661	
High	U8454_U8446_A7	2		40_C5	45	
		0.8826			1.0537	
High	U8454_U8446_A8	5		U8454_U8446_A8	15	
		0.8816			1.0518	
High	X111616-1.6.6.3	3		TX06A001386	25	
		0.8804			1.0349	
High	U8453_D5	1		U8454_U8446_B7	45	
		0.8776			1.0232	
High	U8453_D1	75		X111616-1.6.9.8	3	
		0.8704			1.0062	
High	Robidoux	45		BOND_CL	1	
		0.8695		U8439_U8554_U84	0.9755	
High	TX07A001420	45		40_D5	65	

		0.8622		0.9632	
High	U8454_U8446_B2	5	U8454_U8446_A7	25	
Mediu	U8439_U8554_U84	0.8609		0.9624	
m	40_D5	25	U8453_E8	5	
Mediu		0.8539		0.9494	
m	U8546_E3	65	U8454_U8446_A5	6	
Mediu		0.8506			
m	U8454_U8446_A10	85	OK05830	0.9414	
Mediu	U8439_U8554_U84	0.8386		0.9364	
m	40_B7	1	U8454_U8446_B2	55	
Mediu	U8439_U8554_U84	0.8340		0.9250	
m	40_B5	7	KS090387K-20	05	
Mediu		0.8274		0.9250	
m	X111616-1.6.10.3	45	X111616-1.6.6.3	05	
Mediu		0.8268		0.9146	
m	OK05108	9	TX07A001420	75	
Mediu		0.8169		0.9129	
m	BOND_CL	3	U8453_D8	7	
Mediu		0.8054		0.8974	
m	X111616-1.6.9.8	1	Onaga	15	
Mediu		0.7971		0.8942	
m	U8453_D2	95	U8454_U8446_A10	6	
Mediu		0.7944	U8439_U8554_U84	0.8635	
m	KanMark	225	40_B5	4	
Mediu		0.7909		0.8153	
m	OK05830	85	KanMark	65	
Mediu		0.7689		0.8118	
m	U8454_U8446_B7	8	U8453_E7	5	
		0.7643			
Low	U8454_U8446_B1	3	Robidoux	0.8051	
		0.7216		0.7957	
Low	U8453_E7	2	U8453_D1	2	
		0.6249		0.7033	
Low	Onaga	55	U8453_D2	45	
		0.6114		0.6760	
Low	U8453_D8	45	U8453_D5	35	
		0.5927		0.6619	
Low	OK1067274	45	U8454_U8446_B10	25	
		0.5657		0.6420	
Low	X111482-1.8.2.2	25	OK1067274	05	
		0.5346		0.6040	
Low	U8454_U8446_B3	25	X111482-1.8.2.2	45	
	U8439_U8554_U84	0.5150		0.4617	
Low	40_D10	2	U8546_E3	45	
		0.4744		0.4194	
Low	U8453_D9	3	U8453_D9	25	
		0.4618		0.3542	
Low	U8454_U8446_B10	5	U8453_D3	55	
Low	TX06A001386	0.451	U8453_C8	0	*

		0.2858	
Low	U8454_U8446_A5	35	*
Low	U8454_U8446_B4	0.2158	*
Low	OK04507	0	*
Low	U8453_C8	0	*

A.2.4 Link to data

https://github.com/JamesRRoss/KSU_DATA_2022