Effect of heat stress and auxin application at flowering on grain yield and QTL associated with heat stress responses in wheat (*Triticum aestivum* L.)

by

Ganegama Lekamge Dhanuja Neranjalee Abeysingha

A thesis submitted in partial fulfillment of the requirements for the degree of

Doctor of Philosophy

in
Plant Science

Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

The reproductive phase of wheat (*Triticum aestivum* L.) is highly sensitive to high-temperature stress. Temperatures above the growth optimum (23ºC) negatively affect the reproductive development, resulting in poor grain yield. Genetic modifications and proper agronomic practices can be used to overcome the negative effects of heat stress. Experiments presented in this thesis test the hypotheses that: 1) heat stress at initial flowering (35 °C for 6 h per day for 6 days) has a negative impact on grain yield and foliar auxin application (4-Cl-IAA, 1µM) has the ability to at least partially negate the negative impact of heat stress, and 2) variation in heat stress response with respect to grain yield among a wheat RIL population will allow for the identification of specific phenotypic traits and quantitative trait loci (QTL) associated with heat stress resistance.

First, a controlled environment experiment was conducted to evaluate the Canadian hard-red spring and/or CIMMYTY derived parents of two recombinant inbred line (RIL) populations of wheat for heat resistance and auxin responsiveness; the first population was derived from a cross between 'Attila' and 'CDC Go', and the second between 'CDC Teal' and 'CDC Go'. The 'Attila' x 'CDC Go' RIL population (171 lines) was selected for in-depth evaluation because 1) grain yield after heat-stress differed in 'Attila' and 'CDC Go', 2) the ability of a one-time foliar 4-Cl-IAA application (prior to heat stress) to ameliorate the negative effects of heat stress on grain yield was observed in 'Attila' and 'CDC Go', and 3) the 'Attila' × 'CDC Go' RIL population was more extensively characterized in the previous field studies than the 'CDC Teal' x 'CDC Go' RILs.

The 'Attila' x 'CDC Go' RILs, the parental RIL cultivars, and seven other Canadian spring wheat cultivars were further evaluated for heat resistance and auxin responsiveness under controlled environmental conditions. 'Attila' showed greater yield stability under heat stress

compared to ‘CDC Go’. Heat stress reduced the grain yield in ‘CDC Go’ by 45%. Heat stress reduced the RIL population mean grain yield with a substantial reduction in fertile spikelets per spike and grain number per spikelet or per fertile spikelet. Within the RIL population, 45% (77 RILs) were categorized as heat-resistant, 20.5% as moderately heat susceptible (35 RILs) and 7.6% (13 RILs) as highly heat susceptible. Strong to minor relationships were observed between yield component traits and grain yield among the standard spring wheat cultivars and the ‘Attila’ × ‘CDC Go’ RILs, and in some cases heat stress affected the strength of the relationships. Auxin treatment increased some yield traits (grain number and weight, fertile spikelets per spike, and grain number per spikelet or per fertile spikelet) under heat stress and/or non-temperature stress conditions in ‘Attila’, ‘CDC Go’, and RILs 18, 46, 70, 80, and 145.

Inclusive composite interval QTL mapping was conducted using phenotypic data of the ‘Attila’ x ‘CDC Go’ RIL population and genotypic data obtained from a previous study conducted using a subset of (1200 SNPs) Wheat 90K SNP array together with *Ppd-D1*, *Vrn-A1*, and *Rht-B1* genes. Whole spike and spike section data from non-temperature stress (NS) and heat stress (HS) treatments identified 73 QTL (NS, 37; HS, 36) on 14 of the 21 chromosomes that individually explained 1.6 to 47.5% phenotypic variation with Logarithm of Odds (LOD) values ranging from 2.5 to 25.8. Eight important QTL clusters associated with two or more important grain yield or yield-related traits were identified on chromosomes 5A, 4B, 2B, 2D and 1B.

Overall, heat stress at early flowering reduced grain yield, with the magnitude of the reduction dependent on the genotype. Relationships between grain yield and other yield-component traits were modified by the heat stress in some cases, stressing the importance of cultivar trait evaluation under environments where the cultivar will be grown. One-time foliar application of auxin prior to heat stress (4-Cl-IAA at 1 μM) at the early flowering stage can

increase the grain yield and/or yield component traits in some genotypes and has the potential for use as an agronomic tool to enhance wheat grain yield. QTL and QTL clusters were identified for non-temperature stress and/or heat stress, with many detected in QTL hotspots in the wheat genome for grain yield and spike architecture.

Preface

All the experiments in this thesis were conceptualized by Dr. Jocelyn Ozga (University of Alberta), with input from Dr. Dennis Reinecke (University of Alberta). I co-designed experiments for implementation. I performed the experiments, and collected, organized, and analyzed the phenotypic data for all experiments. Data interpretation was completed with the help of Dr. Jocelyn Ozga for all experiments. Dr. Muhammad Iqbal (University of Alberta) aided with QTL analysis and interpretation. The auxin (4-Cl-IAA) solutions used in all the experiments were prepared by Dr. Dennis Reinecke. The 'Attila' × 'CDC Go' RIL population seeds were obtained from and genotyping, and the linkage map construction used in the chapter 4 of this thesis was completed by the wheat breeding group of Dr. Dean Spaner (University of Alberta).

Dedication

To my beloved mother Chandra Gunawardena and late father Sugathapala Abeysingha

Acknowledgement

I am extremely thankful and indebted to Dr. J. A. Ozga my supervisor, for her excellent guidance and encouragement academically and emotionally throughout my Ph. D. program and for the financial support. My sincere gratitude and appreciation also go to Dr. Dean Spaner, my co-supervisor for his help, guidance, and financial support during last few years. My gratitude extends to my committee member Dr. Sheri Strydhorst, for her support and insightful comments during this academic journey. My sincere thanks should also go to Dr. Muhammad Iqbal and Dr. Rong-Cai Yang for their support, guidance, and suggestions in statistical and QTL analyses.

I would also like to thank Dr. Dean Spaner and his wheat breeding group for providing the wheat seeds for cultivars and genotypes, genotypic data, and linkage map used in my experiments and QTL analysis. I am thankful to Dr. Dennis Reinecke for preparing the hormone solutions used in my experiments. Special thanks should go to Dr. K. Semagn for helping me with the linkage map figures. I cannot forget the friendly support received from Nikki-Karyssa Scott, Kelly Dunfield, and Holly Horvath during my study period. I would like to thank all the past and current members of my lab including Dakshina, Dilini, Dinithi, Anu, Bin, and Harleen for their love, kindness, help and friendship while working in the lab. Many thanks to Ariana and Joseph who helped me with sample processing and data collection.

I would like to acknowledge the Alberta Wheat and Barley Commission and NSERC for their financial support of this project to my supervisor, Dr. Ozga, and ACIDF, AWC, Sask Wheat, NSERC, and AAFC for their financial support of this project to my co-supervisor, Dr. Dean Spaner. My sincere thanks to the providers of Robert Simonet graduate scholarship that I received through the Faculty of Graduate Studies and Research, University of Alberta for last five years, and the Alberta Wheat Commission for giving me the Graduate Research Scholarship in Crop Science in 2017.

I am grateful to all my teachers I met throughout my life for their help, guidance, and encouragement. I cannot forget all my friends who went through hard times together, cheered me on, and celebrated each accomplishment including: Lalani and Chandana, Chamila and Nandika, Madhavi and Isuru, Dakshina and Anu, and Dilini and Dilahara. Finally, I would like to thank my family members my mother, sister, brother, and all the in-laws, for their love, constant support, and encouragement. I am very grateful to my mother and my late father who made enormous

sacrifices to make me who I am today. Finally, I wish to acknowledge the love, support, and patience of my husband Nimesh and thank to my loving daughters Pasindi and Sasini for being my inspiration.

Table of contents

List of tables

List of figures

List of abbreviations

Abbreviation	Definition
2,4-D	2,4-dichlorophenoxyacetic acid
4-Cl-IAA	4-Chloroindole-3-acetic acid
AFLP	Amplified Fragment Length Polymorphism
ANOVA	Analyses of variance
BBCH	Biologische Bundesanstalt, Bundessortenamt and CHemische Industrie (a scale used to identify the phenological development stages of a plant)
CIM	Composite interval mapping
CTAG	Canadian Triticum Advancement through Genomics
CWRS	Canada Western Red Spring
DArT	Diversity arrays technology markers
DH	Doubled haploid
DNA	Deoxyribonucleic acid
Eps	Earliness per se genes
FLA	Flag-leaf area
FLL	Flag-leaf length
FLW	Flag-leaf width
H_2O_2	Hydrogen peroxide
HD	Heading date
HS	Heat-stress
HSF	Heat-shock transcription factor
IAA	Indole-3-acetic acid
IAM	Indole-3-acetamide
IAOX	Indole-3-acetaldoxime
IPA	Indole-3-pyruvic acid
LOD	Logarithm of odds
LSD	Least significant difference
MAS	Marker-assisted selection
NAA	1-naphthaleneacetic acid

NS	Non-temperature stress
Ppd	Photoperiod response genes
PSII	Photosystem II
PVE	Phenotypic variance
QTL	Quantitative trait loci
RAPD	Random amplification of polymorphic DNA
RFLP	Restriction fragment length polymorphism
Rht	Reduced height
RIL	Recombinant inbred line
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SSR	Simple sequence repeat
TAA1	TRYPTOPHAN AMINOTRANSFERASE genes
TAM	Tryptamine
TAR1 & TAR2	TRYPTOPHAN AMINOTRANSFERASE RELATED genes
Vrn	Vernalization genes

Chapter 1-General introduction

1.1 Origin of wheat (*Triticum aestivum* L.)

The major agricultural crops grown world-wide have been selected from their wild ancestors and successfully transferred to agro-ecosystems to grow mainly as monocultures by improving the yielding ability and nutritional qualities through selection and breeding (Smýkal et al., 2018; Doebley et al., 2006). Modern hexaploid bread wheat (*Triticum aestivum* L.) cultivars have evolved through three ploidy events to contain A, B, and D genomes before use in agricultural fields around the world (Preece et al., 2017).

Origin and domestication of diploid (2n, einkorn; *Triticum monococcum* L., genome AA) and tetraploid (4n, emmer; *T. turgidum* L. ssp. *dicoccon*, genome AABB) wheat is believed to have occurred in the Fertile Crescent in western Asia (modern day southern Iraq, Syria, Lebanon, Jordan, Israel, and northern Egypt) approximately 10,000 years ago (Brown et al., 2009; Preece et al., 2017). The domestication of hulled emmer wheat was the first step that ultimately resulted in the evolution of free-threshing tetraploid durum wheat (*T. turgidum* ssp. *durum*, 2n = 4x = 28, genome AABB). Common wheat, *Triticum aestivum* L. (2n=6x=42, genome AABBDD) is believed to originate from the spontaneous amphiploid hybridization of tetraploid *T. turgidum* wheat with diploid *Aegilops tauschii Coss.* (2n = 2x = 14, genome DD) around 8,000 years ago (Venske et al., 2019). Thereafter, domestication and breeding for domestic use has reduced the naturally evolved survival strategies of wheat making wheat cultivars more vulnerable to environmental stresses than their wild progenitors (Reif et al., 2005).

Wheat has a large genome (~16 Gb) as it contains A, B, and D genomes derived from the above mentioned three progenitor species (Brenchley et al., 2012) compared to that of, rice (400 Mb) and *Arabidopsis thaliana* (130-140 Mb) (Martínez-Pérez et al., 1999). Hexaploid wheat contains 21 chromosomes in the 2N state (7 chromosomes each from A, B, and D genomes; Martínez-Pérez et al., 2003).

1.2 Wheat production and uses

Wheat is one of the key staple crops for global food security, providing more than 35 % of the cereal caloric intake in the developing world, 74 % in the developed world, and 41 % globally from direct consumption (Shiferaw et al., 2013). Wheat is milled to produce flour, which

is used to make a variety of food products around the world, including bread, cereals, pasta, noodles, rolls, pastries, cakes, and cookies (Giraldo et al., 2019). Generally, wheat grains used for human consumption must follow strict quality requirements including higher protein content (Canadian Grain Commission, 2021). Feed-grade wheat is used for cattle, poultry, and other livestock feed (Giraldo et al., 2019). Moreover, wheat is used for industrial purposes such as ethanol and biofuel production (Spiertz & Ewert, 2009). Wheat grain use for animal feed and industrial purposes contains high starch and low protein content and generally the cultivars produce higher grain yield (Canadian Grain Commission, 2021). Currently, wheat is one of the most widely grown crops at more than 218 Mha, with a total world production of 772.6 Mmt (Statista, 2022) making it the third largest crop in the world behind corn and rice (Asseng et al., 2011). The European Union followed by China, India, Russia, United States, and Canada are major wheat producing countries/group of countries of the world. Canada is the world's sixth-largest producer (21.6 mmt produced in 2020-2021 crop year) and one of the largest exporters of wheat (Agriculture and Agri-food Canada, 2021). The prairie provinces (Saskatchewan, Manitoba, and Alberta) dominate durum and spring wheat production, producing approximately 95% of these wheat types in Canada (2018-2019 field year: USDA, 2019). Saskatchewan produces 44% of all Canadian wheat, followed by Alberta at 33%, and Manitoba at 16% (USDA, 2019). Canadian wheat classes are categorized as: 1) western Canadian or eastern Canadian by the regions in, which the varieties are grown; 2) hard or soft based on the kernel hardness; 3) red or white based on the grain (bran colour); and 4) winter (sown in the fall and harvested in summer; 10-month cycle) or spring wheat (planted in April or May and harvested in August to October; 4-5 month cycle), depending on the season it is planted (Canadian Grain Commission, 2021). There are ten Canadian western wheat classes (Canada Western Red Spring, Canada Western Hard White Spring, Canada Western Amber Durum, Canada Western Red Winter, Canada Western Soft White Spring, Canada Western Extra Strong, Canada Prairie Spring White, Canada Prairie Spring Red, Canada Northern Hard Red, and Canada Western Special Purpose) and seven Canadian eastern wheat classes (Canada Eastern Red Spring, Canada Eastern Hard Red Winter, Canada Eastern Soft Red Winter, Canada Eastern Amber Durum, Canada Eastern White Winter, Canada Eastern Other Wheat, and Canada Eastern Feed; Canadian Grain Commission, 2021) and each class has specific characteristics related to their end-use functionality for bread, noodles, pastries, confections, and other food or feed uses.

1.3 Wheat floral spike architecture

The floral spike (elongated, unbranched, determinate inflorescence) consists of a rachis (central axis) and spikelets (Fig. 1.1A). Spikelets are attached to the rachis by a rachilla in an alternate arrangement (Whitford et al., 2013). At the base of each spikelet there are bract-like organs called glumes, which protect the delicate florets growing inside the spikelet (Fig. 1.1C; Kirby, 1974). A spikelet consists of multiple florets (3-6 potentially fertile florets; De Vries, 1971; Kirby, 1988). Wheat flowers are mostly cleistogamous, and pollen is shed before or just after flowers start opening (Whitford et al., 2013). Each floret consists of a lemma (awns are attached to the lemma in awned wheat), palea, stamens (3), a pistil, and two lodicules which are located between the lemma and the ovary base (Fig. 1.1C). The lemma and palea envelop the stamen and pistil (Whitford et al., 2013). The pair of lodicules expand rapidly at the time of anthesis and, push away the rigid lemma allowing anthers and stigma to emerge (Zajączkowska et al., 2021). Although, the wheat spike exhibits determinate growth as the inflorescence apex culminates with a terminal spikelet, each spikelet has an indeterminate growth habit (De Vries, 1971; Kirby, 1988).

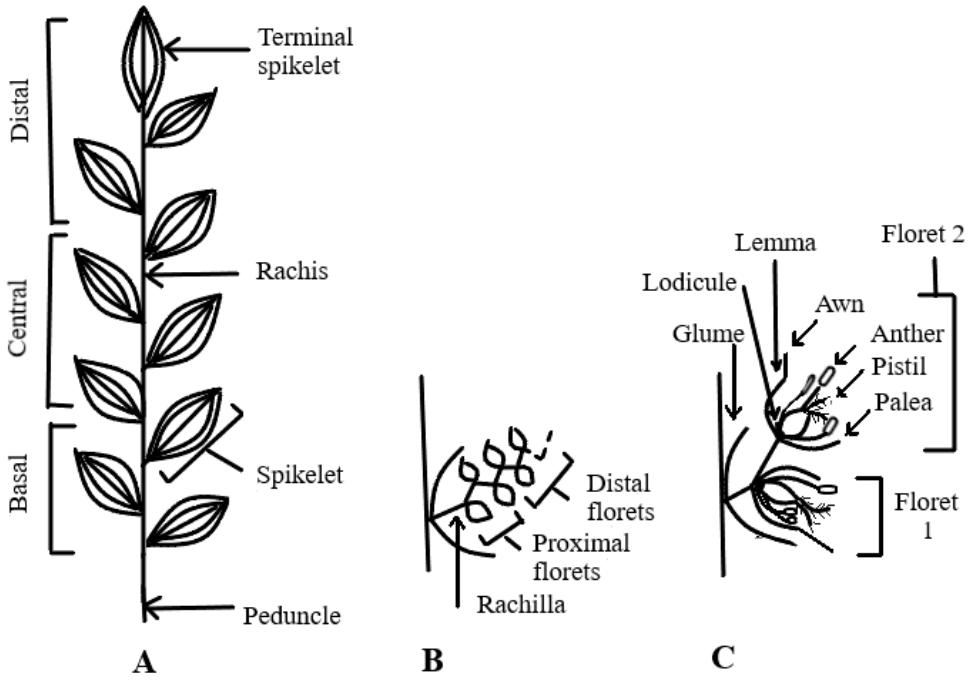

Figure. 1.1. Wheat spike and floret architecture. A) wheat spikelets are alternatively arranged along the rachis. In this thesis, spikelets are grouped into three sections: basal, central, and distal depending on the spikelet position along the rachis. B) floret arrangement within a spikelet. C) parts of a typical wheat floret within a spikelet.

1.4 Reproductive development of wheat

Wheat is an annual plant with a determinate growth habit, which has a marked transition from vegetative to reproductive growth phases. The pre-anthesis phase of wheat can be categorized into three phases based on morphological changes of the shoot apical meristem: the vegetative phase, the early reproductive phase, and the late reproductive phase (Gol et al., 2017). The main shoot stem continues to produce tillers in the vegetative phase, until the plant transitions to the early reproductive phase. At this transition, production of new leaf primordia ceases, and spikelet formation begins. The transition period is complete when the shoot apex forms two ridges (double ridge stage) on the sides of the elongating shoot apex (Fig. 1.2A; Bowden et al., 2008). The lower ridge is a leaf primordium that ceases its growth during early development, while the upper ridge (spikelet primordium) differentiates to form all the floret organs: glume, lemma, palea, stamen and

pistil of the floret (Hyles et al., 2020). Spikelet primordia development ends with the development of the terminal spikelet (Fig. 1.2B) and the duration between the beginning of spikelet primordia initiation to terminal spikelet development is an important determinate of the spikelet number per spike, indicating genotypes with a longer flowering time may initiate a greater number of spikelets per spike until the terminal spikelet is formed (Slafer & Rawson, 1994; Gol et al., 2017). However, the number of spikelets per spike is not an important trait to determine the final grain number unless they successfully transition to produce fertile florets (Phillipp et al., 2018). Therefore, events in the late reproductive phase also play an important role in determining final grain yield. The late reproductive phase is characterized by two important developmental processes happening at the same time: stem elongation and the differentiation and maturation of florets, which are highly influenced by environmental conditions compared to the vegetative and early reproductive phases (Kirby, 1988; Gol et al., 2017). The balance of photoassimilate translocation between the elongating stem and the developing florets is a critical factor for determining the final grain number, where less partitioning to the florets encourages floret abortion (Kirby, 1988; Ochagavía et al., 2018). In general, 6-12 floret primordia initiate during the early reproductive phase, however, normally less than half survive to reach the fertilization stage at anthesis (González et al., 2003). Floret death or degeneration coincides with the period of maximum stem and spike growth rate, suggesting that death of floret primordia is due to competition between the spike and stem for limited assimilates (Kirby, 1988; González et al., 2003). Under this scenario, genotypes with semi-dwarf plant structures have an added advantage in supplying more photoassimilates to the developing spikes and florets/grains than the genotypes with tall plant structures (Jobson et al., 2019). Furthermore, a long spike growth duration (from terminal spikelet initiation to anthesis) enhanced the spike dry weight, fertile floret number, and grain number and weight (Miralles et al., 2000; González et al., 2003). In general, the duration of early and late reproductive phases is positively associated with the final spikeklet number and number of fertile spikelets per spike, respectively.

If a floral spike is divided into three sections (basal, central, and distal Fig. 1.1A), anthesis begins in the central section and continues towards the basal and distal sections during a three- to five-day period (De Vries, 1971). Within the spikelets of the central section, proximal florets are fertilized two to four days earlier than the distal florets (Fig. 1.1B; De Vries, 1971). Hence, the grains formed in the central spike section gain a substantial developmental advantage for

assimilates over those in the basal and distal sections under resource limiting conditions. Also, developing grains of the proximal florets have a greater developmental advantage for assimilates than the distal florets. As each spikelet can produce more than one grain, the 'spikelet' has become one of the most essential components in the improvement of wheat yield and spike architecture (total spikelets or fertile spikelets per spike; grains per spikelet or per fertile spikelet; Guo et al., 2018).

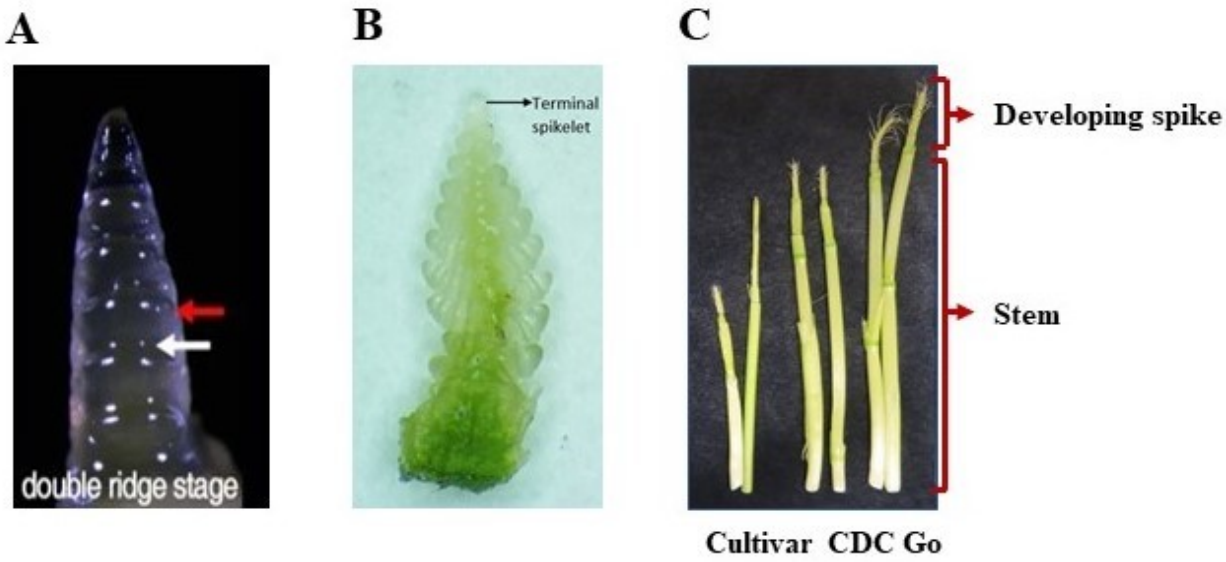

Figure. 1.2. Images of the wheat shoot apex at the pre-anthesis early and late reproductive phases of growth. A) image of a shoot apex at double ridge stage of the pre-anthesis early reproductive stage (white arrow shows the bracteal primordium and red arrow shows the spikelet primordium). B) shoot apex with a well-developed terminal spikelet at the pre-anthesis early reproductive stage. C) wheat seedling dissected to expose the floral spike that was within the stem (BBCH 39-45) at pre-anthesis late reproductive stage. (Image A was reproduced from Li et al., 2018 and image B was reproduced from Kumarapeli, 2021 by permission).

1.5 Genetics of flowering time in wheat

Flowering time is a quantitative trait controlled by multiple genes in wheat. Pre-anthesis developmental phases in wheat are mainly controlled by vernalization (*Vrn*), photoperiod (*Ppd*) response, and earliness per se genes (*Eps*; Kamren et al., 2013).

Vernalization (induction of the flowering process by exposing to a chilling treatment) is an important process in winter wheat to initiate the transition from the vegetative to the reproductive phase, but it has minimal to no effect on spring wheat (Kamren et al., 2013). The wheat vernalization trait is controlled by *Vrn-A1*, *Vrn-B1*, *Vrn-D1*, and *Vrn-D5* alleles located at the major vernalization loci on the long arms of group five chromosomes (Iqbal et al., 2007b). Winter wheat contains recessive alleles at all these loci (*vrn-A1*, *vrn-B1*, *vrn-D1*, and *vrn-D5*) making them sensitive to vernalization, whereas presence of one or more dominant alleles at these loci results in spring growth habit (Iqbal et al., 2007b; Iqbal et al., 2011). *Vrn-B1*, *Vrn-D1*, and *Vrn-D5* show lower sensitivity to the vernalization (Iqbal et al., 2007b). Flowering in wheat is also influenced by photoperiod genes that consist of a homeo-allelic series of loci, *Ppd-D1*, *Ppd-B1*, and *Ppd-A1* that are located on the short arms of chromosome 2 of the D, B, and A genomes, respectively (Pérez-Gianmarco et al., 2019). Dominant alleles at the *Ppd* loci make the genotypes insensitive to day length, whereas recessive (*ppd-D1*, *ppd-B1*, and *ppd-A1*) alleles at these loci make flowering time photoperiod-sensitive (Kamren et al., 2013). Photoperiod and vernalization response genes adjust the flowering time by responding to the environmental conditions to optimize reproductive success (Kamren et al., 2013). Earliness per se genes (that influence flowering times of varieties whose requirements of vernalization, and photoperiod have been fulfilled), affect the flowering time by reducing the vegetative growth phase independent from environmental stimuli (Kamren et al., 2013).

1.6 Effect of heat stress on yield and yield component parameters of wheat

If current rate of global warming continues, global temperatures are predicted to increase by 1.5 °C between 2030 and 2052 (Masson-Delmotte et al., 2018). Crop modelling studies have suggested that 1 °C increase in average global surface temperatures can reduce the global wheat yield by 4.1 to 6.4% (Asseng et al., 2015; Liu et al., 2016). The optimum temperature for normal growth and development of wheat is between 17 to 23 ºC (Porter & Garwith, 1999). Temperatures beyond these ranges can alter the plant growth and development including plant water relations, photosynthetic capacity, metabolic activities, enzyme production and activities, plant hormone production and activities, and reproductive organ growth and development in wheat, and all these events can reduce crop yield (Farooq et al., 2011). Heat stress can be defined as an increase in temperature beyond the optimum threshold level for a period of time, which is sufficient to cause

negative effects on plant growth and development (Akter & Islam, 2017). The intensity, duration, and the rate of temperature change will determine the impact of the above-optimal temperature period on plant growth and development (Carew et al., 2017). The effects of heat stress on wheat growth and development varies at different phenological stages, but the reproductive phase is overall more sensitive to heat stress than the vegetative phase (Fischer & Maurer, 1976). Many studies confirmed that the critical period for determining the number of spikelets/fertile spikelets/grains per spike is between 20 days before to 10 days after anthesis, and grain weight is also sensitive to temperature during this time range (Saini & Aspinall, 1982; Fischer, 1985; Calderini & Reynolds, 2000; Liu et al., 2020). Several phenological stages such as spikelet initiation, floral organ differentiation, male and female sporogenesis, and pollination and fertilization can be negatively affected by the heat-stress during this period, leading to lower grain set and yield (Saini & Aspinal, 1981; Saini & Aspinall, 1982; Saini et al., 1984).

Previous studies showed heat stress increases the rate of spike development and reduces the time for pre-anthesis reproductive development, which affects the production of spikelets per spike and grains per spikelet (Saini & Aspinall, 1982; McMaster, 1997; Porter & Gawith, 1999). Grain number per spike was substantially affected when temperatures increased from 20 ^{o}C to 30 ^{o}C between spikelet initiation and anthesis due to a reduction in grain set in wheat (Saini & Aspinall, 1982). Heat stress-induced changes in the normal functions of somatic and gametophytic tissues can lead to pollen and/or ovule sterility, resulting in grain set reduction (Saini & Aspinall, 1982). A number of stages in male reproductive organ development or subsequent function can be negatively affected by heat stress including microsporogenesis (meiosis-to-microspore transition), pollen release, pollen adhesion to the stigma, and pollen tube formation. With respect to microsporogenesis, tapetal programed cell death is essential for proper microspore development and pollen maturation and fertility (Browne et al., 2018). Upon exposure to heat stress in a number of plant species like wheat, barley and rice, anthers typically show a premature disappearance of the tapetal cell layer together with severe alterations in microspore development (Storme & Geelen, 2014). In wheat, for example, short periods of high temperature (3 d at 30 °C) led to premature degeneration of the tapetum layer (Saini et al., 1984). Also, impaired transport of photosynthate to anther tissues under heat stress conditions may be due to decreased hexose supply by the tapetum or reduced uptake by the pollen was observed in wheat and identified as a reason for increased pollen mortality (Dwivedi et al., 2017; Rieu et al., 2017). Heat stress exposure on

growth chamber grown sorghum resulted in increased accumulation of reactive oxygen species (ROS) in pollen grains leading to pollen membrane damage and production of desiccated pollen grains lowering the seed set (Djanaguiraman et al., 2018).

Some studies indicate female reproductive organs in cereals may be more protected against heat stress than the male reproductive organs (rice, Shi et al., 2018; maize, Wang et al., 2019) as they are enclosed in other floral tissues. However, smaller, or complete absence of embryo sacs, and reduced nucellar development were observed in wheat when temperature increased for three days from 20 ºC to 30 ºC at the onset of meiosis (Saini et al., 1983). Additionally, even though heat-stressed stigmas had similar numbers of germinated pollen grains compared to the non-stressed control, in 7% of the pollen grains, the pollen tubes did not grow normally and reach the ovary for fertilization (Saini et al., 1983). Furthermore, the heat sensitive rice cultivar Moroberekan showed lower sugar concentration in unpollinated and pollinated pistils under heat-stressed condition (38 ºC for 6 h per day for 3 days) and it was associated with reduced spikelet fertility (Li et al., 2015). The combination of high temperature (32/24 ºC; day/night) and draught (total water withdrawal) for 5 days at gametogenesis reduced the flowering and grain filling duration and pollen viability, shriveled the stigmatic branches, and enhanced the generation of ROS and reactive nitrogen species (RNS) in pistils leading to lower floret fertility in wheat (Fábián et al., 2019).

1.7 Application of chemical protectants to improve heat stress tolerance

Heat stress tolerance can be improved through advanced agronomic practices and genetic modifications to stabilize grain production. Use of water conservation techniques, proper nutrient management, adjusting the sowing time to escape heat stress during reproductive development, and application of chemical protectants can be effectively used as agronomic practices to alleviate the adverse impact of heat stress in wheat (Akter & Islam, 2017). In recent years use of chemical protectants to enhance the heat tolerance level of wheat genotypes has gained interest. Proline, glycine betaine, ascorbic acid, and phytohormones like cytokinin, and auxin have been tested to determine if their application can ameliorate the harmful and adverse effects of heat stress (Akter & Islam, 2017).

Proline and glycine betaine application considerably reduced the ROS production, improved the accumulation of soluble sugars, and protected the developing tissues from heat stress

effects in cereals (Wahid et al., 2012). Glycine betaine activates signaling molecules such as calcium-dependent protein kinases, which could activate stress-responsive and heat-shock transcription factor genes (Zulfiqar et al., 2022). The activated stress-responsive genes may boost the natural defense system by enhancing the activities of enzymatic antioxidants, such as superoxide dismutase, catalase, and peroxidase, which may alleviate the negative impact of ROS triggered by heat stress (Zulfiqar et al., 2022). Exogenous glycine betaine also protected photosystem II (PSII) in heat-stressed plants of wheat (Chowdhury et al., 2020). Ascorbic acid is a small, water-soluble antioxidant molecule, which acts as a primary substrate in the cyclic pathway for enzymatic detoxification of H_2O_2 making plants less vulnerable to various stresses (Foyer et al., 1997). Ascorbic acid (400 mM) applied at the pre-anthesis stage before imposing heat stress (42 ºC for 2 h for 5 days) increased the pollen viability in wheat and this was associated with a significant increase in the transcript of heat shock proteins and antioxidant enzymes in pollen (Kumar et al., 2013).

Exogenous phytohormone treatments including application of auxins, cytokinins, salicylic acid, and brassinosteroids have been reported to ameliorate heat stress effects in plants during the reproductive development stage (Sobol et al., 2014; Kaur et al., 2022). Application of cytokinins to *Arabidopsis* plants before imposing heat stress (36 °C for 36 h) prevented flower abortion (Sobol et al., 2014). Salicylic acid, a potent signaling molecule, is involved in the induction of plant defense strategies associated with stress conditions (Kaur et al., 2022). Exogenous salicylic acid treatment improved the activities of antioxidant enzymes including catalase, superoxide dismutase, and ascorbate peroxidase and non-enzymatic components including free and total ascorbate and decreased lipid peroxidation, resulting in enhanced abiotic stress tolerance in cereals and *Arabidopsis* (Kaur et al., 2022). Salicylic acid (1–50 mmol L^{-1} before imposing heat stress) was reported to increase soluble sugars and proline content, grain yield, spikelet number per panicle and grain set in rice under heat stress (40 ºC at the pollen mother cell meiosis stage for 10 days; Zhang et al., 2017). Exogenous brassinosteroids at the 3-leaf stage (5 ng/L) increased the production of antioxidant enzymes including superoxide dismutase (SOD), and peroxidases in the leaves of heat-stressed (38 /30 °C) rice seedlings (Cao & Zhao, 2008). Similarly, brassinosteroids application to wheat plants at pre-and post-flowering stages resulted in increased ascorbate peroxidase and SOD enzyme activities and provide a greater tolerance potential to survive under heat stress (Kumari & Hemantaranjan, 2019).

In this thesis, I will focus on testing the use of auxin application at initial flowering as a management tool to stabilize wheat grain yield under heat stress conditions.

1.8 Role of auxins in reproductive growth and development

Auxins are a class of growth hormone that regulate many processes in plant vegetative and reproductive growth and development (Thimann, 1938; Masuda, 1990; Went, 1942; Estelle, 1996). Studies suggest that the ubiquitous form of auxin in plants, indole-3-acetic acid (IAA), is synthesized via two different biosynthesis pathways, the tryptophan-dependent pathway, and the tryptophan-independent pathway (Zhao, 2012). The tryptophan-dependent pathway through indole-3-pyruvic acid (IPA) has been recognized as the major pathway in plants (Zhao, 2012). In the IPA pathway, the auxin IAA is synthesized from tryptophan in two steps. The first step involves the removal of the amino group by the TRYPTOPHAN AMINOTRANSFERASE (TAA) family of aminotransferases coded for by *TAA* and TAA-related (*TAR*) genes to produce IPA (Fig. 1.3). This is followed by oxidative decarboxylation of IPA catalyzed by the YUC family of flavin-containing monooxygenases coded for by YUC genes to generate IAA (Fig. 1.3; Zhao, 2012, 2014). In Arabidopsis, there are 3 members of TAA/TAR genes and 11 members of the YUC family of genes (Matthes et al., 2019).

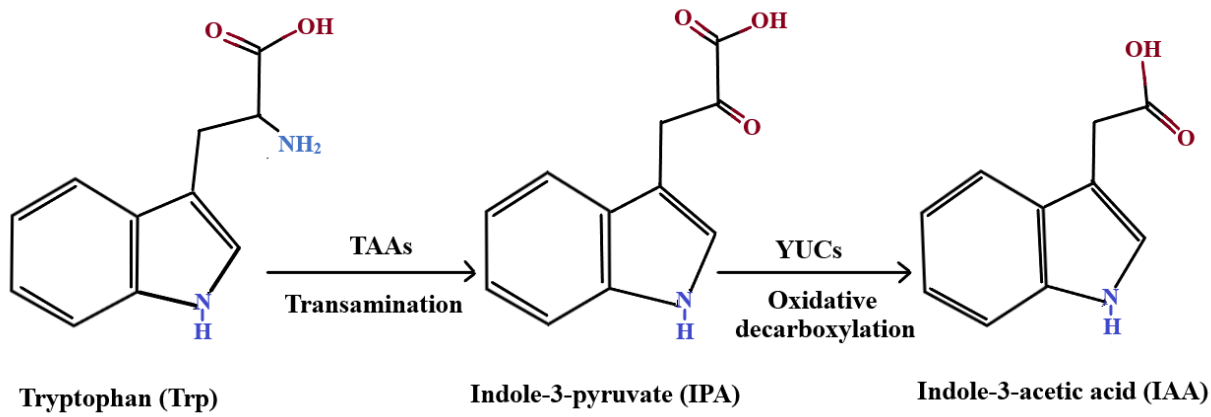

Figure. 1.3. A complete tryptophan-dependent auxin biosynthesis pathway in plants (modified from Zhao et al., 2014).

Auxins are involved in regulating reproductive development of plants (Aloni et al., 2006; Cheng et al., 2006; Cecchetti et al., 2008). Using triple and quadruple mutants of the four YUC genes (YUC1, YUC2, YUC4, and YUC6), Cheng et al. (2006) showed spatially and temporally regulated auxin biosynthesis by the *YUC* genes is essential for the formation of floral organs and vascular tissues in *Arabidopsis*. Auxin accumulation was required for stamen primordia formation and outgrowth, filament elongation, and the coordination of timing and progression of anther maturation and dehiscence as well as the generation of functional male gametophytes in *Arabidopsis* (Cecchetti et al., 2008). In rice, endogenous IAA levels in the spikelets at the anthesis stage was significantly and positively correlated with pollen viability (Sharma et al., 2018). Furthermore, auxin is important in female reproductive organ growth and development in plants as well. Auxin involvement in growth and morphogenesis of female tissues during gynoecium development, gametophyte formation, and endosperm cellularization in *Arabidopsis* and cereals has identified using auxin reporter genes (Shirley et al., 2019). Auxins also have been implicated in the modulation of photoassimilate partitioning into reproductive organs (Bangerth, et al., 1985; Cole & Patrick, 1998). A positive correlation between grain dry matter accumulation and IAA grain concentration was detected in the spring wheat cultivars Solo and Kolibi during the grain filling period (Bangerth et al.,1985). Furthermore, an agar gel of IAA (1×10^{-7} M) injected into the flag internode lacuna of durum wheat (*Triticum turgidum* cv. Franshawi) promoted the transport of ^{14}C-labelled photoassimilate from the flag leaf to the grain, and grain dry matter accumulation throughout the grain filling phase (Cole & Patrick, 1998).

Abiotic stresses can alter auxin biosynthesis in developing reproductive organs in plants, and it can negatively affect the grain yield. Heat stress significantly reduced the transcript levels of several auxin biosynthesis genes in developing anthers including *YUC2, YUC6* and *TAA1* in *Arabidopsis*, and two YUC genes in barley (Sakata et al., 2010). Lower transcript levels of those genes were associated with reduced auxin production in developing pollen mother cells and tapetal cells (particularly at the uninuclear microspore stage) and premature abortion of microspores in *Arabidopsis* and barley (Sakata et al., 2010). Auxin biosynthesis in the anther tissues was also affected by the duration of the heat stress treatment. Auxin levels significantly decreased in anther parietal and epidermal cells after 3 days of heat-stress treatment, and further reduction occurred in the pollen mother cells and tapetum cells after an additional 2-day heat-stress in barley (Sakata et al., 2010). Multiple applications of auxin (indole-3-acetic acid [IAA], 1-naphthaleneacetic acid

[NAA], or 2,4- dichlorophenoxyacetic acid [2,4-D] at 0.1-100 µM) at 18, 19, 21, and 23 days after sowing in barley and a single application of IAA and NAA at 0.1 or 1 µM just before increasing the temperatures to 31°C in *Arabidopsis*, were able to reverse the high temperature-induced pollen abortion (Sakata et al., 2010). IAA application (at 25, 50 and 100 mg/L) to cowpea (*Vigna sinensis* L.) ten days before and after flowering was reported to increase the number and weight of pods and number and weight of seeds with the lower IAA concentrations tested (25 and 50 mg/L; El-Saeid et al., 2010). Endogenous and exogenous auxin (IAA) affected the expression of some abiotic stress-controlled genes including *RAB18*, *RD22*, and *RD29A* in *Arabidopsis* seedlings, and this response was correlated with improved drought stress resistance in IAA-treated seedlings (Shi et al., 2014). In our previous study, 'CDC Go', a semi-dwarf (*Rht-B1b*) cultivar, responded to auxin application (one-time foliar application of 4-Cl-IAA) by producing higher grain number and weight under non-stress (NS) and heat-stress (HS) conditions (34-35 ºC for 6 h per day for 6 days during initial flowering) when grown in a greenhouse (19 and 24 % higher grain number and 20 and 33% higher grain weight under HS and NS, respectively) and 6-8% grain yield increase under field conditions (at 1µM), where higher mid-season temperatures occurred (Abeysingha et al., 2021). Given the importance of auxin in reproductive development, use of exogenous auxin as an agronomic management practice to stabilize grain production under heat stress conditions is an interesting area of research to explore.

1.9 Using QTL markers as breeding tools to improve grain yield under non-temperature stress or heat-stress conditions

Plant breeding is the science of changing the traits of plant species to create desirable plant types that adapt to human needs more efficiently through conventional or marker-assisted plant breeding techniques (Thomas, 2020). The core of conventional plant breeding is phenotypic selection for superior genotypes (for traits including higher grain yield, disease resistance, and abiotic stress resistance). High genetic variability is the basis for improvement and development of new cultivars (DePauw & O'Brien, 2016). Advances in biochemistry and molecular biology during the past few decades have helped to overcome the constraints of conventional plant breeding by providing breeders with many powerful genetic markers including classical markers (ex: morphological markers, cytological markers, and biochemical/protein markers) and DNA markers (Jiang, 2013). The genetic markers are used to identify genes or genomic regions of

interest for crop improvement (commonly called marker-assisted selection). Direct use of classical markers like cytological and biochemical/protein markers has been limited in genetic mapping and plant breeding (Jiang, 2013). A DNA marker is a small region of DNA sequence with a known position on the genome that is transmitted from one generation to the next showing polymorphism between different individuals. With the advent of this marker-assisted selection technology, plant breeding has become more efficient as they can be effectively used to understand the plant genome organization and genetic variation (polymorphism) and selection can be applied earlier in the crop life cycle. Restriction fragment length polymorphism (RFLP; Botstein et al., 1980), random amplification of polymorphic DNA (RAPD; Williams et al., 1990), amplified fragment length polymorphism (AFLP; Vos et al., 1995), microsatellite or simple sequence repeat (SSR; Song et al., 2005), and single nucleotide polymorphism (SNP; Jordan & Humphries, 1994) are some popular DNA markers. However, these genotyping methods are constrained by their dependence on gel electrophoresis. To overcome this constraint hybridization-based genotyping methods including diversity arrays technology markers (DArT; Jaccoud et al., 2001) have been developed. The 'Attila' × 'CDC Go' RIL population used in this thesis was analyzed using DArT markers and 90K Illumina iSelect SNP array platform (Wang et al., 2014) under both conventional and organic management systems (Asif et al., 2015; Zou et al., 2017a and b).

1.9.1 DArT markers

DArT is a microarray hybridization technique which, involves the use of methylation-sensitive restriction enzymes to digest genomic DNA, thereby reducing the genome complexity and enriching the low copy sequences for marker development (Semagn et al., 2006). This technology enables the simultaneous typing of several hundred polymorphic loci spread over the genome without any previous sequence information and has been shown to be reproducible and cost effective (Jaccoud et al., 2001; Wenzl et al., 2004). For these reasons, DArT markers have been efficiently used in the analyses of genetic diversity, population structure, association mapping and construction of linkage maps in plant genetic studies, as anonymous genomic markers. DArT arrays have been developed for a variety of plant/crop species including wheat (Francki et al., 2009; Wenzl et al., 2010). However, the dominant inheritance of this technique (cannot distinguish homozygous dominant allele from heterozygous allele) is a disadvantage when using DArT markers (Semagn et al., 2006).

1.9.2 SNP markers

Single nucleotide polymorphisms (SNPs) are polymorphisms that are caused by a single nucleotide mutation that gives rise to different alleles containing alternative bases at a given position (Semagn et al., 2006). SNPs are more prevalent, more stable, and have higher frequency of occurrence throughout the genome than other DNA-based markers like RFLP, RAPD and DArt, while having a co-dominant inheritance, making it the predominant marker type in genetic studies (Semagn et al., 2006). Unlike other DNA-based markers, SNPs are directly based on known sequence polymorphisms and, therefore, require extensive DNA sequencing data (Semagn et al., 2006). Recently, a series of high-density SNP genotyping arrays have been identified for marker-assisted selection in bread wheat; for example, the Illumina Wheat 9K iSelect SNP array; Cavanagh et al., 2013), the Illumina Wheat 90K iSelect SNP genotyping array (Wang et al., 2014), the Axiom Wheat 660K SNP array, and the Axiom HD Wheat genotyping (820K) array (Winfield et al., 2016). A total of 77,126 out of 81,587 SNPs in the Wheat 90K SNP genotyping array showed reliable physical positions in the Chinese Spring wheat reference genome V1.0 (Sun et al., 2020). While, the final number of SNPs retained for QTL mapping could be less than 2000 due to co-segregation of SNPs, the 90K SNP array provides a greater opportunity to identify new QTL not identified using other genotyping platforms like DArT (Perez-Lara et al., 2016; Zou et al., 2017a and b).

1.9.3 QTL analyses

Quantitative traits like grain yield, grain number, and days to anthesis normally show continuous variation, controlled by one or more genes (with major or minor effects on the trait), and are modified by environmental conditions (Khan, 2015). The genomic region associated with the expression of a quantitative trait is referred to as a quantitative trait locus (QTL). A QTL may be a single gene or may be cluster of linked genes that affect the trait (Khan, 2015). QTL mapping is generally based on biparental populations (parents must genetically be different with regard to the trait of interest) in which, the genotypic data (based on molecular marker) and phenotypic data are statistically analyzed to detect all possible marker loci where allelic variation correlates with the phenotype (Miles & Wayne, 2008). Single marker analyses were prominent when the markers and the marker location information were insufficient (Knott, 2005). Later, QTL interval mapping was introduced and the use of it became more feasible with improvements

in molecular techniques (Lander & Botstein, 1989). The single interval mapping method assessed the likelihood for a single putative QTL at each location on the genome. However, QTL located elsewhere on the genome can have an interfering effect. Consequently, the power of detection may be compromised, and the estimation of locations and effects of QTL may be biased (Jansen, 1993). Therefore, composite interval mapping (CIM) was introduced (Jansen & Stam, 1994). The basis of this method is an interval test that attempts to separate and isolate individual QTL effects by combining interval mapping with multiple regression. It controls the genetic variation in other regions of the genome, thus reducing background "noise" that can affect QTL detection (Jansen & Stam, 1994). Therefore, CIM determines both the location and effect size of the QTL more accurately than single interval mapping (Jansen & Stam, 1994). CIM can be extended to deal with data coming from multiple cross populations and for joint analysis of multiple traits. Appropriate experimental designs and QTL analysis methods are available for the detection and estimation of QTL x QTL and QTL x environment interactions (Wang et al., 2019).

Improved grain yield has been a major focus of most wheat breeding programmes around the world. QTL analyses have been used to investigate the genomic regions associated with yield and yield component traits in wheat to detect candidate genes (a gene believed to be related to a trait of interest), which can be incorporated into a cultivar by breeding. Several studies that detected important QTL related to grain weight, grain number, fertile spikelets per spike, total spikelets per spike, days to flowering/anthesis, plant height and flag leaf characteristics in wheat are noted below.

QTL related to grain yield and yield component traits, including tiller number, grain weight per spike, 50-grain weight, and spikelet number per spike, were identified adjacent to the *Vrn A1* location of chromosome 5A using RFLP markers on 118 single-chromosome recombinant lines derived from a cross between Chinese spring (Cappelle-Desprez 5A) and Chinese spring (Triticum spelta 5A; Kato et al., 2000). It was shown that grain yield QTL were correlated with yield component QTL in this study. The QTL, GNI1, which increases the grain number, was detected on chromosome 2A in wheat (Sakuma et al., 2019). Some stable QTL in wheat related to grain number per spike have been identified on chromosomes 1A, 2A, 2B, 3A, 3D, 4B, 6A, and 7A (Huang et al., 2004; Deng et al., 2011; Jia et al., 2013; Assanga et al., 2017; Liu et al., 2018).

QTL for heading and flowering date have been detected across 21 wheat chromosomes (Li et al., 2018; Assanga et al., 2017). A major QTL for heading date (HD), which explained 40.5% of the phenotypic variance across 3 years, was detected on chromosome 2D using a recombinant inbred line (RIL) population and AFLP and SSR markers (Xu et al., 2005). Seven QTL for HD were identified on chromosomes 1A, 2A, 2B, 5A, 6D, 7A, and 7B in a wheat doubled haploid (DH) population (Kuchel et al., 2006). Floral transition, length of vegetative growth and flowering date related genomic regions (11 regions) were identified in a winter wheat DH population (Baga et al., 2009). A stable flowering time QTL on chromosome 5A adjacent to the *Vrn A1* location and other environment specific QTL on chromosome 4A, 4B, and 6B were detected in a RIL population developed by crossing Canadian wheat cultivar CDC Go and CIMMYT wheat cultivar Attila under conventional and organically managed field conditions (Zou et al., 2017b). The flowering time QTL on chromosome 5A was coincident with maturity time and plant height QTL, indicating there may be a correlation between those traits (Zou et al., 2017b). These studies confirm that flowering/heading time is a quantitative trait and controlled by several genes with major and minor effects.

Reduced plant height is an important trait associated with a reduced incidence of lodging, increased grain number per spike, and an improved harvest index, leading to increased grain yield and quality in grain crops. During the green revolution, dwarfing genes were introduced into hexaploid wheat and currently most of the cultivars growing around the world contain different dwarfing genes (Peng et al., 2011; Zhang et al., 2013; Chen et al., 2015) including *Rht-B1b* on chromosomes 4B and *Rht-D1b* on chromosomes 4D. A very consistent major effect QTL adjacent to *Rht-D1b* gene on chromosome 4D that accounted for 38% of the phenotypic variance for plant height was detected across five environments in a RIL population derived from Canadian wheat cultivars ‘Cutler’ and ‘AC Barrie’ (Perez-Lara et al., 2016). A QTL related to plant height on chromosome 4B was detected in ‘Attila’ x ‘CDC Go’ RIL population adjacent to the *Rht-B1* gene and it was co-located with maturity time- and grain protein content-related QTL (Zou et al., 2017b). Other studies also detected QTL related to plant height on chromosomes 2B, 4A, 4B, 5B, 7A, and 7B in wheat (Jia et al., 2013; Milner et al., 2016; Assanga et al., 2017).

Flag leaf-related traits, including the flag-leaf length (FLL), flag-leaf width (FLW), and flag-leaf area (FLA), are also important traits that are associated with wheat yield potential and are significantly correlated with the grain yield (Wang et al., 2011; Wu et al., 2016; Liu et al., 2018).

A QTL (TaFLW1) associated with FLW was finely mapped on chromosome 5A (Xue et al., 2013). QTL for flag-leaf-related traits have also been detected on chromosomes 1B, 2D, 3A, 3B, 4A, 4B, 4D, 5B, 6B, 7A, and 7D in wheat (Jia et al., 2013; Fan et al., 2015; Wu et al., 2016; Liu et al., 2018).

Most QTL detected under non-stress environments also control the yield and yield-related traits under heat-stress environments. Many unique QTL related to heat tolerance in wheat have also been detected. Heat stress-tolerance-related loci were reported on chromosomes 3A, 3B, 4A, 4B and 5A in tetraploid wheat (Sun & Quick, 1991). Heat-tolerant QTL analyzed using heat susceptibility index (HSI, an indicator of heat response) were detected on chromosomes 1B, 5B and 7B by examining 144 RILs derived from a cross between wheat cultivars Kauz and MTRWA116 (Mohammadi et al., 2008). The RIL population developed by crossing 'Halberd' (heat tolerant) x 'Cutter' (heat susceptible) was used to identify heat tolerance QTL associated with yield component traits under heat stress environments (38 °C day/18 °C night) using HSI over a two-year period, where five QTL (located on chromosomes 1A, 2A, 2B, and 3B) were simultaneously detected in both years under heat stress (Mason et al., 2010). Genomic regions associated with heat tolerance were detected on chromosomes 2A, 2D, 4A and 5A, and a consistent QTL was found on chromosome 2D based on photosynthetic rate analysis in a wheat RIL population developed by crossing the heat-resistant lineWH1021 with heat-sensitive lineWH711 (Sangwan et al., 2019).

1.10 Cultivar descriptions of 'Attila', 'CDC Go', and 'CDC Teal'

1.10.1 'Attila'

'Attila' is an awned semi-dwarf (84 cm) hard-red spring bread wheat cultivar developed by the International Maize and Wheat Improvement Center, Mexico, as a heat-resistant wheat cultivar for warmer environments (Yang et al., 2002). 'Attila' is widely grown in southeast Asia (Rosewarne et al., 2008). 'Attila' is an important parent in the rust resistance breeding program of CIMMYT and shows a moderate level of slow rusting resistance to both leaf rust and stripe rust (Rosewarne et al., 2008). Under Canadian field conditions, 'Attila' produces flowers in ~ 54 days, matures in 92-106 days, and produces a grain yield of 4.5-6 Mg ha^{-1} in the western Canadian prairies (Reid et al., 2011; Asif et al., 2015; Zou et al., 2017b).

1.10.2 'CDC Go'

'CDC Go' is a semi-dwarf hard-red spring bread wheat cultivar that commercially grown in Canada. This cultivar is designated under the market class of Canada Western Red Spring (CWRS). It is a high yielding cultivar (~ 5 Mg ha^{-1}; Asif et al., 2015; Zou et al., 2017b). It has an average plant height of 85 cm with awned spikes that are nearly 8.6 cm long (Canadian Food Inspection Agency, 2019). This cultivar produces flowers in ~ 50 days and matures in 96-102 days in the western Canadian prairies (Asif et al., 2015; Zou et al., 2017b). Strong stems and the semi-dwarf growth habit of this cultivar leads to a good lodging resistance rating. 'CDC Go' is resistant to bunt, moderately resistant to leaf rust and stem rust, and moderately susceptible to loose smut in the wheat growing areas of western Canada (SaskSeed Guide, 2019).

1.10.3 'CDC Teal'

'CDC Teal' is a semi-dwarf hard-red spring bread wheat cultivar under the market class of CWRS, which is best adapted to the black soil zone of western Canada (Hughes & Hucl, 1993). 'CDC Teal' is a strong strawed cultivar with a good lodging resistance (Hughes & Hucl, 1993), and plant height ranges from 76 to 105 cm depending on the field conditions (Hughes & Hucl, 1993; Chen et al., 2015). Flowering time is ~ 59 days and it matures in ~ 90-98 days (Hughes & Hucl 1993; Chen et al., 2015). This cultivar yielded ~ 4.9 Mg ha^{-1} under western Canadian field conditions (Chen et al., 2015). It shows good resistance to leaf and stem rusts, moderately resistant to loose smut and intermediately resistant to common bunt in the wheat growing areas of western Canada (Hughes & Hucl 1993).

1.11 Thesis hypotheses to be tested and experimental objectives

1.11.1 Hypotheses to be tested

The main hypotheses tested in this thesis are:

1. Heat stress has a negative impact on wheat grain yield and QTL that are associated with heat-stress resistance with respect to grain yield can be identified using an RIL population that segregates for heat resistance.
2. Foliar auxin application has the ability to at least partially restore the yielding ability of hard red spring wheat exposed to high-temperature stress conditions during flowering and this ability is genotype dependent.

1.11.2 Experimental objectives

1. To determine the responses of the RIL parents (‘Attila’, ‘CDC Go’, and ‘CDC Teal’) to:

    a) heat stress

    b) one time foliar 4-Cl-IAA (1µM) application under non-temperature stress and heat-stress conditions at early flowering.

   Thus, allowing for the selection of a RIL population that would be suited for an in-depth study on heat tolerance and the effect of 4-Cl-IAA (1µM) application on heat tolerance.
2. To use seven standard Canadian wheat cultivars, RIL parents (‘Attila’ and ‘CDC Go’) and an ‘Attila’ x ‘CDC Go’ RIL population to:

    a) Identify the most relevant yield components that would result in grain yield improvement under non-temperature stress and heat-stress conditions,

    b) Identify heat-resistant and heat-sensitive genotypes, and

    c) Detect the ability of a one-time foliar application of 4-Cl-IAA (1µM) at the early flowering stage of wheat to improve grain yielding
3. To identify the QTLs associated with grain yield and yield components which are specific to, or common with, non-temperature stress and/or heat-stress conditions at flowering.

Chapter 2 - Screening RIL parental cultivars for responses to heat-stress and auxin application on grain yield parameters

2.1 Introduction

Genotype and environment strongly influence the yield performance of wheat (Studnicki et al., 2016). The predicted climate change scenarios indicate unpredictability in the timing, duration, and intensity of heat stress, which can negatively affect wheat grain yields worldwide and threaten food security (Carew et al., 2017). Identification of heat-stress tolerance in wheat will help future breeding programs to maintain grain yields under heat-stress conditions. Apart from cultivar selection, genetic gains in heat tolerance could be accelerated when suitable agronomic practices are incorporated (Studnicki et al., 2016). There are few studies testing on yield stability of existing cultivars under heat-stress environment using different agronomic management practices (Akter & Islam, 2017). Plant growth regulators have been used extensively to aid in efficient production of horticultural crops, but much less so in agronomic crops. The current low cost of plant growth regulators and the need to develop new and revised agronomic management tools has led to a renewed interest in the use of plant growth regulators to aid in agronomic crop production (Hanaa & Safaa, 2019; Neill et al., 2019). Previous studies in Dr. Ozga's lab have shown that the auxin class of plant growth regulators can ameliorate heat stress effects on spring wheat; however, the response was genotype dependent (Abeysingha et al., 2021). In the spring wheat cultivar CDC Go, a single application of auxin (4-Cl-IAA at 1 μM) to the whole plant at the mid to late boot stage (BBCH scale 43-45) increased grain number and/or grain weight under normal and heat-stress conditions (Abeysingha et al., 2021).

Two RIL wheat populations that had 'CDC Go' as a parent were available from Dr. Dean Spaner's group at the University of Alberta for an in-depth study on heat tolerance and the effect of auxin application on heat tolerance in spring wheat. One RIL population was derived from a cross between 'CDC Go' and 'Attila', and the other population was derived from a cross between 'CDC Go' and 'CDC Teal' (see Chapter 1 for cultivar descriptions).

The 'Attila' × 'CDC Go' RIL population was developed as part of the 'Canadian Triticum Advancement through Genomics' (CTAG) project, which is Canada's contribution to the International Wheat Genome Sequencing Consortium (Asif, 2014) to study the competitive ability

of wheat under organic and conventional management systems (Asif et al., 2015). This population has been extensively evaluated under organic and conventional farming systems over a period of seven years in the western Canadian prairies. QTL associated with grain yield and yield components have been mapped in this RIL population grown under both management systems (Asif et al., 2015; Zou et al., 2017a and b). The 'CDC Teal' x 'CDC Go' RIL population was developed to map QTL associated with flowering time and other phenotypic traits and was evaluated over a 3-year period under western Canadian field conditions (Chen et al., 2015). Spring wheat cultivars grown in western Canada mainly contain *Vrn-A1a* and *Ppd-D1* genes to make the cultivars early flowering and early maturing for short growing seasons (Iqbal et al., 2007b). Both parental cultivars 'CDC Go', and 'CDC Teal' carry the *Vrn-A1a* (Iqbal et al., 2007b) and the *Ppd-D1a* genes (Kamran et al., 2013), but carry different alleles at the *Vrn-B1* locus. 'CDC Go' has the dominant allele *Vrn-B1* and 'CDC Teal' has the recessive allele *vrn-B1,* making 'CDC Go' an earlier flowering and earlier maturing cultivar than 'CDC Teal' (Chen et al., 2015).

To determine the responses of the RIL parents ('Attila', 'CDC Go', and 'CDC Teal') to 1) heat stress and 2) one time foliar 4-Cl-IAA (1µM) application under non-temperature stress and heat-stress conditions at early flowering. Thus, allowing for the selection of a RIL population that would be suited for an in-depth study on heat tolerance and the effect of 4-Cl-IAA (1µM) application on heat tolerance.

2.2 Materials and methods

'Attila', 'CDC Go' and 'CDC Teal' were sown (30th January 2017) at an approximate depth of 1.5 cm in 1-L square plastic pots [12.5 (length) x 12.5 (width) x 15 (depth) cm; 2 to 3 grains per pot] containing 4:1 Sunshine #4 potting mix (Sun Gro Horticulture, Vancouver, Canada) and sand. The seedlings were thinned to one seedling per pot approximately 2 weeks after planting. The potting medium was kept moist throughout the experiment, and plants were fertilized weekly with 10: 52: 10 (N: P: K, early in the week) and 12: 2: 14 (later in the week) at 100 ppm. Plants were maintained under non-temperature stress conditions (19ºC/17ºC; day/night) inside a growth chamber at the University of Alberta with a 16 h light/8 h dark photoperiod, with an average light intensity of 373 µmol m^{-2} s^{-1} (Philips Silhouette high output fluorescent bulbs F54T5/835 Holland, Alto Collection). 4-Cl-IAA at 1 µM in aqueous 0.25% (v/v) Agral or aqueous 0.25% [v/v] Agral only control was applied as a one-time foliar spray until liquid ran off the leaves using a hand-held

bottle sprayer when the majority of the plants were at the flag leaf initiation to late boot stage (BBCH 37-45; Fig. A1; Lancashire et al., 1991). Then plants were either exposed to the heat-stress treatment (see details below) or they remained under the non-temperature stress conditions (Fig. A1). The experiment was arranged as a completely randomized design with 4 treatments (2 auxin x 2 temperature treatments), and 9 replications (pots) per treatment.

For the heat-stress treatment, plants were exposed to 35^{o}C for 6 h per day for 6 d within 12 h after treatment application by moving plants from the original growth chamber to a heat-stress growth chamber. In the heat-stress chamber, the light cycle began at 07:00 h with 24^{o}C air temperature. The heat treatment began at 11:00 h with 35^{o}C air temperature and 35^{o}C was reached within 30 min. The 35^{o}C temperature was maintained for 6 h (until 17:30 h). Following the heat treatment, the remainder of the light cycle was maintained at a 24^{o}C air temperature. The dark cycle (began at 23:00 h) was maintained at 20^{o}C. The photoperiod was 16 h light/8 h dark at an average light intensity of 373 μmol m^{-2} s^{-1} (Philips Silhouette high output fluorescent bulbs F54T5/835 Holland, Alto Collection). After six days, the heat stress-treated plants were returned to the original growth chamber and maintained under non-temperature stress conditions to develop to maturity.

Data was collected for the first three floral spikes, and subsequent tillers were removed (when tillers were 5-10 cm tall). Each floral spike was harvested when its peduncle was dry, and the grains were at the mature-dry stage. Subsequently, the spikes were dried at 40^{o}C for two days to obtain a consistent grain moisture content.

Data was collected for: the number of days from planting to anthesis (anthesis = at least 5-8 florets showed visible anthers in spike 1), flag-leaf area (estimated as flag-leaf length from the collar region to the tip of the leaf blade × width measured at mid-leaf length of spike 1 × 0.7) as described by Quarrie and Jones (1979), and plant height (the height from the soil surface to the top of spike 1 excluding awns). After harvesting, each spike was divided into three sections based on the spikelet number per spike, the basal (bottom third of the spike), the central (middle third of the spikelets), and the distal (top third of the spikelets) to determine the spikelet and grain data within each section. Spikelet and grain data were also calculated for whole spike. A spikelet with no grains at maturity was labeled as a non-fertile spikelet for estimating the number of fertile spikelets (defined as spikelets that produced mature grains) per spike or per spike section.

2.2.1 Statistical analyses

Data analyses were performed using the Proc GLM and Proc MIXED procedures of SAS 9.3 (SAS Institute Inc. Cary, NC, USA, 2010). For each data parameter, normality and homogeneity of variance were tested using Shapiro-Wilk and Levene's tests, respectively. Statistical significance of the temperature and auxin treatments were determined using 3-factor factorial analyses of variance (ANOVA; 3 cultivars × 2 auxin treatments × 2 temperature treatments) for the following data types: days to anthesis, flag-leaf area and spike height of spike 1, and for the individual and average spike data of spike 1, 2 and 3 (spikelets, fertile spikelets, grain number and weight per spike). The effect of temperature and auxin treatments on spikelets, fertile spikelets, grain number and weight, within each spike section (basal, central, and distal; for the individual and average spike data of spikes 1, 2, and 3) was analyzed using a 4-factor ANOVA (3 cultivars × 2 auxin treatments × 2 temperature treatments × 3 sections within the spike). Mean separations were determined using the Least Significant Difference (LSD) test. Statistical significance was declared at $P \leq 0.05$. ANOVA F-values and probabilities for main effect means and interactions related to these analyses are given in Appendix D (Tables D1-D6).

2.3 Results

2.3.1 Effect of heat stress on reproductive parameters

'CDC Go' was the first cultivar to reach anthesis (55 days), followed by 'CDC Teal' (64 days), then 'Attila' (74 days) when grown under non-temperature stress condition (Table 2.1; Fig. A2A). Heat stress only affected the flowering time of 'Attila', reducing the number of days to anthesis by 4 days (Table 2.1; Fig. A2A).

The cultivar 'Attila' produced the highest number of total spikelets per spike in the first three spikes (26, 27, and 27, respectively), followed by 'CDC Teal' (21, 22, and 22, respectively), and then 'CDC Go' (19, 18, and 19, respectively) under non-temperature stress conditions (Tables 2.1, 2.2, and 2.3). The heat-stress treatment had minimal to no effect on the number of spikelets per spike (Tables 2.1, 2.2, 2.3, and 2.4). 'Attila' also produced the highest number of fertile spikelets per spike in the first three spikes (21, 22, and 20, respectively) compared to 'CDC Go', and 'CDC Teal' (approximately 15-17 fertile spikelets in the first three spikes; Tables 2.1, 2.2, and 2.3). Heat-stress treatment did not affect the number of fertile spikelets in the first floral spike of 'Attila'; however, it reduced the number of fertile spikelets in spikes 2 and 3 by 18 and 15%,

respectively (Tables 2.1, 2.2, and 2.3), and a 19% reduction was observed when averaged across the first three spikes (Table 2.4). The heat stress-induced decrease in the number of fertile spikelets in 'Attila' was observed in the basal and distal spike sections in spike 2 and the basal spike section in spike 3 (Table 2.5). A 24% reduction in the number of fertile spikelets per spike in the basal section of 'Attila' was observed when averaged across the first three spike sections under heat-stress conditions (Table 2.6). Heat stress did not affect the number of fertile spikelets in 'CDC Go' or 'CDC Teal' (Tables 2.1, 2.2, 2.3, and 2.4).

At the spikelet level, 'Attila' and 'CDC Go' produced more grains per spikelet than 'CDC Teal'; however, 'Attila' produced the highest number of grains per fertile spikelet, followed by 'CDC Go' then 'CDC Teal' under non-temperature stress condition (average of first three spikes, Table 2.4). Heat stress reduced the number of grains per spikelet and per fertile spikelet in 'Attila' (26 and 14%) and 'CDC Go' (13 and 10%) but did not affect that in 'CDC Teal' (average of first three spikes, Table 2.4). 'Attila' still produced the highest number of grains per fertile spikelet compared to the other cultivars when exposed to the heat-stress treatment (Table 2.4).

With respect to grain yield, 'Attila' produced the highest grain number per spike (56, 60, and 54) followed by 'CDC Go' (34, 35, and 39), then 'CDC Teal' (30, 30, and 30) under non-temperature stress conditions (spikes 1, 2, and 3, respectively; Tables 2.1, 2.2, 2.3; Fig. A3). When averaged across the first three spikes, 'Attila' produced 37% more grains per spike than 'CDC Go' and 47% more grains than 'CDC Teal' under non-temperature stress conditions (Table 2.4). The heat-stress treatment reduced the grain number per spike of 'Attila' in all three spikes assessed (27, 30, and 31%, spikes 1, 2, and 3, respectively, Tables 2.1, 2.2, 2.3; Fig. A3; 30%, average of three spikes, Table 2.4; Fig. A3). The grain number per spike was not affected by the heat-stress treatment in 'CDC Go' or 'CDC Teal' (Tables 2.1, 2.2, 2.3; Fig. A3). Although grain number was reduced in 'Atilla' by the heat-stress treatment, it still produced the highest grain number per spike compared to 'CDC Go' (20% higher) and 'CDC Teal' (30% higher; Table 2.4).

Similar to grain number, grain weight per spike was also higher in 'Attila' (2.3 g, 2.5 g, and 2.4 g) compared to 'CDC Go' (1.5 g, 1.6 g, and 1.7 g) and 'CDC Teal' (1.3 g, 1.3 g, and 1.4 g) under non-temperature stress conditions (spikes 1, 2, and 3, respectively; Tables 2.1, 2.2, 2.3; Fig. A4). When averaged across the first three spikes, 'Attila' exhibited 35% higher grain weight than 'CDC Go' and 42% higher grain weight than 'CDC Teal' under non-temperature stress conditions (Table 2.4). The heat-stress treatment reduced the grain weight per spike of 'Attila' (27,

30, and 35%, spikes 1, 2, and 3, respectively, Tables 2.1, 2.2, 2.3 and Fig. A4; 31%, average of three spikes, Table 2.4; Fig. A4). The grain weight per spike was not affected by the heat-stress treatment in 'CDC Go' or 'CDC Teal' (Tables 2.1, 2.2, 2.3; Fig. A4). Grain weight per spike was still higher in 'Attila' compared to 'CDC Go' (17% higher) and CDC Teal (23% higher; Table 2.4) when plants were exposed to the heat treatment.

Spikelets in the basal and the central sections of the spike produced the majority of the grain yield (grain number and weight) in all three cultivars when grown under non-temperature stress or heat-stress conditions (Table 2.5). Exposure to heat stress reduced the grain number and weight in the spikelets from the basal (44% and 42% lower, respectively) and the central (20% and 24% lower, respectively) sections of 'Attila', and the basal section of 'CDC Go' (19% lower for both parameters; Table 2.6). Grain yield in the spike sections of 'CDC Teal' was not affected by the heat-stress treatment (Table 2.6).

2.3.2 Effect of 4-Cl-IAA treatment on reproductive parameters

4-Cl-IAA application reduced the number of days to anthesis in the first floral spike of 'Attila' by 4 days, under non-temperature stress and heat-stress conditions (Table 2.1; Fig. A2A). 4-Cl-IAA application followed by heat stress exposure reduced the number of days to anthesis by 3 days in 'CDC Teal' (Table 2.1; Fig. A2A). The number of days to anthesis in 'CDC Go' was not affected by the 4-Cl-IAA treatment (Table 2.1; Fig. A2A).

4-Cl-IAA treatment had minimal to no effect on the number of spikelets per spike under non-temperature stress or heat-stress conditions for all cultivars (Tables 2.1, 2.2, 2.3, and 2.4). In 'Attila', heat stress reduced the number of fertile spikelets per spike, and 4-Cl-IAA application ameliorated the negative effect of heat stress on this parameter (spikes 2 and 3, and average across first three spikes: Tables 2.2, 2.3, and 2.4). When assessed within the spike sections, 4-Cl-IAA treatment increased the number of fertile spikelets in the distal spike section of 'Attila' in the plants exposed to heat stress (Table 2.6).

4-Cl-IAA treatment minimally affected grain number (spike 2 was 13% lower compared to the 0 μM control) and did not affect grain weight in 'Attila' under non-temperature stress conditions (Tables 2.1, 2.2, 2.3; Figs. A3 and A4). However, in 'Attila' the heat stress-induced reduction in grain number and weight was ameliorated by 4-Cl-IAA application prior to the heat-stress treatment (spikes 1, 3, and averaged across the first three spikes; compare means of 0 μM

with 1 µM 4-Cl-IAA exposed to the heat-stress treatment; Tables 2.1, 2.2, 2.3, 2.4; Fig. A3 and A4). When assessed within the spike, 4-Cl-IAA application increased the grain number in the basal, central, and the distal spike sections of the first three spikes in 'Attila' (Table 2.5).

2.3.3 Effect of heat stress and 4-Cl-IAA on plant height and flag-leaf area

'Attila' had the shortest plant height (79 cm; estimated from the length of the main tiller), followed by 'CDC Go' (85 cm), then 'CDC Teal' (95 cm) when grown under the non-temperature stress condition (Table 2.7; Fig. A2B). The heat-stress treatment reduced the plant height of 'Attila' by 13% and 'CDC Teal' by 8% but did not affect on 'CDC Go' (Table 2.7). The plant height was not affected by 4-Cl-IAA treatment in any of the cultivars (Table 2.7; Fig. A2B).

Heat-stress treatment reduced the flag-leaf area of spike 1 by 23% in 'Attila' and by 26% in 'CDC Teal' (Table 2.7; Fig. A2C). 4-Cl-IAA application negated the negative effect of the heat-stress treatment on flag-leaf growth of 'Attila' and to some extent for 'CDC Teal' (Table 2.7; Fig. A2C). Plant height and flag-leaf area were not affected by the heat-stress or 4-Cl-IAA treatments in 'CDC Go' (Table 2.7; Figs. A2B and A2C).

2.4 Discussion

2.4.1 Effect of heat stress on reproductive parameters

Yield traits including days to anthesis, total spikelets and fertile spikelets per spike, grains per spikelet and per fertile spikelet, and grain number and weight per spike, are considered to be important grain yield components in wheat. Heat stress during the reproductive growth stage can negatively affect the productivity success of a number of these yield components (Farooq et al., 2011; Bheemanahalli et al., 2019).

Flowering time, maturity time and the yielding ability of a wheat genotype must be compromised to a certain extent when selecting cultivars to grow under areas that have short growing seasons. 'CDC Go' is a highly adaptable cultivar for growing under the short growing seasons in the prairie provinces of Canada (Iqbal et al., 2007a). Vernalization and photoperiod genes carried by 'CDC Go' (Iqbal et al., 2007a; Kamran et al., 2013; Chen et al., 2015) facilitate early flowering in this cultivar compared to 'CDC Teal' and 'Attila'. 'CDC Go' was 9 and 19 days earlier under non-temperature stress, and 8 and 16 days earlier under heat-stress conditions than observed for 'CDC Teal' and 'Attila', respectively: Table 2.1, Fig. A2A). Similarly, previous

studies showed ‘CDC Go’ flowered approximately 1 day (Chen et al., 2015) and 4 days (Zou et al., 2017b) earlier than ‘CDC Teal’ and ‘Attila’, respectively, under western Canadian field conditions.

In the current experiment, the later flowering cultivar Attila produced the highest number of total and fertile spikelets per spike, and number of grains per fertile spikelet, in the first three spikes under non-temperature stress conditions. Pre-anthesis reproductive development of wheat is commonly categorized into two stages, the early reproductive phase when spikelet primordia are initiated, and the late reproductive phase when the stem internodes elongate and the floret primordia develop into flowers (Gol et al., 2017). The heat-stress treatment was given during the late reproductive stage, after spikelet primordia initiation; therefore, it did not affect the number of spikelets per spike in the first three spikes of the tested cultivars Attila, CDC Go, and CDC Teal.

Among the phenotypic traits measured in this study, the ability to maintain grain number and grain weight when the plant is exposed to heat stress are considered two of the most important traits when selecting heat stress-resistant cultivars for cultivation (Akter & Islam, 2017). We observed that the later flowering cultivar Attila produced the highest grain number and weight under non-temperature stress conditions. The heat-stress treatment reduced the number of grains per spikelet or per fertile spikelet in ‘Attila’ and ‘CDC Go’; however, ‘Attila’ still produced more grains per fertile spikelet than ‘CDC Go’, and ‘CDC Teal’ (Table 2.4), and it produced the highest grain yield even after approximately 30% reduction under the heat-stress conditions (Table 2.4, Figs. A3 and A4), making it a potential cultivar to grow in areas where high mid-season temperatures are possible. Previous studies also suggested that ‘Attila’ is a heat-resistant wheat cultivar that performs well in warmer environments (Yang et al., 2002). Among the cultivars CDC Go and CDC Teal, ‘CDC Go’ produced a higher grain number and showed a higher grain weight producing trend compared to ‘CDC Teal’ under the non-temperature stress conditions in the current experiment. Previous studies also showed ‘Attila’ outyielded ‘CDC Go’ when averaged across 2008-2014 (7 years) field data (Zou et al., 2017b) and ‘CDC Go’ outyielded ‘CDC Teal’ when averaged across 3 years of field data (Chen et al., 2015) under western Canadian field conditions. However, under heat-stress temperature conditions in the current experiment, both ‘CDC Go’, and ‘CDC Teal’ produced similar grain yield (grain weight and number). Previous studies also showed the ability of different genotypes to tolerate flowering-time heat stress is dependent on the genotype (Farooq et al., 2011; Nazir et al., 2021). A longer late reproductive

phase and/or greater floral ear to stem mass ratio increases the number of fertile spikelets per spike in wheat (Kirby, 1988; Miralles et al., 2000; González et al., 2011), and are both likely factors leading to higher grain yield (number of fertile spikelets, grains, and grain weight per spike) in 'Attila' compared to the other two cultivars. These data also suggest that the ability to produce more spikelets per spike and more fertile florets per spikelet are traits that are important for producing higher grain yields under heat-stress conditions.

As described in chapter one, heat stress negatively affects the reproductive development processes in wheat including differentiation of floral organs, anther and pollen development, pistil development, and pollination and fertilization, resulting in poor grain set in wheat (Rawson & Bagga, 1979; Saini & Aspinal, 1982; Bheemanahalli et al., 2019). A reduction in extruded anther (non-dehisced) length, but no change in pollen viability, was observed in the main tiller spike of 'Attila' and 'CDC Go' under heat stress (35 ^{o}C for 6 h per day for 8 days) at BBCH 37 (flag leaf just visible; Kumarapeli, 2021). Some heat resistant wheat cultivars maintain pollen viability by increasing the antioxidant capacities in developing anthers, which helps to detoxify ROS (reactive oxygen species) and attenuate heat stress-induced oxidative membrane damage (Dwivedi et al., 2019). Although this may be one possible mechanism functioning in 'Attila' anthers under heat-stress conditions, heat stress did significantly reduce the number of fertile spikelets and grain yield in this cultivar. Reduction in pollen vigor, changes in anther dehiscence, and abnormalities developed in the pistil may be a few of the reasons for heat stress-induced grain yield reduction in 'Attila'.

2.4.2 Effect of heat stress on plant height and flag-leaf area

'Attila' produced the shortest stems compared to the other cultivars tested, suggesting reduced resource competition between the stem and the developing spikes and grains in this cultivar. Under field conditions, 'CDC Go' was also taller (2 cm) than 'Attila' (Zou et al., 2017 b) and approximately 14 cm shorter than 'CDC Teal' (Chen et al., 2015). Heat stress reduced the plant height in 'Attila' and 'CDC Teal' suggesting heat stress can negatively affect the vegetative growth, which can ultimately reduce the photosynthetic capacity.

The flag-leaf is the main photoassimilate supplier for developing spikes in wheat. Only the flag-leaf area of the first tiller was assessed in this study, and all three cultivars were similar under non-temperature stress conditions (Table 2.7; Fig. A2C). Similar to plant height, flag-leaf

area reduction was observed under the heat-stress condition in 'Attila' and 'CDC Teal' suggesting heat stress can negatively affect the flag-leaf growth and ultimately reduce the photosynthetic capacity.

2.4.3 Effect of 4-Cl-IAA application on reproductive parameters, plant height and flag-leaf area

Reduction in auxin concentration in the developing anthers under heat-stress conditions is known to reduce the anther length and production of mature pollen, and lead to early anther dehiscence (before pollen maturation and anther filament elongation) in *Arabidopsis* and barley leading to a poor seed/grain set (Cecchetti et al., 2008; Sakata et al., 2010). Auxin application before imposing the heat-stress treatments may help in the development of normal reproductive organs (Sakata et al., 2010) and increase the photoassimilate availability and the photoassimilate partitioning into the developing grains (Darussalam et al., 1998) and possibly lower the floret primordia death and increase the grain set and weight in wheat. Previous studies showed that auxin applied prior to imposing a heat-stress treatment can ameliorate the heat stress effects on yield and yield component parameters in wheat and barley (Abeysingha et al., 2021; Sakata et al., 2010). In the current experiment, 4-Cl-IAA application (1 μM) increased the number of fertile spikelets per spike, grains per fertile spikelet, grain number and grain weight in 'Attila' when grown under heat-stress conditions (Tables 2.1, 2.2, 2.3, and 2.4; Figs. A3 and A4). An increasing trend in grain number and grain weight with 4-Cl-IAA application (1 μM) was also observed for 'CDC Go' when grown under heat-stress conditions, but it was not significant (Table 2.4; Fig A3 and A4). Our previous studies showed a single foliar application of auxins (4-Cl-IAA or 4-Me-IAA at 1 μM) at mid to late-boot stage (BBCH scale 43-45) increased the grain number and/or grain weight in non-temperature stressed and heat-stressed plants of 'CDC Go' under greenhouse conditions (Abeysingha et al., 2021). A 4-Cl-IAA-induced (at 1 μM) grain yield increase was also observed in 'CDC Go' grown in the field (Saskatoon, SK, Canada) under moderately high mid-season temperature conditions (Abeysingha et al., 2021). Although the growth conditions vary markedly in light intensity and duration, temperature, and relative humidity among these studies, the general consensus is that wheat responses to exogenous auxin (4-Cl-IAA at 1 μM) with respect to yield are genotype specific (Abeysingha et al., 2021), and 'CDC Go' and 'Attila' have the ability to respond to auxin application leading to increased grain yield under heat-stress conditions.

Furthermore, 4-Cl-IAA increased the flag-leaf area of 'Attila'suggesting auxin application before imposing the heat stress can ammeliorate the negative effects of heat stress on vegetative parameters as well.

2.4.4 Parental cultivar selection for in-depth analysis of heat stress and 4-Cl-IAA responses in an RIL population

When considering the available RIL populations for study, 'the RIL population derived from the cross between 'Attila' and 'CDC Go' has been extensively evaluated under conventionally- (from 2008 to 2014) and organically- managed (from 2008 to 2010) field conditions in the western Canadian prairies (Asif et al., 2015; Zou et al., 2017a and b). Furthermore, the 'Attila' × 'CDC Go' RIL population was genotyped with 579 polymorphic markers using diversity array technology (DArT), 1200 informative single-nucleotide polymorphic (SNP) markers from a 90K SNP array, and three gene-specific markers (*Rht-B1, Ppd-D1*, and *Vrn-A1*) to detect QTL under organic and conventionally managed field conditions (Asif et al., 2015; Zou et al., 2017a and b). The RIL population derived from the cross between 'CDC Go' and 'CDC Teal' were evaluated under field conditions from 2011-2013 and it was genotyped with 341 DArT polymorphic markers and a *Vrn-B1* marker (Chen et al., 2015).

The 'Attila' × 'CDC Go' RIL population was selected for in-depth evaluation described in chapters 3 and 4 for the following reasons: 1) grain yield after heat-stress differed in 'Attila' and 'CDC Go', 2) the ability of a one-time foliar 4-Cl-IAA application (prior to heat stress) to ameliorate the negative effects of heat stress with respect to grain yield was observed in 'Attila' and 'CDC Go', and 3) the 'Attila' × 'CDC Go' RIL population was more extensively characterized in the field than the 'CDC Go' x 'CDC Teal' RIL population.

2.5 Conclusions

The number of days to anthesis, number of total and fertile spikelets per spike, number of grains per total and fertile spikelet, grain number per spike, grain weight per spike, plant height, and flag-leaf area of the three cultivars tested were differentially affected by heat stress imposed at the flag leaf initiation to late boot stage. 'Attila' had better grain yield under non-temperature stress and heat-stress conditions, followed by 'CDC Go' and then, 'CDC Teal'. A one-time foliar application of auxin (4-Cl-IAA at 1 μM) stabilized the wheat grain yield of 'Attila', and 'CDC

Go' showed a positive trend in increasing grain yield when grown under heat-stress conditions. In consideration of the current experiment results and the data availability for the 'Attila' × 'CDC Go' and 'CDC Go' × 'CDC Teal' RIL populations, the former RIL population was selected for further analyses related to heat stress and auxin responses in wheat.

2.6 Tables

Table 2.1 Effect of auxin (4-Cl-IAA) and heat stress on reproductive parameters of the main tiller spike (spike 1) of wheat cultivars Attila, CDC Go, and CDC Teal.

Cultivar	4-Cl-IAA conc. †	Heat trt. ‡	Grain weight (g)	Grain number	Total spklts* per spike	Fertile spklts per spike	Grains per spklt	Grains per fertile spklt	Days to anthesis
Attila	1 µM	+	2.095& a§	57 a	25 a	20 a	2.21 a	2.76 a	70 b†
Attila	1 µM	-	2.108 a	49 bc	24 b	19 ab	2.06 abc	2.58 ab	70 b
Attila	0	+	1.700 b	41 cd	25 ab	18 abc	1.67 de	2.33 bc	70 b
Attila	0	-	2.324 a	56 ab	26 a	21 a	2.17 ab	2.70 a	74 a
CDC Go	1 µM	+	1.463 bc	31 e	20 de	17 bcd	1.61 def	1.96 ef	54 f
CDC Go	1 µM	-	1.409 c	35 de	19 e	15 d	1.79 cd	2.22 cd	57 e
CDC Go	0	+	1.244 c	33 e	19 e	15 d	1.61 def	1.98 def	54 f
CDC Go	0	-	1.472 bc	34 de	19 e	16 cd	1.84 bcd	2.13 cde	55 ef
CDC Teal	1 µM	+	1.236 c	29 e	22 c	16 cd	1.29 f	1.79 f	61 d
CDC Teal	1 µM	-	1.375 c	29 e	21 cd	15 d	1.44 ef	1.99 def	63 cd
CDC Teal	0	+	1.305 c	28 e	22 c	16 bcd	1.33 f	1.77 f	62 cd
CDC Teal	0	-	1.350 c	30 e	21 cd	15 d	1.38 ef	1.95 ef	64 c
Temperature mean		+	1.507 n	36 m	22 m	17 m	1.62 n	2.10 n	62 n
		-	1.673 m	39 m	22 m	17 m	1.78 m	2.26 m	64 m
4-Cl-IAA mean	1 µM		1.614 p	38 p	22 p	17 p	1.74 p	2.22 p	62 q
	0		1.566 p	37 p	22 p	17 p	1.67 p	2.14 p	63 p
Cultivar x Temperature			NS‡	NS	NS	NS	NS	NS	NS‡
Cultivar x 4-C-IAA			NS	NS	NS	NS	NS	NS	S
Temperature x 4-Cl-IAA			S	S	NS	NS	NS	NS	NS
Cultivar x Temperature x 4-Cl-IAA			NS	S	NS	NS	NS	S	NS

† An aqueous solution of 1 µM auxin (4-Cl-IAA) in 0.25 % Agral, or 0.25 % Agral alone (control) was applied to plants at the booting to anthesis stage.

‡ (+) Heat-stress temperature treatment; (-) non-temperature stress treatment.

* spklts = spikelets; & Data are means, n=9.

§ Means followed by a different letter are significantly different among cultivars, auxin treatments, and temperature treatments (a-f), among temperature treatment means (m, n), and among auxin treatment means (p, q) within each parameter by the LSD test, $P \leq 0.05$.

¶ NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 2.2 Effect of auxin (4-Cl-IAA) and heat stress on reproductive parameters of spike two of wheat cultivars Attila, CDC Go, and CDC Teal.

Cultivar	4-Cl-IAA conc. †	Heat trt. ‡	Grain weight (g)	Grain number	Total spklts* per spike	Fertile spklts per spike	Grains per spklt	Grains per fertile spklt
Attila	1 µM	+	2.053& bc§	55 ab	26 a	21 a	2.16 ab	2.64 a
Attila	1 µM	-	2.204 ab	52 b	26 a	20 ab	1.97 abc	2.62 a
Attila	0	+	1.746 cd	42 c	26 a	18 bc	1.64 de	2.34 b
Attila	0	-	2.500 a	60 a	27 a	22 a	2.26 a	2.75 a
CDC Go	1 µM	+	1.492 de	34 de	19 c	16 cd	1.81 cde	2.07 cd
CDC Go	1 µM	-	1.454 def	36 cd	19 c	16 cd	1.89 bcde	2.22 bc
CDC Go	0	+	1.387 ef	31 def	19 c	16 cd	1.62 ef	1.94 de
CDC Go	0	-	1.555 de	35 cd	18 c	16 cd	1.92 bcd	2.24 bc
CDC Teal	1 µM	+	1.253 ef	28 ef	21 b	15 d	1.31 g	1.82 de
CDC Teal	1 µM	-	1.190 f	27 f	21 b	14 d	1.23 g	1.83 de
CDC Teal	0	+	1.280 ef	28 ef	22 b	16 cd	1.27 g	1.77 e
CDC Teal	0	-	1.401 ef	30 def	22 b	16 cd	1.35 gf	1.87 de
Temperature mean		+	1.535 n	40 m	22 m	17 m	1.64 n	2.10 n
		-	1.717 m	36 n	22 m	17 m	1.77 m	2.26 m
4-Cl-IAA mean	1 µM		1.608 p	39 p	22 p	17 p	1.73 p	2.20 p
	0		1.644 p	38 p	22 p	17 p	1.68 p	2.15 p
Cultivar x Temperature			S	NS	NS	NS	NS	NS
Cultivar x 4-C-IAA			NS	NS	NS	NS	NS	NS
Temperature x 4-Cl-IAA			S	S	NS	S	S	S
Cultivar x Temperature x 4-Cl-IAA			NS	S	NS	NS	NS	NS

† An aqueous solution of 1 µM auxin (4-Cl-IAA) in 0.25 % Agral, or 0.25 % Agral alone (control) was applied to plants at the booting to anthesis stage.
‡ (+) Heat-stress temperature treatment; (-) non-temperature stress treatment.
* spklts = spikelets; & Data are means, n=9.
§ Means followed by a different letter are significantly different among cultivars, auxin (4-Cl-IAA) treatments, and temperature treatments (a-g), among temperature treatment means (m, n), and among auxin (4-Cl-IAA) treatments means (p, q) within each parameter by the LSD test, $P \leq 0.05$.
¶NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 2.3 Effect of auxin (4-Cl-IAA) and heat stress on reproductive parameters of spike three of wheat cultivars Attila, CDC Go, and CDC Teal.

Cultivar	4-Cl-IAA conc. †	Heat trt. ‡	Grain weight (g)	Grain number	Total spklts* per spike	Fertile spklts per spike	Grains per spklt	Grains per fertile spklt
Attila	1 µM	+	2.018 bc	51 a	26 a	20 a	1.98 a	2.55 ab
Attila	1 µM	-	2.069 ab	48 a	25 a	19 ab	1.87 a	2.46 abc
Attila	0	+	1.572 de	37 bc	26 a	17 bc	1.47 bc	2.28 bcd
Attila	0	-	2.414 a	54 a	27 a	20 a	2.01 a	2.65 a
CDC Go	1 µM	+	1.509 de	34 bcd	19 c	17 bc	1.79 a	2.07 de
CDC Go	1 µM	-	1.429 de	33 bcd	19 c	15 c	1.78 ab	2.18 cde
CDC Go	0	+	1.518 de	34 bcd	19 c	16 c	1.77 ab	2.06 de
CDC Go	0	-	1.669 cd	39 b	19 c	17 bc	2.04 a	2.32 bcd
CDC Teal	1 µM	+	1.244 e	28 d	21 b	15 c	1.33 c	1.89 e
CDC Teal	1 µM	-	1.423 de	30 cd	21 b	15 c	1.41 c	1.95 e
CDC Teal	0	+	1.266 e	28 d	22 b	15 c	1.30 c	1.91 e
CDC Teal	0	-	1.426 de	30 cd	22 b	15 c	1.4 c	2.05 de
Temperature mean		+	1.521 n	36 n	22 m	17 m	1.62 n	2.12 n
		-	1.738 m	39 m	22 m	17 m	1.75 m	2.27 m
4-Cl-IAA mean	1 µM		1.644 p	37 p	22 p	17 p	1.69 p	2.18 p
	0		1.615 p	37 p	22 p	17 p	1.66 p	2.21 p
Cultivar x Temperature			NS	NS	NS	NS	NS	NS
Cultivar x 4-C-IAA			NS	NS	NS	NS	NS	NS
Temperature x 4-Cl-IAA			S	S	NS	NS	S	NS
Cultivar x Temperature x 4-Cl-IAA			NS	NS	NS	NS	NS	NS

† An aqueous solution of 1 µM auxin (4-Cl-IAA) in 0.25 % Agral, or 0.25 % Agral alone (control) was applied to plants at the booting to anthesis stage.

‡ (+) Heat-stress temperature treatment; (-) non-temperature stress treatment.

* spklts = spikelets; & Data are means, n=9.

§ Means followed by a different letter are significantly different among cultivars, auxin (4-Cl-IAA) treatments, and temperature treatments (a-f), among temperature treatment means (m, n), and among auxin (4-Cl-IAA) treatment means (p, q) within each parameter by the LSD test, $P \leq 0.05$.

¶ NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 2.4 Effect of auxin (4-Cl-IAA) and heat stress on reproductive parameters of wheat cultivars Attila, CDC Go, and CDC Teal (average of the first three spikes).

Cultivar	4-Cl-IAA conc. †	Heat trt. ‡	Grain weight (g)	Grain number	Total spklts* per spike	Fertile spklts per spike	Grains per spklt	Grains per fertile spklt
Attila	1 µM	+	2.055& b§	54 ab	26 ab	20 a	2.12 a	2.65 a
Attila	1 µM	-	2.127 b	49 b	25 b	19 ab	1.97 ab	2.55 a
Attila	0	+	1.661 c	40 c	25 b	17 bc	1.59 ef	2.31 b
Attila	0	-	2.411 a	57 a	26 a	21 a	2.15 a	2.70 a
CDC Go	1 µM	+	1.488 cde	34 def	19 d	17 cd	1.74 cde	2.03 cd
CDC Go	1 µM	-	1.431 cdef	35 cde	19 d	16 cd	1.82 bcd	2.21 bc
CDC Go	0	+	1.383 def	32 def	19 d	16 cd	1.67 de	2.00 de
CDC Go	0	-	1.565 cd	36 cd	19 d	16 cd	1.93 abc	2.23 bc
CDC Teal	1 µM	+	1.245 f	28 f	21 c	15 cd	1.31 g	1.83 ed
CDC Teal	1 µM	-	1.329 def	29 ef	21 c	15 d	1.36 g	1.93 ed
CDC Teal	0	+	1.284 ef	28 f	22 c	16 cd	1.30 g	1.82 e
CDC Teal	0	-	1.392 def	30 ef	22 c	15 cd	1.37 fg	1.96 ed
Temperature mean		+	1.519 n	36 n	22 m	17 m	1.62 n	2.11 n
		-	1.709 m	39 m	22 m	17 m	1.77 m	2.26 m
4-Cl-IAA mean	1 µM		1.612 p	38 p	22 p	17 p	1.72 p	2.20 p
	0		1.616 p	37 p	22 p	17 p	1.67 p	2.17 p
Cultivar x Temperature			S	NS	NS	NS	NS	NS
Cultivar x 4-C-IAA			NS	NS	NS	NS	NS	NS
Temperature x 4-Cl-IAA			S	S	NS	S	S	S
Cultivar x Temperature x 4-Cl-IAA			S	S	NS	NS	S	NS

† An aqueous solution of 1 µM auxin (4-Cl-IAA) in 0.25 % Agral, or 0.25 % Agral alone (control) was applied to plants at the booting to anthesis stage.

‡ (+) Heat-stress temperature treatment; (-) non-temperature stress treatment.

* spklts = spikelets; & Data are means, n=9.

§ Means followed by a different letter are significantly different among cultivars, auxin (4-Cl-IAA) treatments, and temperature treatments (a-g), among temperature treatment means (m, n), and among auxin (4-Cl-IAA) treatments means (p, q) within each parameter by the LSD test, $P \leq 0.05$.

¶ NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 2.5 Effect of auxin (4-Cl-IAA) and heat stress on grain yield parameters at the basal, central, and the distal sections of spikes 1, 2, and 3 of wheat cultivars Attila, CDC Go, and CDC Teal.

				Spike 1				Spike 2				Spike 3			
Cultivar	Spike section	4-Cl-IAA Conc. †	Heat trt. ‡	Grain weight (g)	Grain number	Total splklts * per spike	Fertile spklts per spike	Grain weight (g)	Grain number	Total spklts per spike	Fertile spklts per spike	Grain weight (g)	Grain number	Total spklts per spike	Fertile spklts per spike
Attila	Basal	1 µM	+	0.791[&] cde[§]	20 b	8.5 a	6.8 b	0.787 de	21 c	8.6 a	6.6 cd	0.706 ef	17 c	8.6 ab	6.6 cd
Attila	Basal	1 µM	-	0.994 ab	22 ab	7.3 b	7.4 ab	0.949 bc	22 bc	8.6 a	7.3 bcd	0.893 cd	20 abc	8.6 ab	7.0 bc
Attila	Basal	0	+	0.657 e	16 cd	8.4 a	6.4 bc	0.705 e	15 d	8.6 a	6.0 d	0.586 f	13 d	8.2 b	5.4 de
Attila	Basal	0	-	1.123 a	25 a	8.7 a	7.7 ab	1.124 a	26 a	8.9 a	8.0 abc	1.070 ab	24 a	8.9 a	7.3 abc
Attila	Central	1 µM	+	0.943 bc	25 a	8.5 a	8.4 a	0.948 bc	25 ab	8.6 a	8.4 ab	0.967 bcd	24 a	8.6 ab	8.3 ab
Attila	Central	1 µM	-	0.904 bc	21 b	8.4 a	7.9 a	1.013 ab	23 abc	8.6 a	8.3 ab	0.980 abc	23 ab	8.6 ab	8.5 a
Attila	Central	0	+	0.821 cd	20 b	8.4 a	8.0 a	0.849 cd	21 c	8.6 a	8.3 ab	0.809 de	19 bc	8.2 b	7.8 abc
Attila	Central	0	-	1.010 ab	24 a	8.7 a	8.6 a	1.084 ab	26 a	8.9 a	8.9 a	1.148 a	24 a	8.9 a	8.6 a
Attila	Distal	1 µM	+	0.361 f	12 d	8.4 a	5.1 cd	0.318 f	10 e	8.4 a	5.9 de	0.345 g	10 de	8.4 ab	5.0 ef
Attila	Distal	1 µM	-	0.210 fg	6 e	8.3 a	3.8 d	0.242 f	7 ef	8.9 a	4.4 fg	0.196 g	5 f	8.4 ab	3.9 fg
Attila	Distal	0	+	0.223 fg	6 e	8.0 a	3.8 d	0.193 f	6 f	8.4 a	3.7 g	0.177 g	5 f	8.8 ab	3.3 g
Attila	Distal	0	-	0.191 g	6 e	8.4 a	4.6 d	0.288 f	8 ef	9.0 a	5.1 ef	0.196 g	6 ef	8.9 a	4.0 fg
CDC Go	Basal	1 µM	+	0.688 i	15 ij	7.0 i	6.3 i	0.570 j	13 j	6.1 jk	5.3 i	0.660 ij	15 ij	6.3 ij	5.9 ij
CDC Go	Basal	1 µM	-	0.654 i	16 i	6.2 j	6.0 i	0.665 i	16 ij	6.1 jk	6.0 i	0.669 ij	15 ij	6.1 j	5.9 ij
CDC Go	Basal	0	+	0.513 j	13 ij	6.3 ij	5.6 i	0.599 ij	13j	6.2 jk	5.8 i	0.664 ij	14 ij	6.4 ij	5.9 ij
CDC Go	Basal	0	-	0.695 i	16 ij	6.3 ij	6.1 i	0.731 i	16 i	6.0 k	5.9 i	0.775 i	17 i	6.1 j	6.0 ij
CDC Go	Central	1 µM	+	0.609 j	13 ij	7.0 i	6.8 i	0.611 ij	13 ijk	6.1 jk	6.0 i	0.638 ij	14 ij	6.3 ij	6.2 i
CDC Go	Central	1 µM	-	0.615 j	14 ij	6.2 j	6.2 i	0.621 ij	15 ijk	6.1 jk	6.1 i	0.581 j	13 j	6.1 j	5.8 ijk
CDC Go	Central	0	+	0.535 j	13 ij	6.3 ij	6.1 i	0.573 j	12 k	6.2 jk	5.7 i	0.641 ij	14 ij	6.4 ij	6.1 i
CDC Go	Central	0	-	0.619 j	14 ij	6.3 ij	6.2 i	0.622 ij	13 ijk	6.0 k	5.8 i	0.681 ij	15 ij	6.1 j	6.1 i
CDC Go	Distal	1 µM	+	0.166 k	4 k	6.2 j	3.6 j	0.311 k	8 l	6.7 ij	5.1 ij	0.211 k	6 k	6.4 ij	4.4 kl
CDC Go	Distal	1 µM	-	0.141 k	4 k	6.7 ij	3.2 j	0.168 l	5 l	6.9 i	4.2 j	0.179 k	5 k	6.6 ij	3.1
CDC Go	Distal	0	+	0.196 k	6 k	6.4 ij	3.9 j	0.215 kl	6 l	6.4 jk	4.3 j	0.213 k	6 k	6.2 j	4.4 kl

CDC Go	Distal	0	-	0.158 k	5 k	6.2 j	3.9 j	0.201 kl	6 l	6.4 jk	4.2 j	0.214 k	6 k	6.9 i	4.7 jkl
CDC Teal	Basal	1 µM	+	0.608 s	14 s	7.2 s	6.9 s	0.612 st	13 st	7.1 s	6.6 s	0.642 stu	14 st	7.1 s	6.7 s
CDC Teal	Basal	1 µM	-	0.678 s	15 s	6.9 s	6.7 s	0.649 s	14 s	7.2 s	6.7 s	0.743 st	16 st	7 .0 s	7.0 s
CDC Teal	Basal	0	+	0.625 s	13 s	7.2 s	6.8 s	0.648 s	14 st	7.2 s	6.6 s	0.652 stu	14 st	7.2 s	6.7 s
CDC Teal	Basal	0	-	0.683 s	15 s	6.9 s	6.8 s	0.714 s	15 s	7.3 s	7.1 s	0.776 s	16 s	7.2 s	6.9 s
CDC Teal	Central	1 µM	+	0.571 s	12 s	7.2 s	6.9 s	0.575 t	13 st	7.1 s	7.0 s	0.556 u	13 st	7.1 s	7.0 s
CDC Teal	Central	1 µM	-	0.604 s	13 s	6.9 s	6.7 s	0.499 t	11 t	7.2 s	6.3 s	0.598 tu	12 t	7.0 s	6.4 s
CDC Teal	Central	0	+	0.610 s	13 s	7.2 s	7.2 s	0.567 t	12 st	7.2 s	7.1 s	0.569 u	13 st	7.2 s	6.8 s
CDC Teal	Central	0	-	0.597 s	13 s	6.9 s	6.8 s	0.628 st	13 st	7.3 s	7.2 s	0.601 tu	12 t	7.2 s	6.6 s
CDC Teal	Distal	1 µM	+	0.057 t	2 t	7.2 s	1.9 t	0.066 u	2 u	7.2 s	1.9 t	0.046 v	1 u	7.1 s	1.4 t
CDC Teal	Distal	1 µM	-	0.093 t	3 t	7.6 s	1.9 t	0.042 u	1 u	7.0 s	1.1 t	0.082 v	2 u	7.3 s	2.0 t
CDC Teal	Distal	0	+	0.071 t	3 t	7.3 s	2.4 t	0.065 u	2 u	7.4 s	2.1 t	0.045 v	1 u	7.2 s	1.4 t
CDC Teal	Distal	0	-	0.070 t	2 t	7.6 s	2.1 t	0.058 u	1 u	7.4 s	1.6 t	0.049 v	1 u	7.1 s	1.2 t
Cultivar x spike section				S‡	S	NS	S	S	S	NS	S	S	S	NS	S
Cultivar x spike section x temperature				S	NS	NS	NS	NS	NS	NS	NS	S	NS	NS	NS
Cultivar x spike section x 4-Cl-IAA							NS	NS	NS	NS	NS	NS	NS	NS	NS
Cultivar x spike section x temperature x 4-Cl-IAA				NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

† An aqueous solution of 1 µM auxin (4-Cl-IAA) in 0.25 % Agral, or 0.25 % Agral alone (control) was applied to plants at the booting to anthesis stage.
‡ (+) Heat-stress temperature treatment; (-) non-temperature stress treatment.
* spklts = spikelets; & Data are means, n=9.
§ Means followed by a different letter are significantly different among auxin (4-Cl-IAA) treatments, temperature treatments and spike sections within cultivars (a-g for 'Attila'; i-l for 'CDC Go' and s-v for 'CDC Teal') within each parameter by the LSD test, $P \leq 0.05$.
¶ NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 2.6 Effect of auxin (4-Cl-IAA) and heat stress on grain yield parameters at the basal, central, and the distal spike sections (average of first three spikes) of wheat cultivars Attila, CDC Go, and CDC Teal.

Cultivar	Spike section	4-Cl-IAA Conc. †	Heat trt‡	Grain weight (g)	Grain number	Total spklts* per spike	Fertile spklts per spike
Attila	Basal	1 µM	+	0.762& de§	20 c	8.6 abc	6.7 cd
Attila	Basal	1 µM	-	0.945 c	21 c	8.2 c	7.2 bc
Attila	Basal	0	+	0.640 e	14 d	8.3 bc	5.8 de
Attila	Basal	0	-	1.106 a	25 a	8.8 a	7.7 abc
Attila	Central	1 µM	+	0.953 c	24 ab	8.6 abc	8.4 a
Attila	Central	1 µM	-	0.966 bc	22 bc	8.5 abc	8.2 ab
Attila	Central	0	+	0.820 d	20 c	8.3 bc	8.0 ab
Attila	Central	0	-	1.080 ab	25 ab	8.8 a	8.7 a
Attila	Distal	1 µM	+	0.341 f	11 e	8.5 abc	5.4 ef
Attila	Distal	1 µM	-	0.216 g	6 f	8.5 abc	4.0 g
Attila	Distal	0	+	0.201 g	6 f	8.3 bc	3.6 g
Attila	Distal	0	-	0.225 fg	7 f	8.8 ab	4.6 fg
CDC Go	Basal	1 µM	+	0.639 ij	14 ij	6.5 j	5.9 i
CDC Go	Basal	1 µM	-	0.662 ij	15 ij	6.2 j	6.0 i
CDC Go	Basal	0	+	0.592 j	13 jk	6.3 j	5.7 i
CDC Go	Basal	0	-	0.734 i	16 i	6.2 j	6.0 i
CDC Go	Central	1 µM	+	0.619 j	14 j	6.5 j	6.3 i
CDC Go	Central	1 µM	-	0.606 j	14 ij	6.2 j	6.0 i
CDC Go	Central	0	+	0.583 j	13 jk	6.3 j	6.0 i
CDC Go	Central	0	-	0.641 ij	14 ij	6.2 j	6.0 i
CDC Go	Distal	1 µM	+	0.230 k	6 l	6.4 j	4.4 j
CDC Go	Distal	1 µM	-	0.163 k	5 l	6.7 i	3.7 j
CDC Go	Distal	0	+	0.208 k	6 l	6.4 j	4.2 j
CDC Go	Distal	0	-	0.191 k	6 l	6.5 j	4.3 j
CDC Teal	Basal	1 µM	+	0.620 stu	14 st	7.2 s	6.7 s
CDC Teal	Basal	1 µM	-	0.690 st	15 s	7.0 s	6.8 s
CDC Teal	Basal	0	+	0.642 stu	14 st	7.2 s	6.7 s
CDC Teal	Basal	0	-	0.724 s	15 s	7.2 s	6.9 s
CDC Teal	Central	1 µM	+	0.568 u	13 st	7.2 s	7.0 s
CDC Teal	Central	1 µM	-	0.567 u	12 t	7.0 s	6.5 s
CDC Teal	Central	0	+	0.582 tu	13 st	7.2 s	7.0 s
CDC Teal	Central	0	-	0.609 tu	13 st	7.2 s	6.9 s
CDC Teal	Distal	1 µM	+	0.057 v	2 u	7.2 s	1.7 t
CDC Teal	Distal	1 µM	-	0.073 v	2 u	7.3 s	1.7 t
CDC Teal	Distal	0	+	0.060 v	2 u	7.3 s	2.0 t
CDC Teal	Distal	0	-	0.059 v	2 u	7.4 s	1.6 t
Cultivar x spike section				S	S	NS	S
Cultivar x spike section x temperature				S	S	NS	NS
Cultivar x spike section x 4-Cl-IAA				NS	NS	NS	NS
Cultivar x spike section x temperature x 4-Cl-IAA				NS	NS	NS	NS

† An aqueous solution of 1 µM auxin (4-Cl-IAA) in 0.25 % Agral, or 0.25 % Agral alone (control) was applied to plants at the booting to anthesis stage.
‡ (+) Heat-stress temperature treatment; (-) non-temperature stress treatment.
* spklts = spikelets; & Data are means, n=9.

§ Means followed by a different letter are significantly different among auxin (4-Cl-IAA) treatments, temperature treatments and spike sections within cultivars (a-g for ‘Attila’; i-l for ‘CDC Go’ and s-v for ‘CDC Teal’) and within each parameter by the LSD test, $P \leq 0.05$.

¶ NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 2.7 Effect of auxin (4-Cl-IAA) and heat stress on plant height and flag-leaf area of the main tiller spike of wheat cultivars Attila, CDC Go, and CDC Teal.

Cultivar	4-Cl-IAA conc. †	Heat trt‡	Plant height (cm)	Flag leaf area (cm^2)
Attila	1 µM	+	69[&] e[§]	37 ab
Attila	1 µM	-	76 d	29 cd
Attila	0	+	69 e	24 d
Attila	0	-	79 d	31 bc
CDC Go	1 µM	+	83 c	37 ab
CDC Go	1 µM	-	84 bc	37 ab
CDC Go	0	+	83 c	36 ab
CDC Go	0	-	85 bc	37 ab
CDC Teal	1 µM	+	87 b	34 abc
CDC Teal	1 µM	-	98 a	36 ab
CDC Teal	0	+	87 b	29 cd
CDC Teal	0	-	95 a	39 a
Temperature mean		+	79 n	33 m
		-	86 m	35 m
4-Cl-IAA mean	1 µM		83 p	35 p
	0		83 p	33 p
Cultivar x Temperature			S	NS
Cultivar x 4-Cl-IAA			NS	NS
Temperature x 4-Cl-IAA			NS	S
Cultivar x Temperature x 4-Cl-IAA			NS	NS

† An aqueous solution of 1 µM auxin (4-Cl-IAA) in 0.25 % Agral, or 0.25 % Agral alone (control) was applied to plants at the booting to anthesis stage.

‡ (+) Heat-stress temperature treatment; (-) non-temperature stress treatment.

[&] Data are means, n=9.

[§] Means followed by a different letter are significantly different among cultivars, auxin (4-Cl-IAA) treatments, and temperature treatments (a-e), among temperature means (m, n) and auxin (4-Cl-IAA) means (p), within each parameter by the LSD test, $P \leq 0.05$.

[¶] NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Chapter 3 - Effect of heat stress and auxin application on grain yield parameters of selected wheat cultivars and an 'Attila' x 'CDC Go' RIL population

3.1 Introduction

Agricultural crop improvement strategies to optimize the Genotype (G) × Environment (E) × Management (M) interactions for a target environment is required to minimize the yield gap under the changing climate (Cooper et al., 2021). Some wheat cultivars are adapted to a broad range of environments (stable cultivars), while others have limited environmental adaptation (Telfer at el., 2018). In agriculture, a crops' adaptation can be manipulated by genetic modification and agronomic practices. Genetic modification involves breeding of varieties that can resist environmental stresses and generate high economic yield. Traits that are associated with yield performance under heat stress can be used to increase the selection efficiency in the genetic development of crops. The cultivars Attila and CDC Go (described in chapters 1 and 2) were differentially adapted to heat stress imposed at the booting flowering stage when evaluated under growth chamber conditions. In this chapter, a population of recombinant inbred lines (RILs) derived from the parents 'Attila' and 'CDC Go', Parental cultivars Attila and CDC Go, as well as a group of seven Canadian spring wheat cultivars, were tested for their ability to adapt to heat stress with the focus on maintaining grain yield. Crop adaptation was also evaluated by applying 4-Cl-IAA as an agronomic management tool to ameliorate the heat stress effects on grain yield and yield component traits. The information from this study will help to identify 1) the most relevant yield components responsible for wheat grain yield improvement when the plants are exposed to heat-stress conditions at flowering, 2) heat-resistant and heat-sensitive genotypes among the RILs, and 3) the ability of a one-time foliar application of 4-Cl-IAA (1μM) at the early flowering stage to improve yielding ability of wheat genotypes under non-temperature stress and heat-stress conditions.

3.2 Materials and methods

3.2.1 Determining the effects of heat stress and 4-Cl-IAA on reproductive and yield component parameters of seven standard wheat cultivars, the RIL parental lines, and the 'Attila' × 'CDC Go' RIL population

Grains of 171 RILs developed from a cross between two spring wheat cultivars Attila and CDC Go, along with both RIL parents, and seven other standard cultivars grown in western Canada (AC Splendor, AC Superb, AC Harvest, Park, AC Andrew, Cutler, and AC Foremost) were planted in root trainer blocks and grown under growth chamber conditions (19ºC light/17ºC dark) at the University of Alberta, Edmonton, Alberta, with a 16 h photoperiod and an average light intensity of 373 μmol m^{-2} s^{-1} (Philips Silhouette high output fluorescent bulbs F54T5/835 Holland, Alto Collection). Each root trainer block had 40 (0.4-L) cells with each cell holding approximately 0.35-L volume of planting medium consisting of 4:1 Sunshine #4 potting mix (Sun Gro Horticulture, Vancouver, Canada) and sand, accommodating 1 plant per cell (Fig. B1). The experiment was arranged in a randomized complete block design with 4 treatments [2 temperatures (heat-stress and non-temperature stress)] x 2 auxin treatments [4-Cl-IAA at 1 μM in aqueous 0.25% (v/v) Agral, and aqueous 0.25% (v/v) Agral only control], replicated 4 times. Each treatment consisted of 1 plant each of the 171 RILs from the 'Attila' × 'CDC Go' RIL population, 7 standard cultivars planted randomly in five 40-cell root-trainer blocks, and 2-3 plants of each RIL parent ('CDC Go' and 'Attila') per block in five 40-cell root-trainer blocks (a total of 200 plants per treatment comprised of 171 RILs + 11 'Attila' + 11 'CDC Go' + 7 standard cultivar plants). One experimental replication consisted of 5 root-trainer blocks per treatment planted at the same time within the growth chamber (4 treatments x 5 blocks = total of 20 root-trainer blocks per replication; Fig. B2). Two grains were planted in one cell and the cells were thinned to one seedling per cell approximately 2 weeks after seeding. The growing medium was maintained moist in all treatments throughout the experiment, and the plants were fertilized weekly with 10: 52: 10 (N: P: K, early in the week) and 12: 2: 14 (later in the week) at 100 ppm. The 40-cell root-trainer blocks were randomized inside the growth chamber at two weeks intervals within a treatment. 4-Cl-IAA at 1 μM in aqueous 0.25% (v/v) Agral or aqueous 0.25% (v/v) Agral only control was applied as a one-time foliar spray to run-off using a hand-held bottle sprayer when the majority of the plants were near or at the booting flowering stage (BBCH 37 [flag leaf initiation] to 49 [1st awns visible]); Lancashire et al., 1991).

For the heat-stress treatment, plants were moved to a growth chamber maintained at 35°C for 6 h per day for 6 days within 12 h after solution application. In the heat-stress growth chamber, the light cycle began at 7:00 h at a 24°C air temperature. The heat treatment began at 11:00 h (35°C air temperature) and was maintained for 6 h (until 17:00 h). Following the heat treatment, the remainder of the light cycle was maintained at 24°C air temperature. The dark cycle (began at 23:00 h) was maintained at 20°C. The photoperiod was 16 h light/8 h dark at an average light intensity of 373 μmol m^{-2} s^{-1} (Philips Silhouette high output fluorescent bulbs F54T5/835 Holland, Alto Collection). After 6 days, the heat stress-treated plants were returned to the original growth chamber and maintained under controlled temperature conditions until maturity. The non-temperature stress control plants remained in the original growth chamber for the entire length of the experiment.

Data were collected for the floral spike of the main tiller and subsequent tillers were removed as they developed (when tillers were 5-10 cm tall). The floral spikes harvesting and drying were done as described in chapter two. The following plant growth parameters were taken on the main tiller spike as described in chapter two: number of days to anthesis, flag-leaf area, plant height, grain weight and number per spike and per spike section, grain number per spikelet and per fertile spikelet, total spikelets per spike, and the number of fertile spikelets per spike. To estimate the heat-stress tolerance in the RIL population, for each RIL the ratio of grain production under heat-stress conditions compared to that under non-temperature stress conditions was calculated (called the heat tolerance ratio; mean grain number or grain weight in spike 1 exposed to the heat-stress treatment divided by the mean grain number or grain weight in spike 1 grown under non-temperature stress conditions) as described in Rehman et al. (2009). In this study, RILs with a grain weight heat tolerance ratio of 0.81 or above were considered as heat resistant, RILs with a ratio of 0.41 to 0.6 were considered as moderately heat sensitive, and RILs with a ratio of 0.4 or below were considered as highly heat sensitive.

3.2.2 Determining the effect of planting medium volume in root trainers and 1-L pots on root fresh and dry weight at the booting-flowering stage (BBCH 43) of 'Attila' and 'CDC Go'

A separate experiment was carried out to determine the effect of planting medium volume on the fresh and dry weight of roots in 'Attila' and 'CDC Go'. Grains of the cultivars Attila and

CDC Go were sown at an approximate depth of 1.5 cm in 1-L (0.9-L planting medium volume) square plastic pots or 0.4-L (0.35-L planting medium volume) root-trainer cells (2 to 3 grains per pot or per root trainer cell) containing 4:1 Sunshine #4 potting mix (Sun Gro Horticulture, Vancouver, Canada) and sand. The seedlings were thinned to one seedling per container or cell approximately 2 weeks after planting. Plants were maintained under 20°C/17°C; day/night temperature conditions inside a growth chamber at the University of Alberta (fertilizer application, photoperiod, and light intensity was maintained as described above). The experiment consisted of 4 treatments (2 growing medium volume x 2 cultivars = 4 treatments), with 15 replications (plants) per treatment. The two cultivars were randomly assigned to containers within each container-type. Plant roots were carefully harvested when the plants were at the end of heading (BBCH 59). To avoid root damage or loss during harvest, the peat-based medium from each pot or root trainer cell was first submerged in water for 30 min and then the roots were lightly shaken to release the planting medium. The roots were then rinsed with water on a fine mesh strainer to further remove planting medium particles followed by manual removal of remaining particles with tweezers. Root fresh weight was measured after blotting excess moisture from the roots with absorbent paper towels. Dry weight was measured after drying the roots at 40°C for three days.

3.2.3 Statistical analyses

The yield parameter data was analyzed in three ways. The first analysis compared data of the nine standard cultivars (analysis 1), the second analysis compared data between the RIL parental cultivars Attila and CDC Go (analysis 2), and the third analysis compared data within the 'Attila' x 'CDC Go' RIL population (analysis 3). All three analyses were performed using PROC UNIVARIATE, PROC GLM or PROC MIXED procedures of SAS 9.3 (SAS Institute Inc. Cary, NC, USA, 2010). For each data parameter, normality and homogeneity of variance were tested using Shapiro-Wilk and Levene's tests, respectively.

Statistical significance of the data for heat-stress and auxin (4-Cl-IAA) treatments for the number of grains per spike, grain weight per spike, number of fertile spikelets per spike, days to anthesis, flag-leaf area, and plant height were determined using 3-factor factorial analyses of variance (ANOVA with the main effects = wheat lines x 2 auxin (4-Cl-IAA) treatments × 2 temperature treatments). Separate ANOVAs were performed comparing the following groupings of wheat lines: 1) 9 cultivars (the 7 standard cultivars described above and 'Attila' and 'CDC Go'

(one plant per treatment per replicate were used); 2) 'Attila' and 'CDC Go' (10 plants per treatment; 4 replicates); or 3) 171 RILs derived from an 'Attila' and 'CDC Go' cross. Correlations between specific traits were analyzed using SAS 9.3 obtaining a Pearson's r value, and the significance level of the relationship was declared at $P \leq 0.05$. Correlation graphs were drawn using OriginPro, Version 2021b. The number of fertile spikelets, and grain number and weight were further analyzed within three sections of the main tiller spike (basal, central, and distal) using a 3-factor ANOVA (main effects = wheat lines x 2 temperature treatments × 3 sections per spike). Three separate ANOVAs were performed that consisted of different plant lines as described above. In all analyses, mean separations were conducted using the least significance difference (LSD) test and statistical significance was declared at $P \leq 0.05$. Statistical significance of the data for root fresh and dry weight of the cultivars Attila and CDC Go was determined using a 2-factor factorial ANOVA (cultivar x growing medium volume). Mean separation was determined using the least significance difference (LSD) test and statistical significance was declared at $P \leq 0.05$. ANOVA F-values and probabilities for main effect means and interactions related to these analyses are given in Appendix D (Tables D7-D11).

3.3 Results

3.3.1 Effects of heat stress on reproductive parameters of nine wheat cultivars

3.3.1.1 The relationship between flowering time and grain yield

Under non-temperature stress conditions, 'CDC Go', 'AC Splender', 'AC Superb', 'Park', and 'AC Harvest' were earlier flowering cultivars (57.6 average days to anthesis), and 'AC Foremost', 'Cutler', 'Attila', and 'AC Andrew' were later flowering cultivars (74.8 average days to anthesis) (Table 3.1). The earlier flowering cultivars also had a lower number of total and fertile spikelets per spike ['Harvest' (18, 15), 'Park' (18, 15), 'AC Splender' (15, 14), 'AC Superb' (15, 10), and 'CDC Go' (16, 12)] compared to the later flowering cultivars ['Cutler' (26, 20), 'AC Andrew' (23, 17), 'AC Foremost' (23, 18), and 'Attila' (22, 20); Table 3.1]. Consistently, the number of total and fertile spikelets per spike was strongly positively correlated with the number of days to anthesis (r = 0.9; Fig. 3.1A and C). However, the positive correlation between these parameters was no longer significant (r =0.5, p = 0.1 and r = 0.4, p = 0.2 respectively) when the cultivars were exposed to the heat-stress treatment (Fig. 3.1B and D). This was due to the variation in the effect of the heat treatment on the number of days to anthesis among the cultivars. Similarly,

grain number and weight were strongly positively correlated with the number of days to anthesis (Fig. 3.2A and C). The strong positive correlation between the grain number and grain weight with the number of days to anthesis was maintained under the heat-stress conditions (Fig.3.2B and D).

3.3.1.2 The relationship between the number of spikelets per spike and grain yield

'Attila' produced the highest grain number (62) and weight (2.35 g), followed by 'Cutler' (49, 1.86 g), 'AC Foremost' (42, 1.55 g), and 'AC Andrew' (40, 1.62 g) in the main tiller spike under non-temperature stress conditions (Table 3.1). The remaining cultivars produced lower grain yield in the main tiller spike [grain number and weight, 'AC Superb' (20, 1.06 g), 'CDC Go' (22, 1.039), 'Park' (28, 1.044), 'AC Splender' (28, 1.219), and 'AC Harvest' (29, 1.15); Table 3.1]. The number of total and fertile spikelets per spike was strongly positively correlated with the grain number (r = 0.8; Fig. 3.3A and C), and grain weight (r = 0.9; Fig. 3.4A and C under non-temperature stress conditions. However, the positive correlation between these parameters was no longer significant or reduced when the cultivars were exposed to the heat-stress treatment due to a general trend for reduced grain number and weight with heat stress among these cultivars (Figs. 3.3 and 3.4; Table B1).

3.3.1.3 The relationship between the number of grains per spikelet and grain yield

At the spikelet level, 'Attila' produced the highest number of grains per spikelet (2.9), and grains per fertile spikelet under non-temperature stress conditions (Table 3.1). The number of grains per spikelet or per fertile spikelet was strongly positively correlated with the total grain number or weight of the spike in the cultivars tested under non-temperature stress and heat-stress conditions (Figs. 3.5 and 3.6). The heat-stress treatment reduced the number of grains per spikelet and grains per fertile spikelet among the cultivars by 18 and 10%, respectively (see temperature main effect means; Table 3.1). 'Attila' produced the highest number of grains per spikelet and per fertile spikelet after heat stress exposure compared to the other cultivars (Table 3.1 and Figs. 3.5 and 3.6). All cultivars had significantly reduced number of grains per fertile spikelet (Table 3.1).

3.3.1.4 The relationship between the grain number and grain weight

As expected, the grain number was strongly positively correlated with the grain weight under non-temperature stress conditions, with the cultivar Attila having the highest grain number

and weight and 'CDC Go' one of the lowest (Fig. 3.7A). In general, the heat-stress treatment reduced the grain number (16%) and weight (15%) of the cultivars (see temperature main effect means; Table 3.1). After heat stress exposure, 'Attila' still produced the same or higher total grain number and weight, and 'CDC Go' the lowest, compared to the other cultivars tested (Table 3.1; Fig. 3.7B). The strong positive correlation between grain number and weight was maintained under heat-stress conditions (Fig. 3.7B) due to a similar trend of grain number and grain weight reduction among the cultivars (Tables 3.1 and B1).

3.3.1.5 The relationship between plant height and flag-leaf area with grain yield

'Cutler' produced the tallest main tiller stem (111 cm), with 'Park', 'AC Splender' and 'Attila' plant height ranging from 93 to 99 cm (Table 3.1). 'AC Superb', 'AC Harvest', 'AC Foremost', 'CDC Go', and 'AC Andrew' produced shorter stems, with the plant height ranging from 80 to 85 cm when grown under the non-temperature stress conditions (Table 3.1). Heat stress reduced the plant height of the taller cultivars 'Cutler' (by 46%), 'AC Splender' (by 12%), and Park (by 11%), but had no effect on the other cultivars (Table 3.1). Plant height was not correlated with grain number or weight under non-temperature stress or heat-stress conditions (Fig. 3.8)

Flag-leaf area (cm^2) was greater in 'Park' (30), 'AC Harvest' (27), 'AC Andrew' (25), 'CDC Go' (25), and 'AC Superb' (24) compared to 'AC Foremost' (19), 'AC Splender' (19), 'Attila' (18), and 'Cutler' (17) when grown under the non-temperature stress conditions (Table 3.1). Flag-leaf area was moderately negatively correlated with the grain weight (r = -0.69, p = 0.04) (Fig. 3.9C) and a non-significant similar correlation trend was observed (r = -0.64, p = 0.06) (Fig. 3.9A) under non-temperature stress conditions. The flag-leaf area was weakly or not negatively correlated with grain number or weight when the cultivars were exposed to the heat-stress treatment due to a general reduction in grain yield in most cultivars (Fig. 3.9B and D).

3.3.2 Effects of 4-Cl-IAA application on reproductive and yield component parameters of nine wheat cultivars

Generally, 4-Cl-IAA treatment had minimal to no effect on the parameters assessed under non-temperature stress or heat-stress conditions across the cultivars, with a few exceptions (Table 3.1). 4-Cl-IAA application reduced the number of days to anthesis by 10 days under non-temperature stress conditions in 'Cutler', and it reduced the number of grains per fertile spikelet

by 33% in ‘AC Splender’ when exposed to the heat stress (Table 3.1). At the whole spike level, heat stress reduced the grain weight in ‘Attila’ and 4-Cl-IAA application negated the negative effect of heat-stress treatment on grain weight (Table 3.1).

3.3.3 Effects of heat stress and 4-Cl-IAA application on reproductive and yield component parameters of wheat cultivars Attila and CDC Go

The RIL parental lines were assessed with greater replication, to confirm their responses to heat stress and 4-Cl-IAA application. Under the non-temperature stress conditions, similar effects on the parameters assessed were observed as noted above. ‘CDC Go’ flowered earlier than ‘Attila’ (reached anthesis 9 days after ‘CDC Go’; Fig. 3.10; Table 3.2. ‘Attila’ produced a greater number of total and fertile spikelets per spike (20 and 17, respectively), and number of grains per spikelet (2) and per fertile spikelet (2.3) than ‘CDC Go’ (16 and 12; 1.4 and 1.8, respectively Fig. 3.11; Table 3.2). At the whole spike level, ‘Attila’ produced higher grain number (46%) and weight (33%) than ‘CDC Go’ (Fig. 3.12; Table 3.2). ‘CDC Go’ main tiller stem was longer (82 cm) than ‘Attila’ (76 cm), but the flag-leaf areas were similar; (Fig. 3.13; Table 3.2).

Heat stress reduced the number of days to anthesis by 3 days in ‘CDC Go’ and by 2 days in ‘Attila’ (Fig. 3.10; Table 3.2). The heat-stress treatment did not affect the total and fertile spikelets per spike in ‘Attila’ but decreased the number of grains per fertile spikelet by 9% (Fig. 3.11; Table 3.2). In ‘CDC Go’, heat stress reduced the number of fertile spikelets per spike by 33%, and the number of grains per spikelet and per fertile spikelet by 50 and 28%, respectively (Fig. 3.11B, C and D; Table 3.2). Heat stress also reduced the grain number (45%, Fig. 3.12, Table 3.2; reduction in the central and distal spike sections, Table 3.3) and weight of the main tiller spike (46%, Fig. 3.12, Table 3.2; in the central and distal spike sections, Table 3.3) in ‘CDC Go’, but did not affect grain number or weight in ‘Attila’ (Fig. 3.12; Tables 3.2 and 3.3).

The heat-stress treatment reduced the plant height of ‘CDC Go’ by 4% and ‘Attila’ by 7% (Fig. 3.13A; Table 3.2), and the flag-leaf area of ‘CDC Go’ (by 17%) (Fig. 3.13B; Table 3.2).

4-Cl-IAA application had no effect on the parameters tested, with a few exceptions (Figs. 3.10-3.13; Table 3.2). 4-Cl-IAA application reduce the grain weight by 21%, number of grains per spikelet by 21% and number of grains per fertile spikelet by 11% in ‘CDC Go’ under non-temperature stress conditions (Fig. 3.11C and D; Table 3.2). 4-Cl-IAA application reduced the plant height of ‘Attila’ by 3% under non-temperature stress conditions and ‘CDC Go’ by 5% when

exposed to the heat stress (Fig. 3.13; Table 3.2). 4-Cl-IAA application increased the flag-leaf area in 'Attila' by 11% under heat-stress condition (Fig. 3.13; Table 3.2).

3.3.4 Effect of heat stress on reproductive parameters of 'Attila' × 'CDC Go' RIL population

3.3.4.1 The relationship between flowering time and grain yield

The RIL population (171 lines) mean days to anthesis (65 days; Table 3.4) was 7% less than 'Attila' (70 days) and 7% higher than 'CDC Go' (61 days) under non-temperature stress conditions (Table 3.2). The number of days to anthesis was strongly positively correlated with the total spikelets per spike (r = 0.72; Fig. 3.14A), moderately positively correlated with fertile spikelets per spike (r = 0.47; Fig. 3.14C) and grain number (r = 0.47; Fig 3.15A), and weakly positively correlated with grain weight (r = 0.28; Fig. 3.15C) under non-temperature stress conditions. The heat-stress treatment did not affect the RIL population mean number of days to anthesis (Table 3.4); however, the population range for the days to anthesis increased by 10 days (Fig. 3.14; Table B2). Moderate to moderately strong positive correlations between the days to anthesis and the total or fertile spikelets per spike (r = 0.59 and 0.53 respectively; Figs. 3.14B and D), grain number (r = 0.61) and weight (r = 0.44) were maintained under the heat-stress temperature conditions (Fig. 3.15B and D).

3.3.4.2 The relationship between the number of spikelets per spike and grain yield

The RIL population mean of total spikelets per spike (19; Table 3.4) was 5% less than the 'Attila' (20) and 19% higher than the 'CDC Go' (16) under non-temperature stress (NS) condition (Table 3.2). Heat stress (HS) did not affect the RIL population mean (Table 3.4 or range of total spikelets per spike (Fig. 3.16A and B; Table B2). Moderate to moderately strong positive correlations were observed between the total spikelets per spike and grain number (NS, r = 0.59; HS, r = 0.63; Fig. 3.16A and B), or grain weight (NS, r = 0.47; HS, r = 0.58; Fig. 3.17A and B) under non-temperature stress and heat-stress conditions in the RIL population. The range of the number of grains per spike in the population was reduced by the heat-stress treatment (Fig. 3.16 A and B).

The RIL population mean of fertile spikelets per spike (15; Table 3.4) was 12% less than the 'Attila' (17) and 25% higher than the 'CDC Go' (12) under non-temperature stress conditions

(Table 3.2). Heat-stress treatment reduced the RIL population mean of fertile spikelets per spike (13) by 13% (Table 3.4) and slightly increased the range (Fig. 3.16C and D; Table B2). The number of fertile spikelets per spike was strongly positively correlated with the grain number (NS, r = 0.90; HS, r = 0.90; Fig. 3.16C and D) and grain weight (NS, r = 0.80; HS, r = 0.84; Fig. 3.17C and D) under non-temperature stress and heat-stress conditions in the RIL population.

3.3.4.3 The relationship between the number of grains per spikelet and grain yield

At the spikelet level, the RIL population mean number of grains per spikelet (1.6; Table 3.4) was 20% less than the 'Attila' (2) and 14% higher than the 'CDC Go' (1.4) under non-temperature stress conditions (Table 3.2). Heat-stress treatment reduced the population mean of grains per spikelet (1.2) by 25% (Table 3.4) but did not affect the range (Fig. 3.18A and B; Table B2). Strong positive correlations were observed between the number of grains per spikelet and the grain number (NS, r = 0.85; HS, r =0.91; Fig. 3.18A and B) or the grain weight (NS, r = 0.73; HS, r =0.81; Fig. 3.19A and B) under non-temperature stress and heat-stress conditions in the RIL population.

The RIL population mean number of grains per fertile spikelet (2; Table 3.4) was 13% less than the 'Attila' (2.3) and 11% higher than the 'CDC Go' (1.8) under non-temperature stress conditions (Table 3.2). Heat stress reduced the population mean number of grains per fertile spikelet (1.8) by 10% (Table 3.4) and slightly increased the range (Fig. 3.18 C and D: Table B2). Strong positive correlations were observed between the number of grains per fertile spikelet and the grain number or the grain weight under non-temperature stress and heat-stress conditions in the RIL population (Figs. 3.18C and D and 3.19C and D).

3.3.4.4 The relationship between the grain number and grain weight

At the whole spike level, the RIL population mean number of grains (31; Table 3.4) was 24% less than the 'Attila' (41) and 41% higher than the 'CDC Go' (22) under non-temperature stress condition (Table 3.2). Heat-stress treatment reduced the RIL population mean grain number in the main tiller spike (23) by 26% (Table 3.4; Fig. 3.20 A) and reduced the population range (Fig. 3.22; Table B2). Within the spike, the central section produced the highest and the distal section produced the lowest number of grains under non-temperature stress and heat-stress conditions in the RIL population (Table 3.5; Fig. 3.20 B). Heat-stress treatment reduced the grain

number in the basal, central, and distal spike sections of the main tiller spike (27%, 25% and 22%, respectively) (Table 3.5; Fig. 3.20 B).

The RIL population mean grain weight (1.2042 g; Table 3.4) was 17% less than the 'Attila' (1.4486 g) and 23% higher than the 'CDC Go' (0.9759 g) under non-temperature stress conditions (Table 3.2). A trend of decreased grain weight in the main tiller spike with heat stress exposure was observed in RIL population, but it was not significant (Table 3.4; Fig. 3.21 A), and the population range for grain weight was slightly smaller (Fig. 3.22; Table B2). However, within the spike, heat stress reduced the grain weight in the central section of the RIL population (Table 3.5). The central spike section produced the highest and the distal spike section produced the lowest RIL population mean grain weight under heat-stress and non-temperature stress conditions (Table 3.5; Fig. 3.21 B). Strong positive correlations were observed between the grain number and the grain weight under heat-stress and non-temperature stress conditions in the RIL population (Fig. 3.22).

3.3.4.5 Effect of heat stress on grain number and grain weight

After heat stress exposure, approximately 39% of the RILs (67 out of 171) were able to maintain a grain number that was at least 80% of that produced under non-temperature stress conditions (heat tolerance ratios of 0.81 to 1.6 for grain number Fig. 3.20C; Table B3). Approximately 18% of the RILs (30 RILs) produced greater grain number on the main tiller spike under heat-stress conditions than that observed under non-temperature stress conditions (heat tolerance ratio 1.01 to 1.6: Fig. 3.20C; Table B3). The parent cultivar 'CDC Go' had a heat tolerance ratio for grain number of 0.55 (only produced 55% of the grain number under heat-stress conditions than that under non-temperature stress conditions; resides in the 0.41-0.6 class interval; Fig. 3.20C). 'Attila' had a heat tolerance ratio for grain number of 0.93 (produced 93% of the grain number under heat-stress conditions than that under non-temperature stress conditions; resides in the 0.81-1 class interval; Fig. 3.20C).

Approximately 45% of the RILs (77 out of 171) were able to maintain a grain weight that was at least 80% of that produced under non-temperature stress conditions (heat tolerance ratios of 0.81 to 1.6 for grain weight Fig. 3.21C; Table B4). Approximately 20% of the RILs (34 RILs) produced greater grain weight on the main tiller spike under heat-stress conditions than that observed under non-temperature stress conditions (heat tolerance ratio 1.01 to 1.6: Fig. 3.21C;

Table B4). The parent cultivar 'CDC Go' had a heat tolerance ratio for grain weight of 0.54 (only produced 54% of the grain weight under heat-stress conditions than that under non-temperature stress conditions; resides in the 0.41-0.6 class interval; Fig. 3.21C). 'Attila' had a heat tolerance ratio for grain weight of 0.96 (produced 96% of the grain weight under heat-stress conditions than that under non-temperature stress conditions; resides in the 0.81-1 class interval; Fig. 3.21C).

In this experiment RILs were divided into heat resistant (heat tolerance ratio 0.81-1.6), moderately heat sensitive (heat tolerance ratio 0.41-0.6), and highly heat sensitive (heat tolerance ratio 0-0.4) based on the grain weight heat tolerance ratio. Accordingly, 77, 35, and 13 RILs were categorized as heat resistant, moderately heat sensitive and highly heat sensitive RILs (Table B4).

3.3.4.6 The relationship between plant height and flag-leaf area with grain yield

The RIL population mean plant height (86 cm; Table 3.4) was higher than that of 'Attila' (76 cm; 12% higher) and 'CDC Go' (82 cm; 5% higher) under non-temperature stress conditions (Table 3.2). Heat-stress treatment reduced the RIL population mean plant height (80 cm) by 7% (Table 3.4) and reduced the population range (Fig. 3.23; Table B2). Moderate to moderately strong positive correlations were observed between the plant height and the grain number (NS, $r = 0.47$; HS, $r = 0.46$) or the grain weight (NS, $r = 0.65$; HS, $r = 0.6$) under non-temperature stress and heat-stress conditions (Fig 3.23).

The RIL population mean flag-leaf area (20 cm^2; Table 3.4) was less than 'Attila' (24 cm^2; 16% less) and 'CDC Go' (23 cm^2; 13% less) under non-temperature stress condition (Table 3.2). Heat-stress treatment did not affect the RIL population mean flag-leaf area (19) (Table 3.4; Fig.3.24). Flag-leaf area was not correlated with the grain number or the grain weight under non-temperature stress conditions, and it was weakly negatively correlated with these parameters under heat-stress conditions in the RIL population (Fig. 3.24).

3.3.5 Effect of 4-Cl-IAA application on reproductive and yield component parameters

4-Cl-IAA application did not affect the RIL population mean for days to anthesis, total spikelets per spike, fertile spikelets per spike, number of grains per spikelet or per fertile spikelet, grain number, grain weight, or plant height under non-temperature stress or heat-stress conditions (Table 3.4). However, analysis of individual RILs showed 4-Cl-IAA application increased the grain number and grain weight under non-temperature stress and heat-stress conditions in a few

RILs (Table 3.6). 4-Cl-IAA application increased grain number in RILs 18 and 70, and the number of fertile spikelets per spike and number of grains per spikelet/fertile spikelet in RIL 18, under non-temperature stress condition (Table 3.6). RILs 46, 80 and 145 exhibited an increase in grain weight, and this was associated with an increase in grain number and number of grains per fertile spikelet for RIL 46 and 145 in response to 4-Cl-IAA application under heat-stress temperature conditions (Table 3.6).

3.3.6 Effect of planting medium volume on root fresh and dry weight at the booting flowering stage (BBCH 43) of 'Attila' and 'CDC Go'

In this experiment, parental lines were grown in root trainers (0.35-L planting medium volume) or pots (0.9-L planting medium volume). 'CDC Go' and 'Attila' produced similar fresh and dry root weights at BBCH 43 when grown in the smaller planting medium volume containers (root trainers; Table 3.7). However, when grown in a 0.9-L planting medium volume, 'CDC Go' produced approximately 40% more root mass (fresh and dry weight) than 'Attila (Table 3.7).

3.4 Discussion

3.4.1 Effects of heat stress on reproductive parameters of the parental cultivars Attila and CDC Go and the RIL population

When parental lines were analyzed with greater replication in the root trainer experiment, the heat-stress treatment reduced the number of days to anthesis in 'CDC Go' by 3 days and 'Attila' by 2 days (Table 3.2). 'Attila' showed greater yield stability in the main tiller spike under heat-stress conditions (heat tolerance ratios of 0.93-0.96 for grain yield) compared to 'CDC Go' (heat tolerance ratios of 0.54-0.55 for grain yield; Figs. 3.20C and 3.21C). The lower heat tolerance ratio for 'CDC Go' was reflected in reduced main tiller grain yield (grain number and weight) by nearly 45% with a substantial reduction in the number of fertile spikelets per spike, grains per spikelet and per fertile spikelet (Table 3.2). These data confirm that the 'Atilla' and 'CDC Go' RIL parental lines vary in heat tolerance, that the root trainer system is appropriate to measure heat-tolerance related phenotypes, and that the RIL population derived from these parents will be useful for further analysis of heat tolerance in this system.

Grain number and grain weight population means in the 'Attila' × 'CDC Go' RIL population were reduced by the heat-stress treatment with a substantial reduction in fertile spikelets

per spike and grain number per spikelet or per fertile spikelet (Table 3.4). Grain number reduction was observed in all three sections of the main tiller spike and grain weight reduction was more evident in the central spike section under heat stress (Table 3.5). The RILs selected as heat resistant in this experiment were minimally affected by the heat-stress treatment (produced more than 80% of grain weight in the main tiller spike). RILs selected as moderately heat-sensitive exhibited between 41 and 60% reduction in grain weight with heat stress, and RILs selected as highly heat-sensitive exhibited greater than 60% grain weight reduction with heat stress (Table B4). Accordingly, 45% of the RILs (77 RILs) were considered as heat-resistant, 20.5% as moderately heat susceptible (35 RILs) and 7.6% (13 RILs) as highly heat susceptible, making the 'Attila' × 'CDC Go' RIL population useful for studying the heat resistant traits in wheat.

3.4.2 The relationship between yield and yield component parameters

When doing cultivar selections to improve a complex and low heritable trait like yield it is important to know direct or indirect influence of other constituent traits and their behavioral changes in targeted environments (Reynolds et al., 2020). Pearson's correlation studies are helpful in measuring the magnitude of the mutual relationships between various closely associated yield component traits with yield and provide reasonable indications for plant breeders to improve economic yield and planning for more efficient breeding programmes. Strong to minor relationships were observed between yield component traits and grain yield in the standard spring wheat cultivar experiment and the 'Attila' × 'CDC Go' RIL population experiment, which will be discussed in detail below.

3.4.2.1 Relationship between days to anthesis and yield parameters

The standard wheat cultivars (experiment 1) showed strong positive correlations between days to anthesis and total or fertile spikelets per spike, grain number, and grain weight under non-temperature stress condition (Figs. 3.1 and 3.2). The flowering time of wheat genotypes is mainly determined by the vernalization and photoperiod response genes (Iqbal et al., 2007b; Kamran et al., 2013). The number of spikelets per spike is strongly influenced by the duration of reproductive development indicating genotypes with longer flowering time has the ability to produce a higher number of spikelets per spike until the terminal spikelet is initiated (Slafer & Rawson, 1994). However, a higher number of spikelets per spike is not a favoured selection target to improve grain

yield unless those genotypes also produce a higher number of grains per spikelet (Phillipp et al., 2018). Even though floret primordia initiation is mainly controlled through developmental processes, the survival of primordia to produce fertile florets seems to be strongly related to the dry matter accumulation and distribution along the developing spikes (Kirby, 1988; Siddique et al., 1989). The strong positive correlations between days to anthesis and fertile spikelets per spike, or grain number and weight in the cultivar experiment under non-temperature stress conditions suggest a longer vegetative phase promotes photoassimilate partitioning into the developing florets, and a longer stem-elongation phase promotes spike dry matter accumulation at anthesis, leading to greater grain production (Slafer et al., 2001).

Moderate to strong positive relationships between days to anthesis and total or fertile spikelets per spike were also observed in the 'Attila' × 'CDC Go' RIL population under non-temperature stress condition (Fig. 3.14). The magnitude of the positive relationship between days to anthesis and grain number was moderately strong, but that of days to anthesis and grain weight was weak in the RIL population under non-temperature stress condition (Fig. 3.15). Genotypes with different combinations of vernalization and photoperiod response genes may be responsible for the variable magnitude of the Pearson's correlation coefficients between days to anthesis and these traits in the RIL population.

Under heat-stress conditions at flowering, the cultivars in experiment 1 showed genotypic-dependent variation in the number of days to anthesis (Table 3.1) and it decreased the positive relationship strength between days to anthesis and total or fertile spikelets per spike (Fig. 3.1). Genotypes with different degrees of sensitivity to increased temperature showed variable developmental rates leading to variation in the days to flowering (heading; Slafer & Rawson, 1994). However, a strong positive correlation between the days to anthesis and grain number or grain weight was maintained among the cultivars under non-temperature stress and heat-stress conditions (Fig. 3.2). In the 'Attila' × 'CDC Go' RIL population, the positive correlation between days to anthesis and grain number or weight increased under heat-stress conditions, a result of an average decrease in grain number and weight and a greater range in days to anthesis among this population (Fig. 3.15).

Different genotypes can have unique developmental and growth responses to specific environmental conditions. For example, 'Cutler' is an early flowering cultivar under field and greenhouse conditions. However, 'Cutler's' flowering time in experiment 1 (76 days; grown in

root trainer blocks with lower planting medium volume and lower inter-plant spacing under growth chamber conditions) was almost double that described by Kamran et al. (2013) under greenhouse conditions (36 days). This suggests that the behaviour of a cultivar under normal growing condition is not necessarily a good predictor of its behaviour under stressful growing conditions.

Knowledge of relationships between flowering time and yield parameters can be useful to improve yielding ability of wheat in breeding programs. Moreover, when selecting wheat cultivars to grow under field conditions in the short growing seasons of Canada, it is important to select early flowering and maturing cultivars with higher yields. However, our data showed that even under favorable conditions a lower number of days to anthesis can result in lower yield likely due to the reduction in time available for photosynthesis and seed nutrient accumulation. Therefore, the use of later flowering cultivars with early sowing dates will be beneficial to obtain higher grain yields under Canadian field conditions. Moreover, early sowing dates may help escape mid and late season heat and drought stress conditions that normally occur in Canadian wheat fields, facilitating higher grain yield (Stelmakh, 1993).

3.4.2.2 Relationship between spikelet traits and grain number and weight

Strong positive correlations were observed between number of grains per spikelet or per fertile spikelet and grain number or weight per spike in the wheat cultivar experiment (Figs. 3.5, and 3.6) under non-temperature stress and heat-stress conditions. In the RIL population, moderately strong positive correlations were observed for the total spikelets per spike and grain number and weight, with strong positive correlations noted for the number of grains per fertile spikelets and grain number and weight (Figs. 3.16 and 3.17) under both temperature conditions. Wheat grain yield improvement strategies are generally highly associated with an increase in grain number per unit area, which is largely determined by the number of spikes per unit area and grains per spike (Shearman et al., 2005; Slafer et al., 2014). Wheat grain number per spike is dependent on the number of spikelets or fertile spikelets per spike and the number of grains per spikelet or per fertile spikelet (Foulkes et al., 2011). In contrast to the spikelets of other cereal crops such as rice and barley, each wheat spikelet has more than one grain (Koppolu et al., 2021). This makes the ‘spikelet’ one of the most essential grain yield components in wheat and improvement of spike architecture (total spikelets or fertile spikelets per spike and grains per spikelet or per fertile spikelet) is an essential target of increasing wheat grain yield (Guo et al., 2018; Wolde et al., 2019).

The current experiments confirm that spikelet traits are important in future breeding programs when focusing to develop high yielding wheat cultivars under non-temperature stress as well as heat-stress conditions.

3.4.2.3 Relationship between grain number and weight

A strong positive relationship between grain number and grain weight was observed in the standard wheat cultivars and the 'Attila' × 'CDC Go' RIL population experiments under non-temperature stress and heat-stress conditions. This positive relationship between grain number and weight per spike in the semi-dwarf wheat lines was observed in previous studies as well and may suggest increasing photoassimilate partitioning into the developing spikes improves spike fertility and the available carbon to fill the grains (Shearman et al., 2005; Phillipp et al., 2018).

3.4.2.4 Relationship between plant height and flag-leaf area and grain yield

Plant height (main tiller) did not correlate with the grain number or grain weight in the wheat cultivar experiment (Fig. 3.8). These data suggest that when comparing cultivars with a limited range in plant height (all cultivars in the experiment are considered to be semi-dwarf), plant height is not an important trait for determining yield under these conditions. The strength of the relationship between plant height and grain yield is better reflected in the RIL population that varies to a greater extent in plant height, where moderate to moderately strong positive correlations were observed with grain yield under non-temperature stress and heat-stress conditions (Fig. 3.23). The RIL correlations suggest that within the height range of the population, greater tiller height led to greater grain yield.

Flag-leaf area (main tiller) had a strong negative correlation with the grain weight and a non-significant but similar trend with grain number in the wheat cultivar experiment under non-temperature stress conditions. This relationship decreased or became non-significant under heat stress conditions (Fig.3.9). In the RIL population, minimal to no correlation was observed between flag-leaf area and grain yield (Fig. 3.24) under non-temperature stress and heat-stress conditions.

These data suggest that flag-leaf area of the main tiller was not an important trait when determining yield in these experiments. However, the number of leaves per plant, total leaf area per plant, and leaf architecture are important yield traits, and they were not assessed in the current experiments.

3.4.3 Effects of 4-Cl-IAA application on reproductive and yield component parameters

Grain yield can be lower under pre-anthesis heat stress due to decreased expression of YUCCA auxin biosynthesis genes and reduced internal auxin levels in developing anthers, which can enhance the abnormal anther and pollen development (Sakata et al., 2010) and decreased assimilate partitioning into the developing spikes (Darussalam et al., 1998). Also, exogenous auxin applied prior to the heat stress treatment could reverse the negative effects of heat stress on yield (Sakata et al., 2010; Abeysingha et al., 2021). In the root trainer experiment, 4-Cl-IAA (1 µM) application did not affect the grain yield parameters in 'Attila'; however, an increasing trend in grain number and weight, fertile spikelets per spike, and grains per spikelet or per fertile spikelet was observed for 'CDC Go' under heat-stress condition, but it was not significant (Table 3.2).

The RIL population means for measured traits were not affected by the 4-Cl-IAA treatment under heat-stress or non-temperature stress conditions (Table 3.4). These results are likely explained by differential responses to 4-Cl-IAA application by a large number of genotypes. Therefore, individual RIL analyses were conducted and some RILs were identified that responded to 4-Cl-IAA by increasing grain number and weight, fertile spikelets per spike, and grain number per spikelet or per fertile spikelet under heat-stress and/or non- temperature stress conditions indicating 4-Cl-IAA application can increase the grain yield in some wheat genotypes (Table 3.6). As the 4-Cl-IAA effect is minor at the individual plant level more replications are needed for a proper understanding of auxin effect on grain yield in the 'Attila' × 'CDC Go' RIL population.

3.4.4 An overview of 'Attila' and 'CDC Go' results in chapters two and three

In chapter two experiments, plants were grown in 1-L pots, which provided more planting medium volume and inter-plant space (~15 cm) than root trainers used in experiments described in this chapter (0.4-L cells with 5 cm inter plant spacing). Planting medium volume (0.35-L versus 0.9-L) affected the root growth of 'Attila' differently than 'CDC Go'. 'CDC Go' produced higher root fresh and dry weight (28 and 29% higher weight respectively) in 1-L pots compared to 'Attila'. However, both cultivars produced similar root fresh and dry weight when grown in 0.4-L root trainer cells. Therefore, 'CDC Go' has the ability to increase root mass and likely associated nutrient uptake during the tillering and flowering stages under non-temperature stress conditions when plant and soil space is not limiting, and this could lead to increased grain yield and minimize nitrate leaching under these conditions. This could also facilitate grain production in 'CDC Go'

when exposed to heat stress at flowering, as noted in chapter 2, where the heat stress had only minor effects on grain production in this cultivar when grown in the 1-L pot system. A greater negative effect of heat stress at flowering on grain yield was observed for 'Attila' when grown in the 1-L pot system (Tables 2.1-2.6). When grown in the 0.4-L root trainer growth systems, which is more root and shoot-space limiting, 'Attila' was more heat tolerant with respect grain yield than 'CDC Go', suggesting that 'Attila' would perform better with respect to grain yield in more root growth limiting environments. These results further support the above-mentioned statement that the behaviour of a cultivar under normal growing condition is not necessarily a good predictor of its behaviour under stressful growing conditions.

3.5 Conclusions

Major conclusions derived from the experiments mentioned in this chapter are 1) the 'Attila' × 'CDC Go' RIL population showed continuous phenotypic variation and significant differences under non-temperature stress and heat-stress conditions for most of the traits measured, and contained a range of heat resistant and heat sensitive RILs making it a suitable population for in-depth genotypic analyses, 2) Relationships between grain yield and other yield-component traits were modified by the heat stress in some cases, stressing the importance of cultivar trait evaluation under environments where the cultivar will be grown, and 3) a one-time foliar application of auxin (4-Cl-IAA at 1 μM) at the booting flowering stage can increase the grain yield and/or yield component parameters in some genotypes under heat-stress and/or non-temperature stress conditions.

3.6 Tables and figures

Table 3.1 Effect of auxin (4-Cl-IAA) and heat stress on phenotypic traits of nine wheat cultivars grown under the controlled environmental conditions.

Cultivar	4-Cl-IAA conc. †	Heat trt‡	Grain weight (g)	Grain number	Days to Anthesis	Plant height (cm)	Flag-leaf area (cm^2)	Total spklts* per spike	Fertile spklts per spike	Grains per spikelet	Grains per fertile spklt
AC Splender	1 µM	+	0.4781& j§	12 j	53 i	81 defghi	17 fghi	13 j	8 m	0.9 hij	1.2 k
AC Splender	1 µM	-	0.8478 ghij	22 hij	56 hi	90 bcde	23 bcdefg	14 ij	12 ghijkl	1.6 defgh	1.8 efghij
AC Splender	0	+	0.8661 fghij	20 hij	55 hi	82 defghi	22 cdefgh	14 ij	11 hijklm	1.5 defghij	1.8 cdefghij
AC Splender	0	-	1.2193 cdefgh	28 efghi	54 hi	93 bc	19 efghi	15 hij	14 cdefghi	1.9 bcd	2.0 cdefghi
AC Superb	1 µM	+	0.8845 fghij	21 hij	55 hi	79 efghi	18 fghi	16 fghij	12 hijklm	1.3 defghij	1.8 efghij
AC Superb	1 µM	-	0.5731 ij	13 j	61 fghi	83 cdefgh	23 abcdefg	15 hij	9 klm	0.9 ij	1.4 jk
AC Superb	0	+	1.0881 defghi	23 ghij	62 fgh	80 efghi	19 efghi	15 ghij	13 defghijk	1.5 defgh	1.8 efghij
AC Superb	0	-	1.059 defghi	20 ij	59 ghi	84 cdefgh	24 abcdef	15 ghij	10 ijklm	1.3 defghij	1.9 cdefghij
AC Harvest	1 µM	+	0.8343 ghij	21 hij	52 i	77 ghi	18 fghi	18 efg	13 defghijk	1.2 fghij	1.6 hijk
AC Harvest	1 µM	-	0.8612 ghij	22 hij	63 fgh	82 cdefghi	26 abcdef	18 efgh	13 defghijk	1.3 defghij	1.7 fghij
AC Harvest	0	+	0.9724 efghij	23 ghij	58 ghi	82 cdefghi	28 abc	17 efghi	13 fghijkl	1.4 defghij	1.8 efghij
AC Harvest	0	-	1.1510 cdefghi	29 defghi	62 fgh	83 cdefgh	27 abcd	18 efgh	15 bcdefgh	1.7 defgh	1.9 cdefghij
Park	1 µM	+	0.6582 hij	19 ij	59 ghi	87 cdefg	23 bcdefg	17 efghi	13 efghijkl	1.1 ghij	1.4 jk
Park	1 µM	-	0.6606 hij	19 ij	63 efg	92 bcd	26 abcde	17 efghi	13 fghijkl	1.1 fghij	1.5 ijk
Park	0	+	0.8543 ghij	22 hij	59 ghi	88 cdef	29 ab	18 efgh	14 cdefghij	1.3 defghij	1.7 ghijk
Park	0	-	1.0439 defghij	28 defghi	60 fghi	99 ab	30 a	18 efgh	15 bcdefgh	1.6 defgh	1.8 defghij
AC Andrew	1 µM	+	1.4508 bcdef	37 bcdefg	71 cde	75 hi	20 defghi	23 ab	17 abcde	1.6 defgh	2.2 cdefg
AC Andrew	1 µM	-	1.51 bcdef	38 bcdef	68 def	82 defghi	25 abcdef	23 ab	17 abcdef	1.7 defgh	2.2 cdef
AC Andrew	0	+	1.1289 defghi	27 fghi	71 bcde	77 fghi	21 cdefgh	23 ab	17 abcdefg	1.2 efghij	1.7 ghijk
AC Andrew	0	-	1.6242 bcd	40 bcde	73 bcd	80 efghi	25 abcdef	23 ab	17 abcde	1.8 cdef	2.3 cde
Cutler	1 µM	+	1.1377 cdefghi	29 defghi	74 abcd	86 cdefgh	19 efghi	21 bcd	13 cdefghijk	1.3 defghij	2.1 cdefgh
Cutler	1 µM	-	1.7784 abc	45 bc	66 defgh	110 a	16 ghi	23 ab	19 ab	1.9 bcde	2.4 bcd
Cutler	0	+	1.726 abcd	38 bcdefg	61 fghi	79 efghi	26 abcdef	22 abc	19 abc	1.7 cdefgh	2.0 cdefghij
Cutler	0	-	1.8578 abc	49 ab	76 abcd	111 a	17 fghi	26 a	20 ab	1.9 bcdef	2.4 bcde

AC Foremost	1 µM	+	1.5187 bcdef	41 bcd	72 bcde	75 hi	15 hi	22 abc	19 ab	1.9 bcdef	2.2 cdefg
AC Foremost	1 µM	-	1.6998 bcd	37 bcdefg	76 abcd	84 cdefgh	19 efghi	22 bc	17 abcdefg	1.7 cdefg	2.2 cdef
AC Foremost	0	+	1.3449 bcdefg	32 cdefgh	76 abcd	86 cdefgh	14 i	22 bc	15 bcdefgh	1.5 defghi	2.1 cdefgh
AC Foremost	0	-	1.5513 bcde	42 bc	75 abcd	85 cdefgh	19 efghi	23 ab	18 abcd	1.9 bcdef	2.4 bc
Attila	1 µM	+	1.9039 ab	46 b	74 abcd	92 bcd	18 fghi	19 cde	16 bcdefgh	2.5 ab	3.0 ab
Attila	1 µM	-	1.7516 abc	49 b	83 a	87 cdefg	23 bcdefg	18 def	17 abcdef	3.0 a	3.2 a
Attila	0	+	1.7200 bcd	43 bc	79 ab	87 cdefg	16 ghi	18 defg	13 defghijk	2.4 abc	3.2 a
Attila	0	-	2.3473 a	62 a	75 abcd	93 bc	18 fghi	22 abc	20 a	2.9 a	3.1 a
CDC Go	1 µM	+	0.5812 ij	12 j	53 i	72 i	20 defghi	15 ghij	9 lm	0.8 j	1.4 jk
CDC Go	1 µM	-	0.7983 ghij	18 ij	57 ghi	84 cdefgh	20 defghi	16 fghij	11 ijklm	1.1 fghij	1.6 ghijk
CDC Go	0	+	0.6742 hij	14 j	53 i	78 fghi	20 defghi	17 efghi	10 jklm	0.8 ij	1.5 jk
CDC Go	0	-	1.0391 defghij	22 hij	53 i	84 cdefgh	25 abcdef	16 fghij	12 hijkl	1.4 defghij	1.8 defghij
Temperature mean		+	1.1012 n	27 n	64 m	81 n	20 n	18 m	14 n	1.4 n	1.9 n
		-	1.2985 m	32 m	64 m	89 m	22 m	19 m	15 m	1.7 m	2.1 m
4-Cl-IAA mean		+	1.1071 q	28 q	63 q	84 p	20 p	18 p	14 q	1.5 q	1.9 p
		-	1.2927 p	31 p	65 p	86 p	22 p	19 p	15 p	1.6 p	2.0 p
Cultivar x Temperature			NS¶	NS	NS	S	NS	NS	S	NS	NS
Cultivar x 4-Cl-IAA			NS	NS	NS	NS	NS	NS	NS	NS	NS
Temperature x 4-Cl-IAA			NS	S	NS	NS	NS	NS	NS	NS	NS
Cultivar x Temperature x 4-Cl-IAA			NS	NS	S	NS	NS	NS	NS	NS	NS

† An aqueous solution of 1 µM auxin (4-Cl-IAA) in 0.25 % Agral, or 0.25 % Agral alone (control) was applied to plants at the flag leaf initiation to end of booting growth stage.
‡ (+) Heat-stress temperature treatment; (-) non-stress-temperature treatment.
* spklts = spikelets; & Data are means, n=4.
§ Means followed by a different letter are significantly different among cultivars, auxin (4-Cl-IAA) treatments, and temperature treatments (a-m), among temperature treatment means (m, n), and among auxin (4-Cl-IAA) means (p, q) within each parameter by the LSD test, with the significance level at $P \leq 0.05$.
¶ NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 3.2 Effects of heat stress and auxin (4-Cl-IAA) application on yield parameters of 'Attila' and 'CDC Go' grown under the controlled environmental conditions.

	4-Cl-IAA conc. †	Heat trt‡	Grain weight (g)	Grain number	Days to anthesis	Plant height (cm)	Flag-leaf area (cm^2)	Total spklts* per spike	Fertile spklts per spike	Grains per spklt	Grains per fertile spklt
Attila	1 µM	+	1.3128& a§	35 b	69 ab	70 e	28 a	20 a	17 a	1.7 b	2.1 b
Attila	1 µM	-	1.3242 a	40 ab	70 a	74 d	27 ab	20 a	17 a	1.9 ab	2.3 a
Attila	0	+	1.3849 a	38 ab	68 b	71 e	25 bc	21 a	17 a	1.8 ab	2.1 b
Attila	0	-	1.4486 a	41 a	70 a	76 c	24 bc	20 a	17 a	2.0 a	2.3 a
CDC Go	1 µM	+	0.5772 d	13 d	59 de	75 cd	21 de	16 b	9 c	0.8 e	1.4 e
CDC Go	1 µM	-	0.7668 c	18 c	60 cd	81 a	21 de	16 b	11 b	1.1 d	1.6 d
CDC Go	0	+	0.5312 d	12 d	58 e	79 b	19 e	16 b	8 c	0.7 e	1.3 e
CDC Go	0	-	0.9759 b	22 c	61 c	82 a	23 cd	16 b	12 b	1.4 c	1.8 c
Temperature mean		+	0.9515 n	24 n	63 n	74 n	23 m	18 m	13 n	1.2538 n	1.7122 n
		-	1.1289 m	30 m	65 m	79 m	23 m	18 m	14 m	1.6217 m	2.0168 m
4-Cl-IAA mean		+	0.9953 p	27 p	64 p	75 q	24 p	18 p	13 p	1.3953 p	1.8450 p
		-	1.0852 p	28 p	64 p	77 p	23 q	18 p	14 p	1.4803 p	1.8840 p
Cultivar x Temperature			S¶	NS	NS	NS	S	NS	S	S	NS
Cultivar x 4-Cl-IAA			NS	NS	NS	NS	NS	NS	NS	NS	NS
Temperature x 4-Cl-IAA			NS	NS	NS	NS	NS	NS	NS	NS	NS
Cultivar x Temperature x 4-Cl-IAA			NS	NS	NS	NS	NS	NS	NS	NS	NS

† An aqueous solution of 1 µM auxin (4-Cl-IAA) in 0.25 % Agral, or 0.25 % Agral alone (control) was applied to plants at the flag leaf initiation to end of booting growth stage.
‡ (+) Heat-stress temperature treatment; (-) non-stress-temperature treatment.
* spklts = spikelets; & Data are means, n=40.
§ Means followed by a different letter are significantly different among cultivars, auxin (4-Cl-IAA), and temperature treatments (a-e), among temperature (m, n) and auxin (4-Cl-IAA) (p, q) treatment means, within each parameter by the LSD test, with the significance level at $P \leq 0.05$.
¶ NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 3.3 Effect of heat stress on grain number and weight in the basal, central, and distal spike sections of the main tiller spike of the wheat cultivars 'Attila' and 'CDC Go' grown under the controlled environmental conditions.

Cultivar	Heat trt‡	Basal grain number	Central grain number	Distal grain number	Basal grain weight (g)	Central grain weight(g)	Distal grain weight (g)
Attila	+	12[&] bc[§]	16 a	10 d	0.499 c	0.587 ab	0.299 d
Attila	-	12 b	17 a	11 bcd	0.510 bc	0.637 a	0.302 d
CDC Go	+	4 fg	5 ef	3 g	0.180 ef	0.245 de	0.105 f
CDC Go	-	6 ef	10 cd	6 e	0.256 de	0.460 c	0.260 d
Temperature mean	+	8 r	10 q	6 s	0.340 r	0.416 q	0.202 t
	-	9 qr	14 p	9 r	0.383 qr	0.549 p	0.281 s
Temperature x Cultivar		S¶	S	S	S	S	S
Cultivar x position		S	S	S	S	S	S
Temperature x position		NS	NS	NS	NS	NS	NS
Cultivar x Temperature x position		NS	NS	NS	NS	NS	NS

‡ (+) Heat-stress temperature treatment; (-) non-stress-temperature treatment imposed at the flag leaf initiation to end of booting growth stage. [&] Data are means, n=40.
[§] Means followed by a different letter are significantly different among cultivars, spike sections and temperature treatments (a-g) and among temperature treatment means and spike sections (p-t) within each parameter by the LSD test, with the significance level at $P \leq 0.05$.
¶ NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 3.4 Effect of auxin (4-Cl-IAA) and heat stress on phenotypic traits of the 'Attila' × 'CDC Go' RIL population grown under the controlled environmental conditions.

	4-Cl-IAA conc. †	Heat trt‡	Grain weight (g)	Grain number	Days to anthesis	Plant height (cm)	Flag-leaf area (cm^2)	Total spklts* per spike	Fertile spklts per spike	Grains per spklt	Grains per fertile spklt
	1 µM	+	0.8784[&] b[§]	22 b	64 a	79 b	20 a	19 a	12 b	1.2 b	1.8 b
	1 µM	-	1.1718 ab	31 a	65 a	85 a	21 a	19 a	15 a	1.6 a	2.1 a
	0	+	0.9196 ab	23 b	64 a	80 b	19 a	19 a	13 b	1.2 b	1.8 b
	0	-	1.2042 a	31 a	65 a	86 a	20 a	19 a	15 a	1.6 a	2 a
RILs x Temperature			S	S	NS	NS	NS	NS	S	S	S
RILs x 4-Cl-IAA			NS	NS	NS	NS	NS	NS	NS	NS	NS
Temperature x 4-Cl-IAA			NS	NS	NS	NS	NS	NS	NS	NS	NS
RILs x Temperature x 4-Cl-IAA			NS	NS	NS	NS	NS	NS	NS	NS	NS

† An aqueous solution of 1 µM auxin (4-Cl-IAA) in 0.25 % Agral, or 0.25 % Agral alone (control) was applied on 171 'Attila' × 'CDC Go' RILs at the flag leaf initiation to end of booting growth stage.
‡ (+) Heat-stress temperature treatment; (-) non-stress-temperature treatment.
* spklts = spikelets; [&] Data are means, n=171 × 4.
[§] Means followed by a different letter are significantly different among auxin (4-Cl-IAA) and temperature treatments (a, b) within each parameter by the LSD test, with the significance level at $P \leq 0.05$.
¶ NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 3.5 Effect of heat stress on grain number and weight in the basal, central, and distal spike sections of the main tiller spike of the 'Attila' × 'CDC Go' RIL population grown under the controlled environmental conditions.

Heat trt‡	Basal grain number	Central grain number	Distal grain number	Basal grain weight (g)	Central grain weight (g)	Distal grain weight (g)
+	7 c§	10.5 b	5.5 d	0.3039 cd	0.4370 b	0.1813 e
-	9.5 b	14 a	7 c	0.4058 bc	0.5789 a	0.2265 de
Temperature x RILs	S¶	S	S	S	S	S
RILs x position	S	S	S	S	S	S
Temperature x position	S	S	S	S	S	S
RILs x Temperature x position	NS	NS	NS	NS	NS	NS

‡ (+) Heat-stress temperature treatment; (-) non-stress-temperature treatment imposed at the flag leaf initiation to end of booting growth stage of 171 'Attila' × 'CDC Go' RILs.
§ Means followed by a different letter are significantly different among the temperature treatments and spike sections within each parameter (a-e) by the LSD test, with the significance level at $P \leq 0.05$.
¶ NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 3.6 Effect of auxin (4-Cl-IAA) and heat-stress on phenotypic traits of selected RILs of the 'Attila' × 'CDC Go' RIL population grown under the controlled environmental conditions.

RIL no.	4-Cl-IAA conc. †	Heat trt‡	Grain weight (g)	Grain number	Days to anthesis	Plant height (cm)	Total spklts* per spike	Fertile spklts per spike	Grains per spikelet	Grains per fertile spklt
18	1 µM	+	0.8566 a	25.3 b	74 a	71 a	18 b	13 b	1.4014 ab	1.9315 ab
	1 µM	-	1.1384 a	39.5 a	77 a	79 a	20 ab	18 a	1.9916 a	2.2297 a
	0	+	0.7791 a	25.3 b	74 a	74 a	21 ab	14 ab	1.2583 b	1.7788 ab
	0	-	0.6191 a	18.8 b	77 a	52 a	21 a	12 b	0.8806 b	1.5379 b
46	1 µM	+	0.6574 f	18 e	55 e	73 e	17 e	10 ef	1.0800 e	1.6480 e
	1 µM	-	1.1166 e	26 e	54 e	91 e	17 e	14 e	1.4866 e	1.8065 e
	0	+	0.2629 g	6 f	53 e	87 e	16 e	13 e	0.3503 f	0.9208 f
	0	-	0.9471 ef	25 e	55 e	89 e	17 e	6 f	1.4835 e	1.8706 e
70	1 µM	+	0.6076 i	20 hi	71 h	73 h	21 h	12 h	0.9349 i	1.7042 h
	1 µM	-	1.1649 h	33 h	79 h	69 h	22 h	16 h	1.6333 h	2.1376 h
	0	+	0.9102 hi	26 hi	70 h	68 h	25 h	14 h	1.0824 hi	1.8384 h
	0	-	0.7536 hi	18 i	77 h	73 h	22 h	13 h	1.0914 hi	1.8598 h
80	1 µM	+	1.0856 j	27 jk	58 j	73 k	19 j	15 j	1.3656 jk	1.7988 jk
	1 µM	-	1.0585 j	30 j	63 j	75 jk	19 j	14 j	1.5971 j	2.1042 j
	0	+	0.544 k	14 k	58 j	70 jk	17 j	10 j	0.8193 k	1.2769 k
	0	-	0.8896 jk	29 j	64 j	77 j	18 j	13 j	1.6239 j	2.1835 j
145	1 µM	+	1.0914 m	24 mn	56 m	74 n	20 m	14 mn	1.2144 mn	1.7609 m
	1 µM	-	1.0944 m	30 m	59 m	77 mn	20 m	16 m	1.5013 m	1.7761 m
	0	+	0.5292 n	11 n	59 m	74 n	19 m	9 n	0.5529 n	1.0708 n
	0	-	1.1089 m	27 m	59 m	83 m	19 m	14 mn	1.4724 m	1.8678 m

† An aqueous solution of 1 µM auxin (4-Cl-IAA) in 0.25 % Agral, or 0.25 % Agral alone (control) was applied on 171 'Attila' × 'CDC Go' RILs at the flag leaf initiation to end of booting growth stage.

‡ (+) Heat-stress temperature treatment; (-) non-stress-temperature treatment.

* spklts = spikelets

§ Means followed by a different letter are significantly different among auxin (4-Cl-IAA) and temperature treatments within each RIL (a, b for RIL 18; e, f, g for RIL 46; h, i for RIL 70; j, k for RIL 80; and m, n for RIL 145) by the LSD test, with the significance level at $P \leq 0.05$.

¶ NS, S = not significant ($P > 0.05$) and significant ($P \leq 0.05$), respectively.

Table 3.7 Effect of planting medium volume in root trainers (0.4-L) and pots (1-L) on root fresh and dry weight at the heading stage (BBCH 59) of 'Attila' and 'CDC Go' when grown under non-temperature stress conditions.

Container	Planting medium volume	Cultivar	Root fresh weight (g)	Root dry weight† (g)
Root trainer	0.4 L	Attila	2.7 c	0.29 c
		CDC Go	2.5 c	0.21 c
Pot	1 L	Attila	5.9 b	0.47 b
		CDC Go	8.2 a	0.66 a

† Root dry weight was measured after drying at 40 °C for two days.

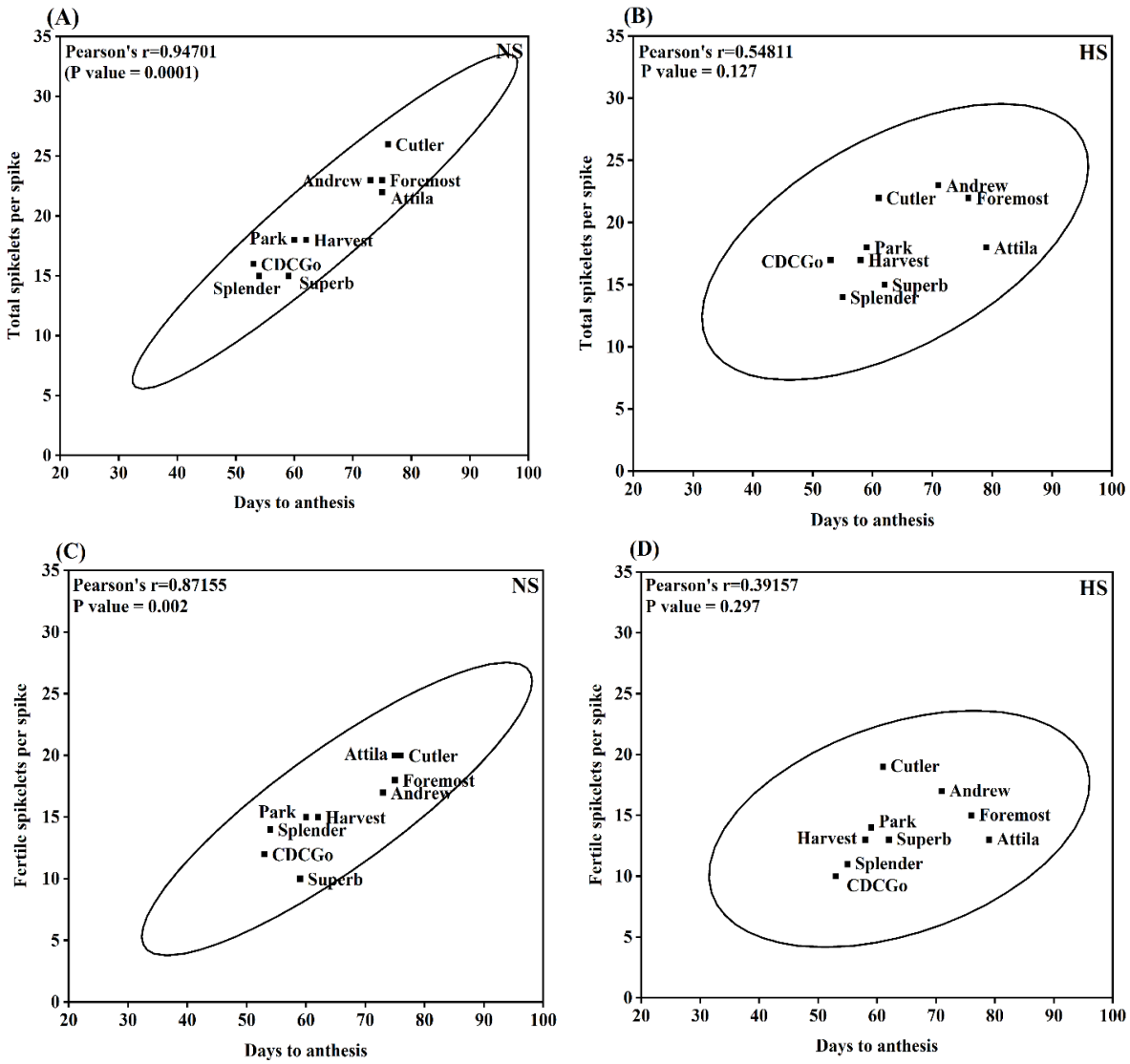

Figure 3.1. Scatter plot correlations of days to anthesis with the number of total or fertile spikelets per spike on the main tiller spike of nine standard wheat cultivars grown under non-temperature stress (NS; A and C) or heat-stress (HS; B and D) conditions.

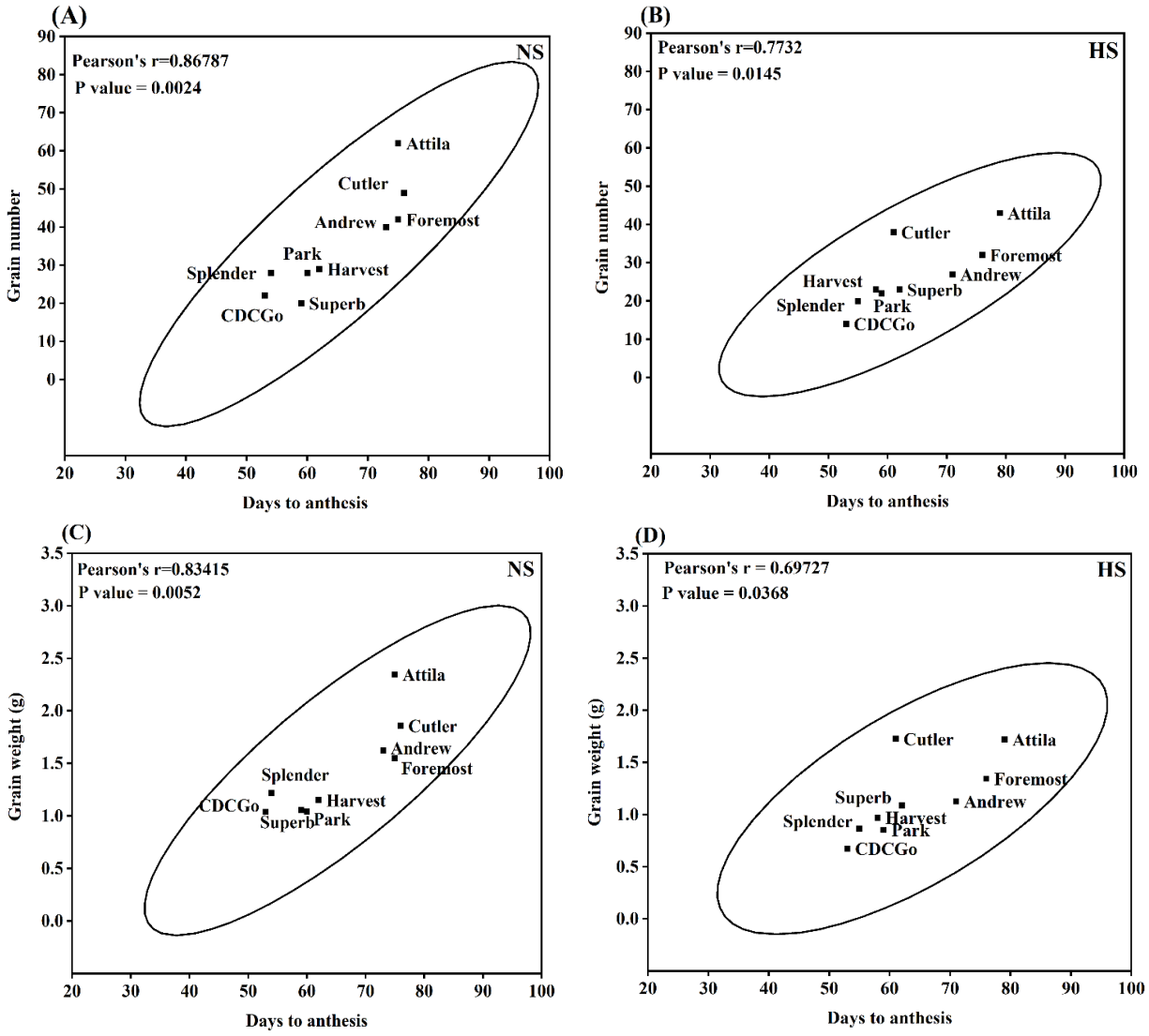

Figure 3.2. Scatter plot correlations of days to anthesis with the number of grains or grain weight on the main tiller spike of nine standard wheat cultivars grown under non-temperature stress (NS; A and C) or heat-stress (HS; B and D) conditions.

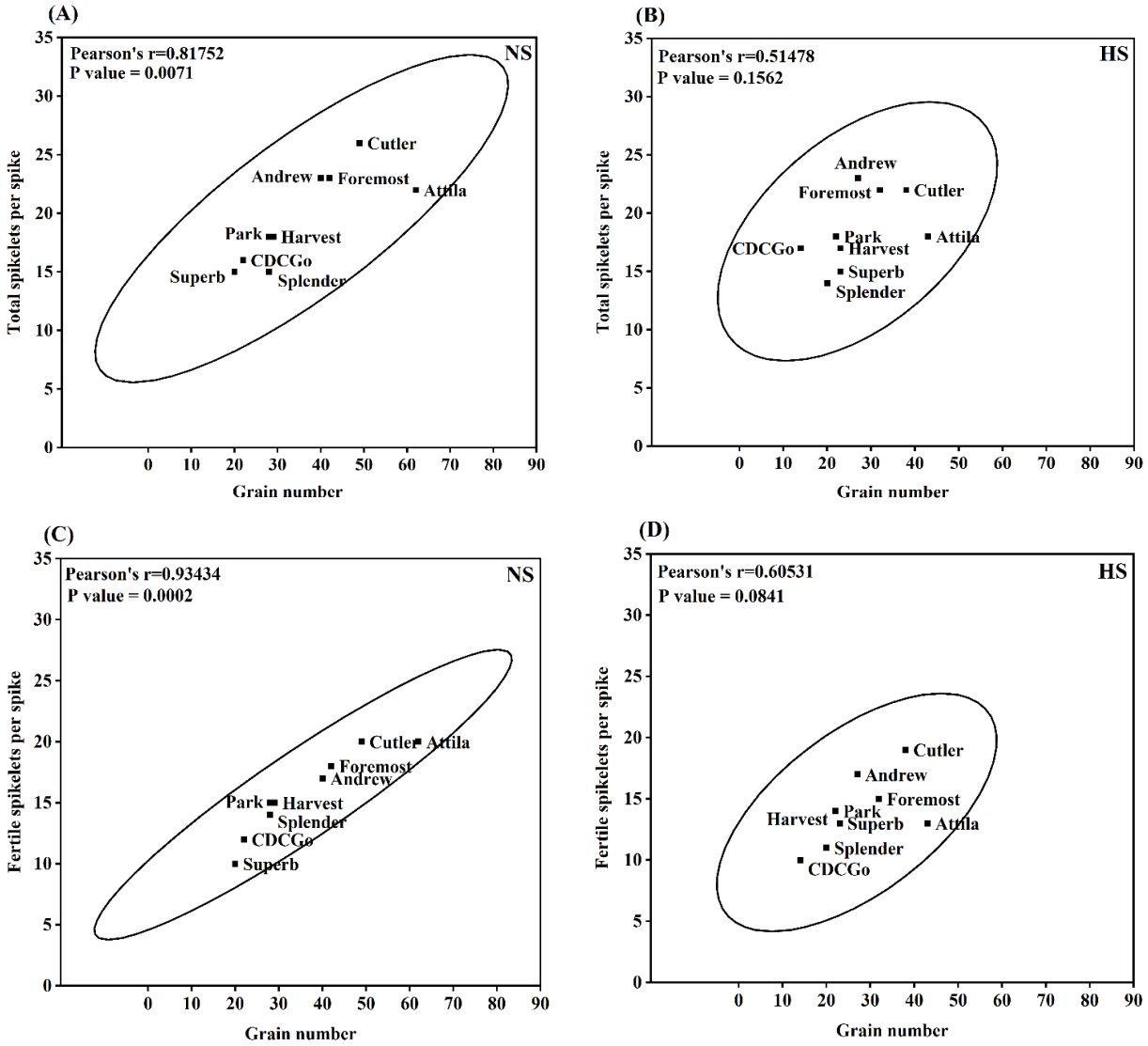

Figure 3.3. Scatter plot correlations of grain number with the number of total or fertile spikelets per spike on the main tiller spike of nine standard wheat cultivars grown under non-temperature stress (NS; A and C) or heat-stress (HS; B and D) conditions.

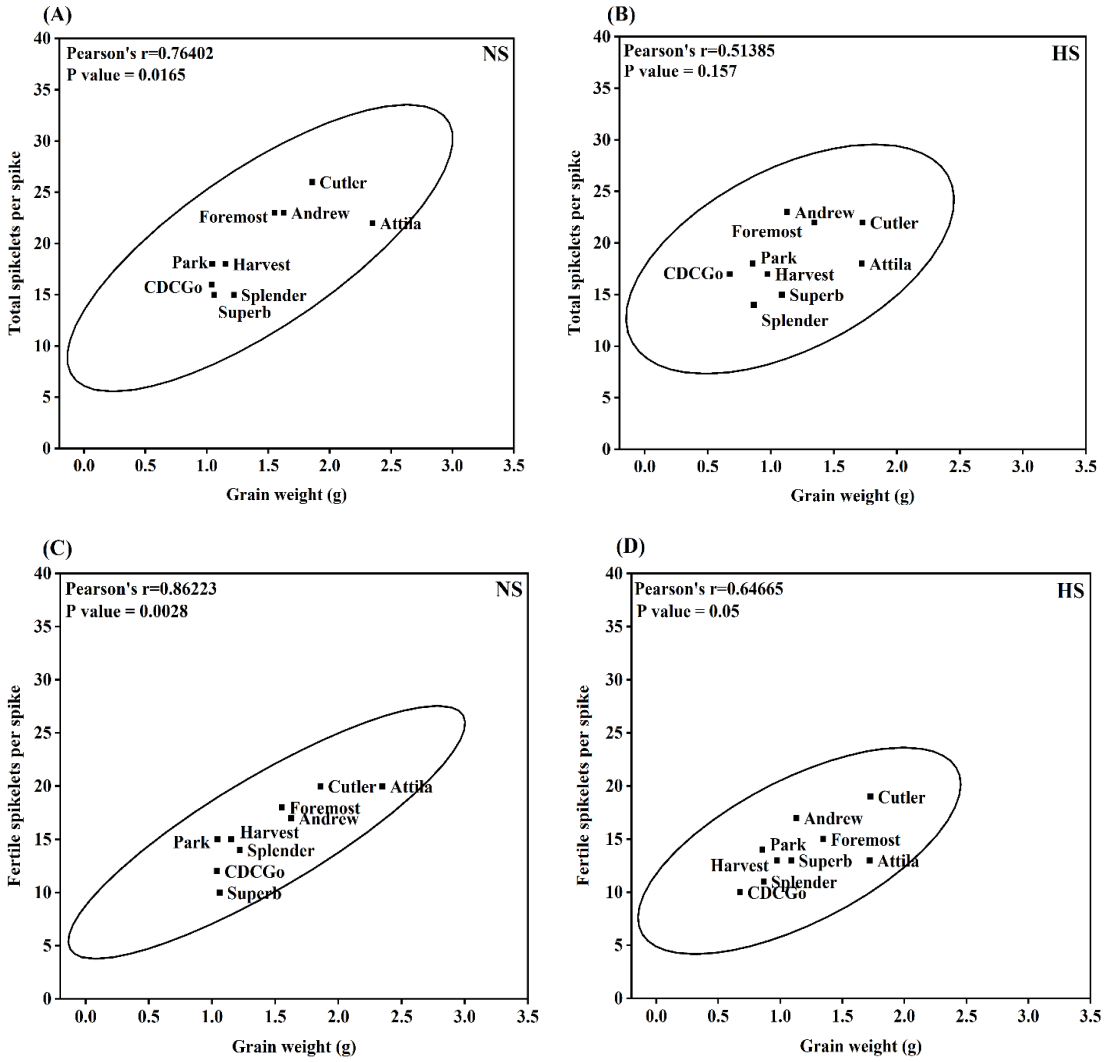

Figure 3.4. Scatter plot correlations of grain weight with the total or fertile spikelets per spike on the main tiller spike of nine standard wheat cultivars grown under non-temperature stress (NS; A and C) or heat-stress (HS; B and D) conditions.

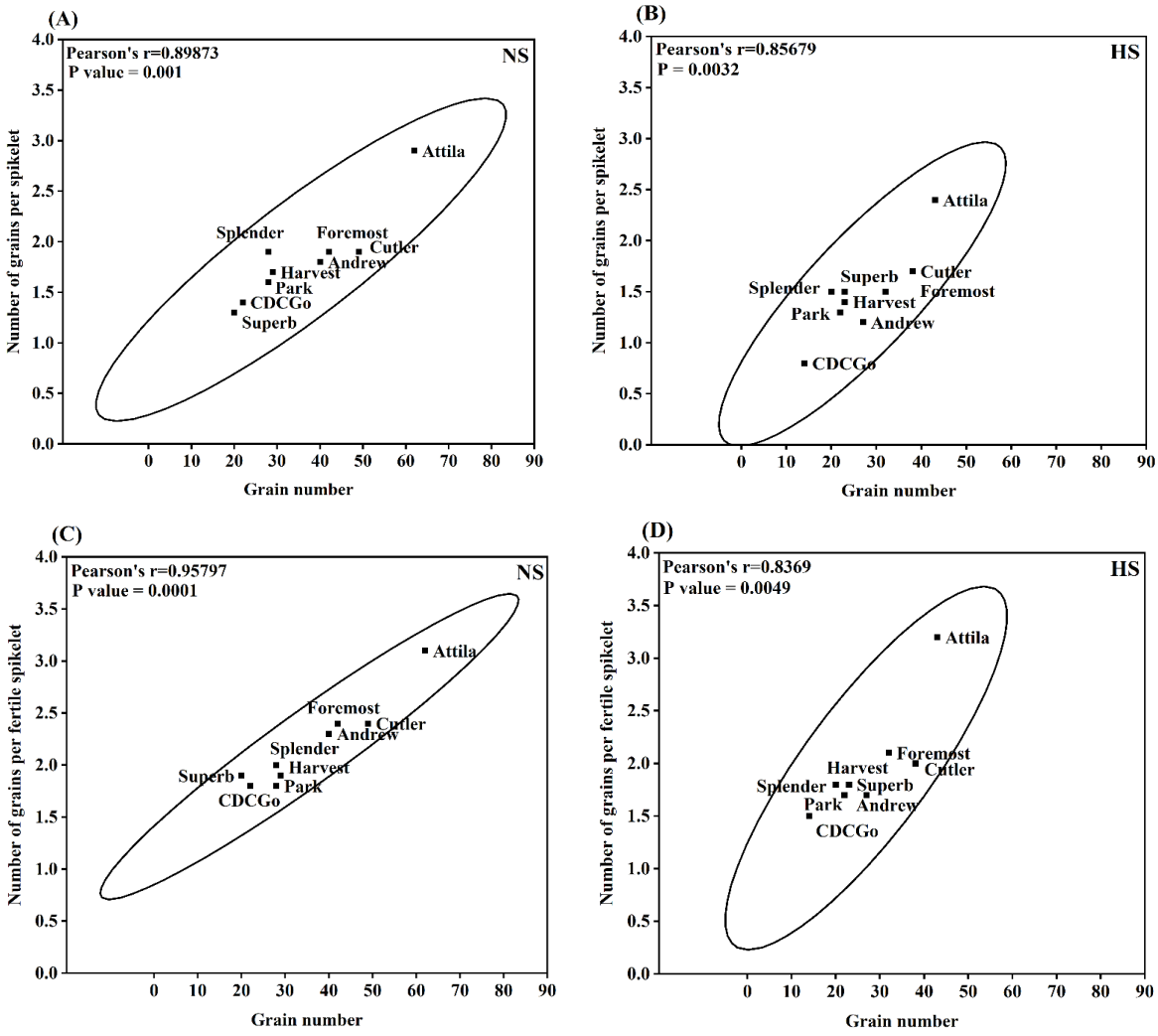

Figure 3.5. Scatter plot correlations of grain number with the number of grains per spikelet or fertile spikelet on the main tiller spike of nine standard wheat cultivars grown under non-temperature stress (NS; A and C) or heat-stress (HS; B and D) conditions.

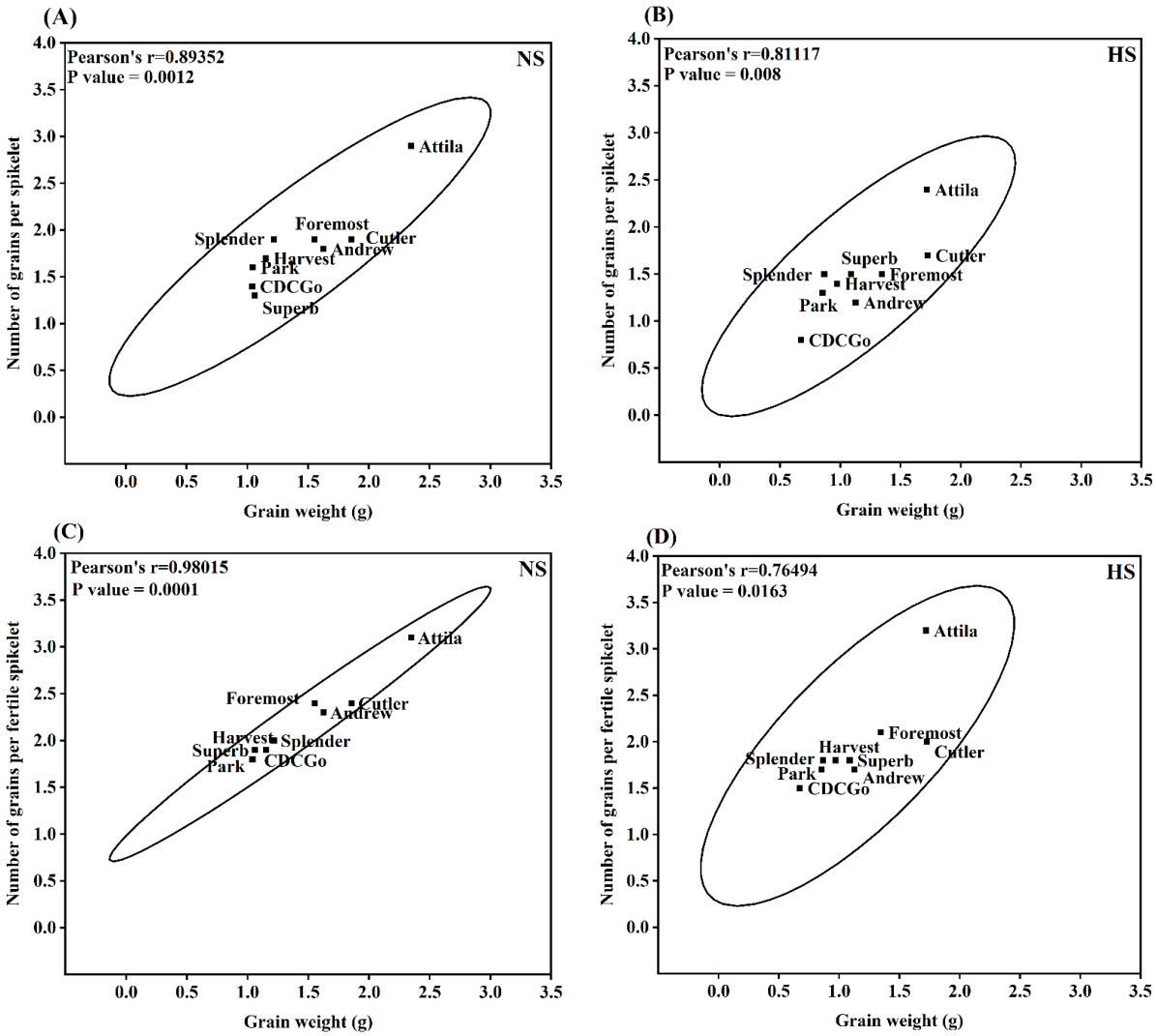

Figure 3.6. Scatter plot correlations of grain weight with the number of grains per spikelet or fertile spikelet on the main tiller spike of nine standard wheat cultivars grown under non-temperature stress (NS; A and C) or heat-stress (HS; B and D) conditions.

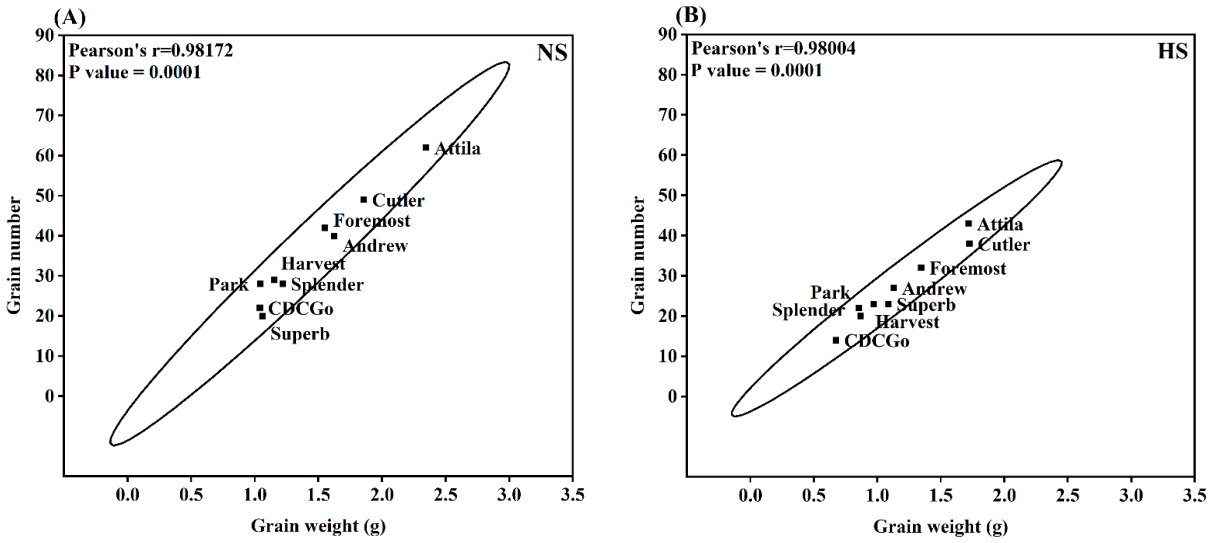

Figure 3.7. Scatter plot correlations of grain number with the grain weight on the main tiller spike of nine standard wheat cultivars grown under non-temperature stress (NS; A) or heat-stress (HS; B) conditions.

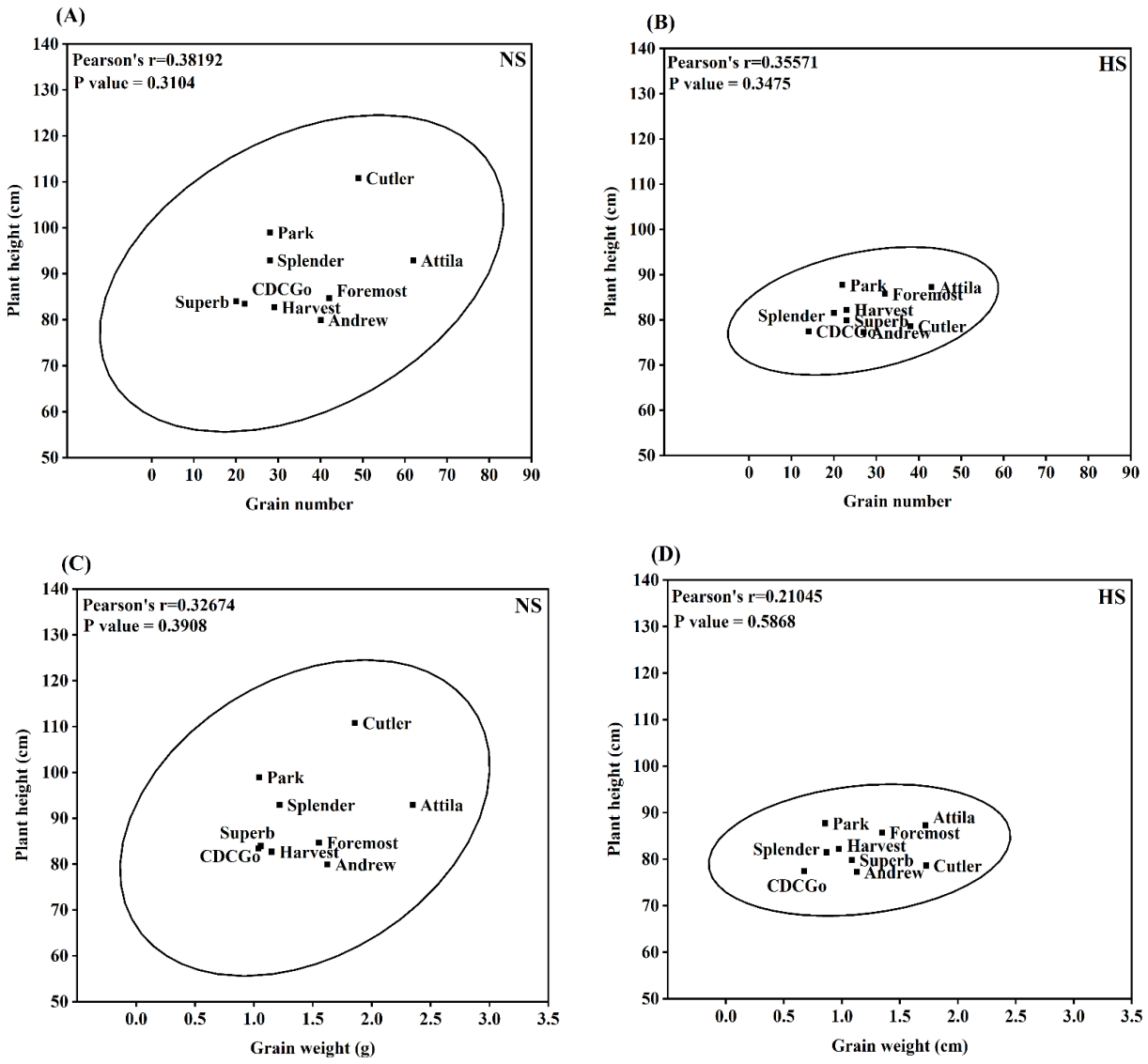

Figure 3.8. Scatter plot correlations of plant height with the grain number or the grain weight of nine standard wheat cultivars grown under non-temperature stress (NS; A and C) or heat-stress (HS; B and D) conditions.

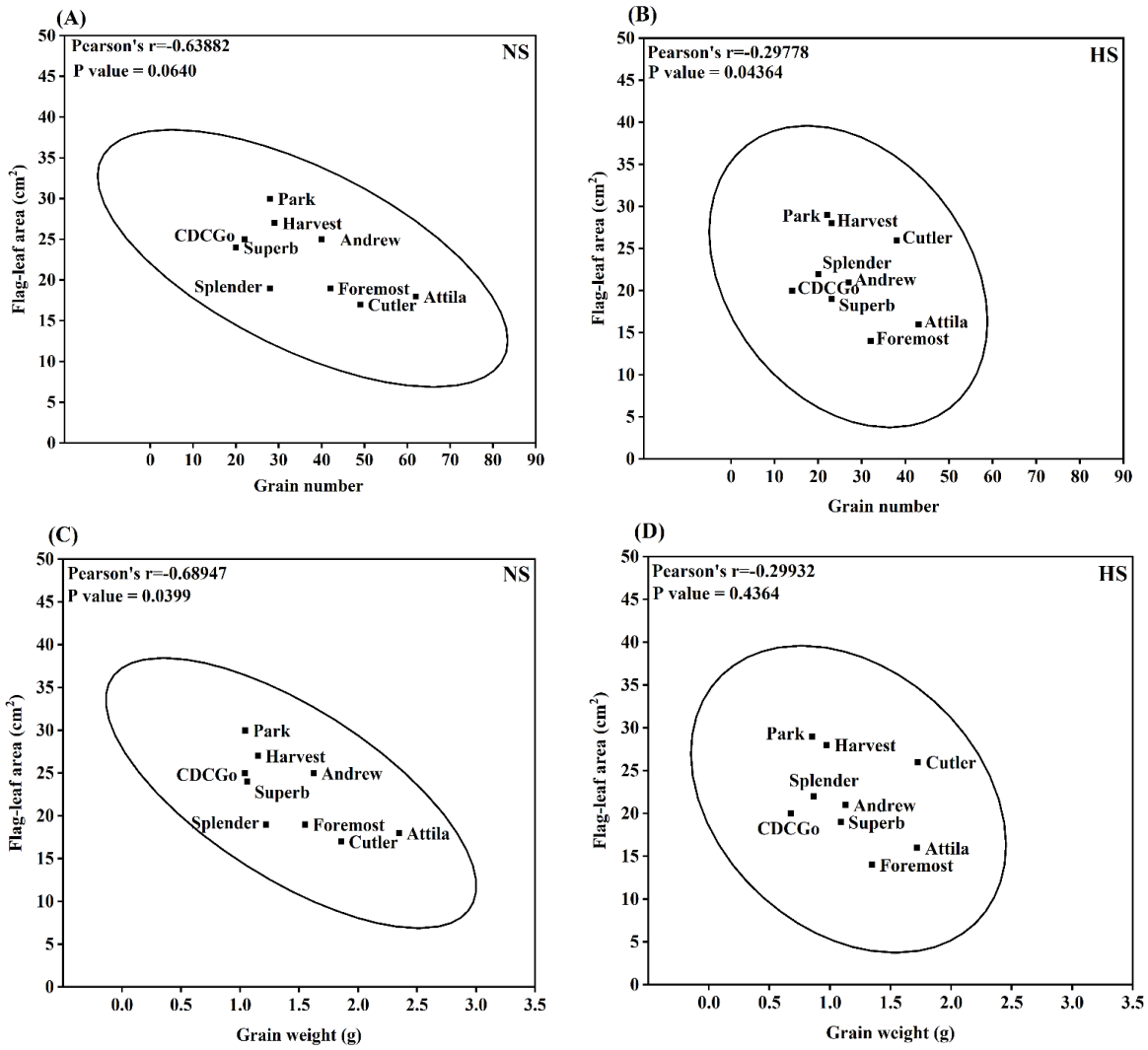

Figure 3.9. Scatter plot correlations of flag leaf area with the grain number or the grain weight of nine standard wheat cultivars grown under non-temperature stress (NS; A and C) or heat-stress (HS; B and D) temperature conditions.

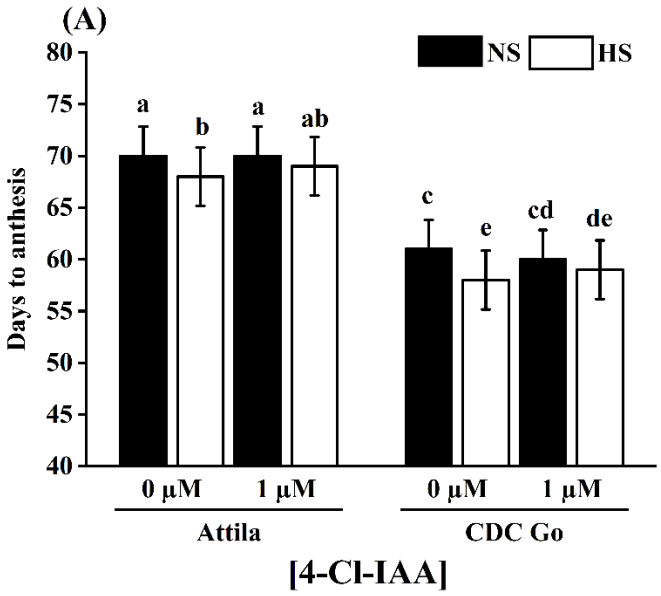

Figure 3.10. Effects of heat stress and 4-Cl-IAA application on days to anthesis of the main tiller spike of 'Attila' and 'CDC Go'. Data are means ± SE, n=40. Different letters indicate significantly different means among treatments and cultivars within each parameter, by the LSD test, at $P \leq 0.05$.

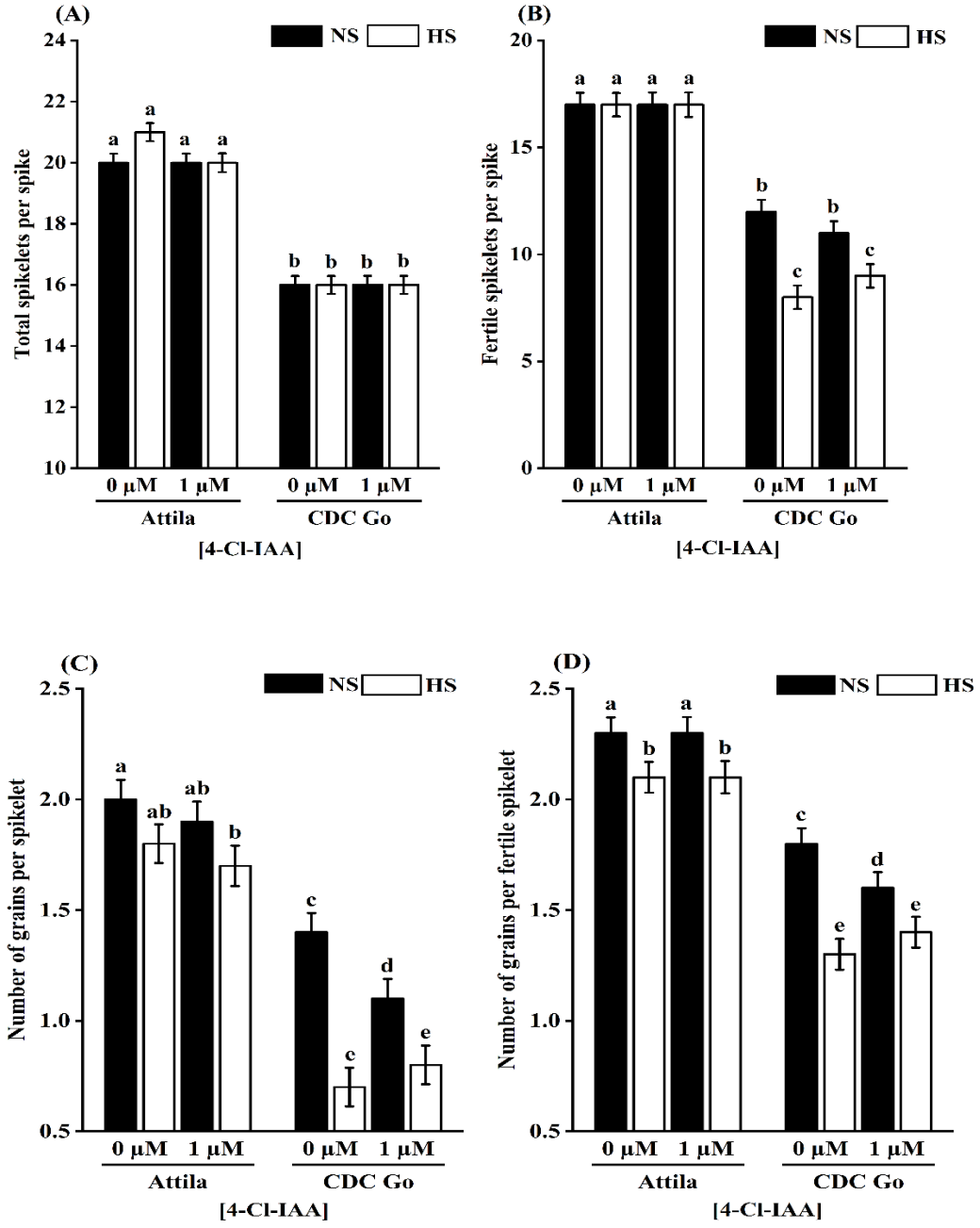

Figure 3.11. Effect of heat stress and 4-Cl-IAA application on (A) total spikelets per spike (B) fertile spikelets per spike (C) number of grains per spikelet and (D) number of grains per fertile spikelet of the main tiller spike of 'Attila' and 'CDC Go'. Data are means ± SE, n=40, Different letters indicate significantly different means among treatments and cultivars within each parameter, by the LSD test, at $P \leq 0.05$.

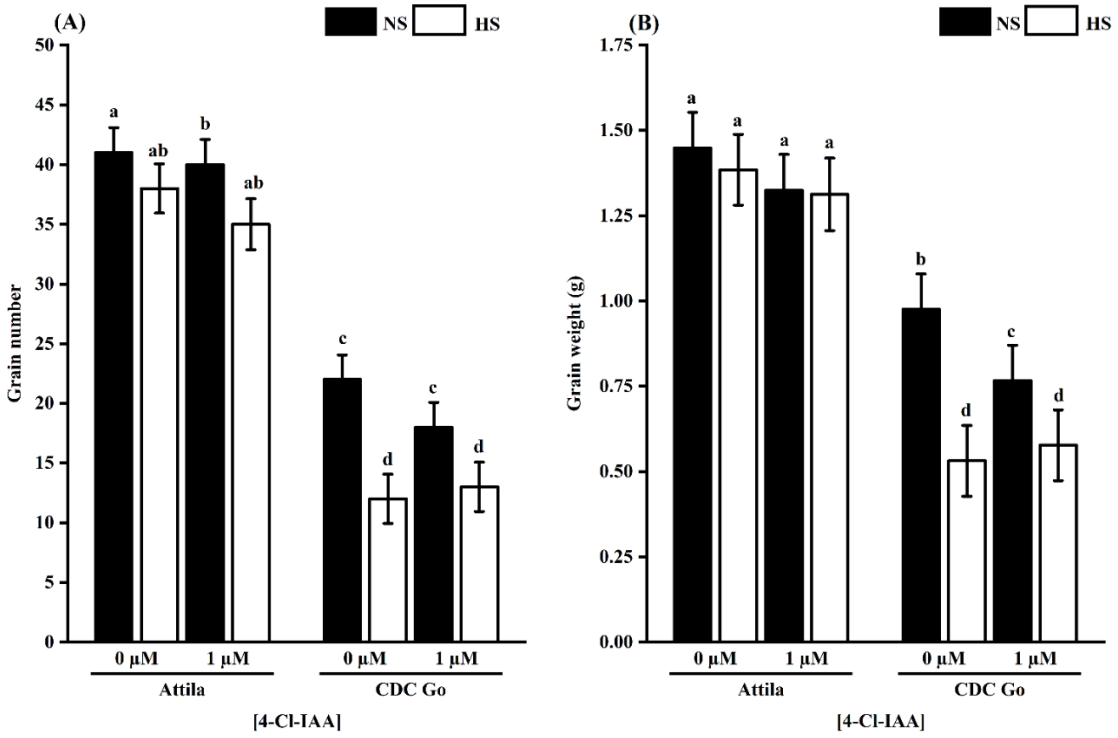

Figure 3.12. Effect of heat stress and 4-Cl-IAA application on (A) grain number and (B) grain weight of the main tiller spike of 'Attila' and 'CDC Go'. Data are means ± SE, n=40, Different letters indicate significantly different means among treatments and cultivars within each parameter, by the LSD test, at $P \leq 0.05$.

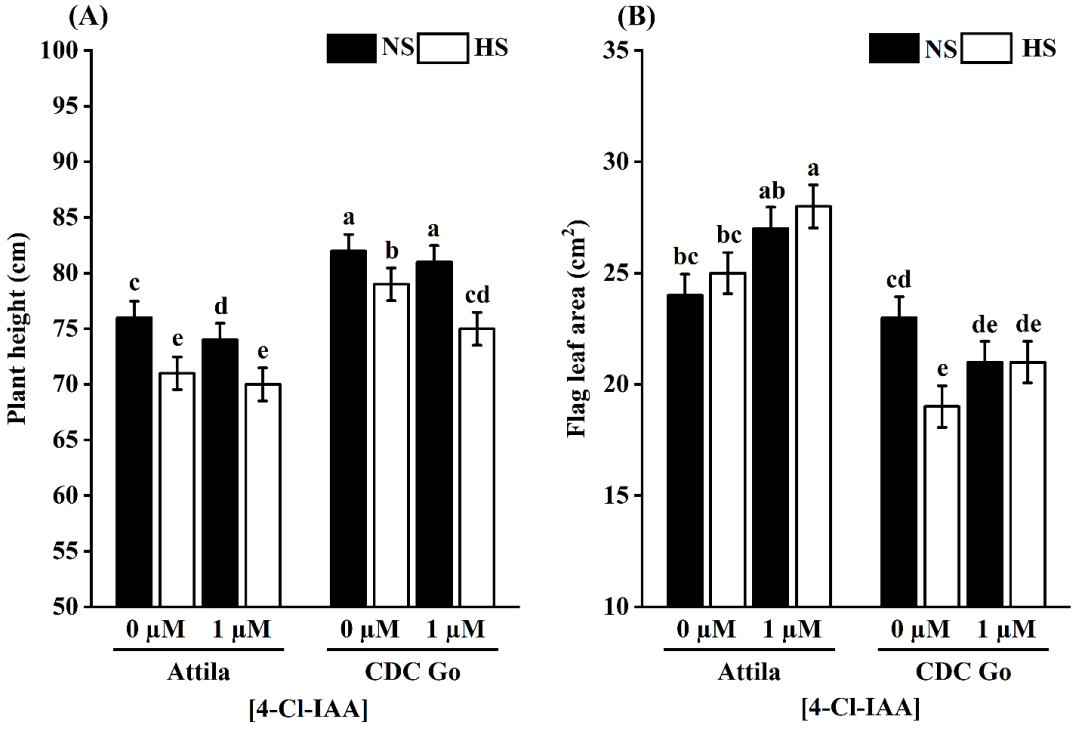

Figure 3.13. Effects of heat stress and 4-Cl-IAA application on (A) plant height and (B) flag-leaf area of the main tiller spike of 'Attila' and 'CDC Go'. Data are means ± SE, n=40. Different letters indicate significantly different means among treatments and cultivars within each parameter, by the LSD test, at $P \leq 0.05$.

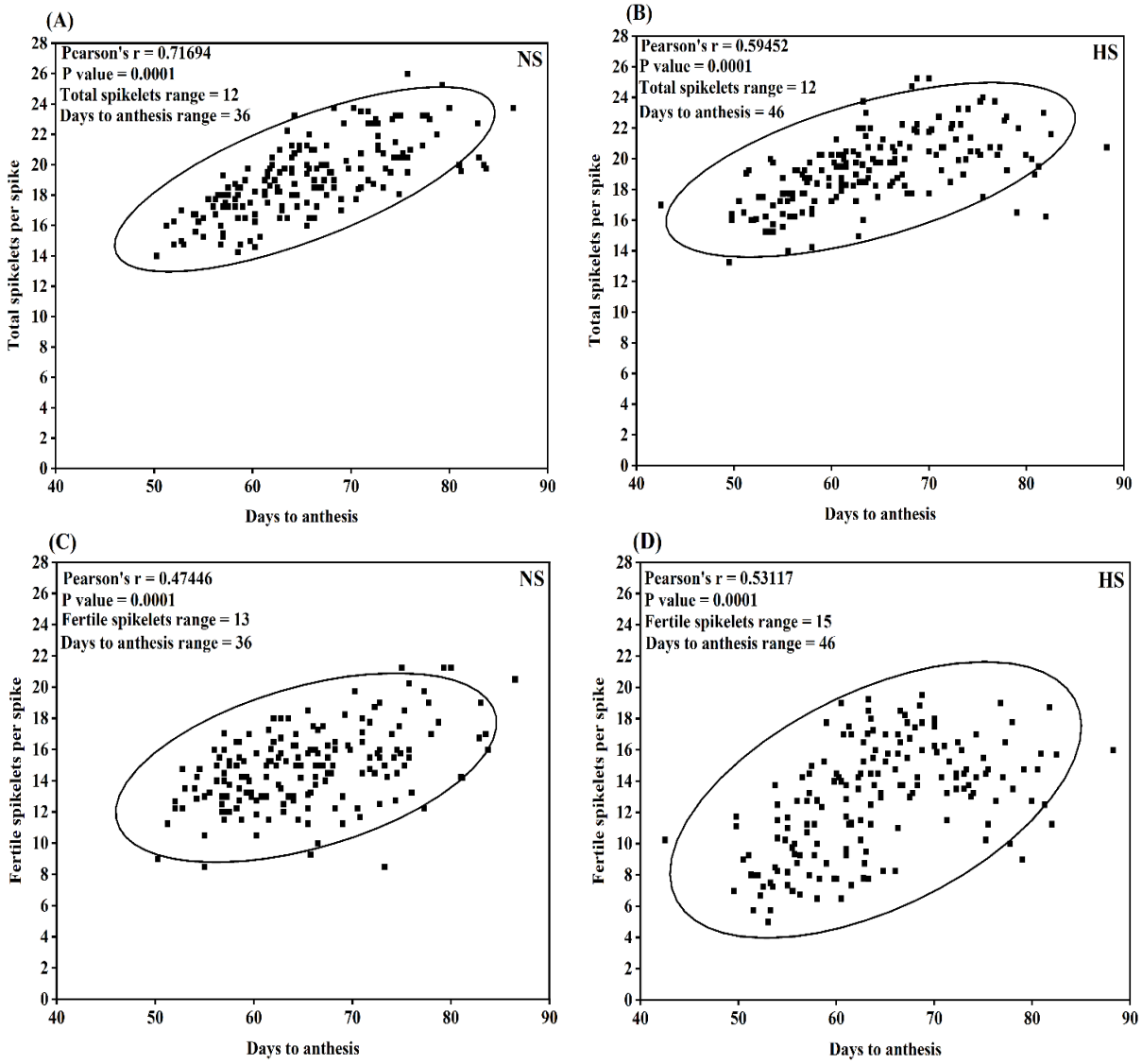

Figure 3.14. Scatter plot correlations of days to anthesis with the number of total or fertile spikelets per spike on the main tiller spike of 'Attila' × 'CDC Go' RIL population grown under non-temperature stress (NS; A and C) and heat-stress (HS; B and D) conditions.

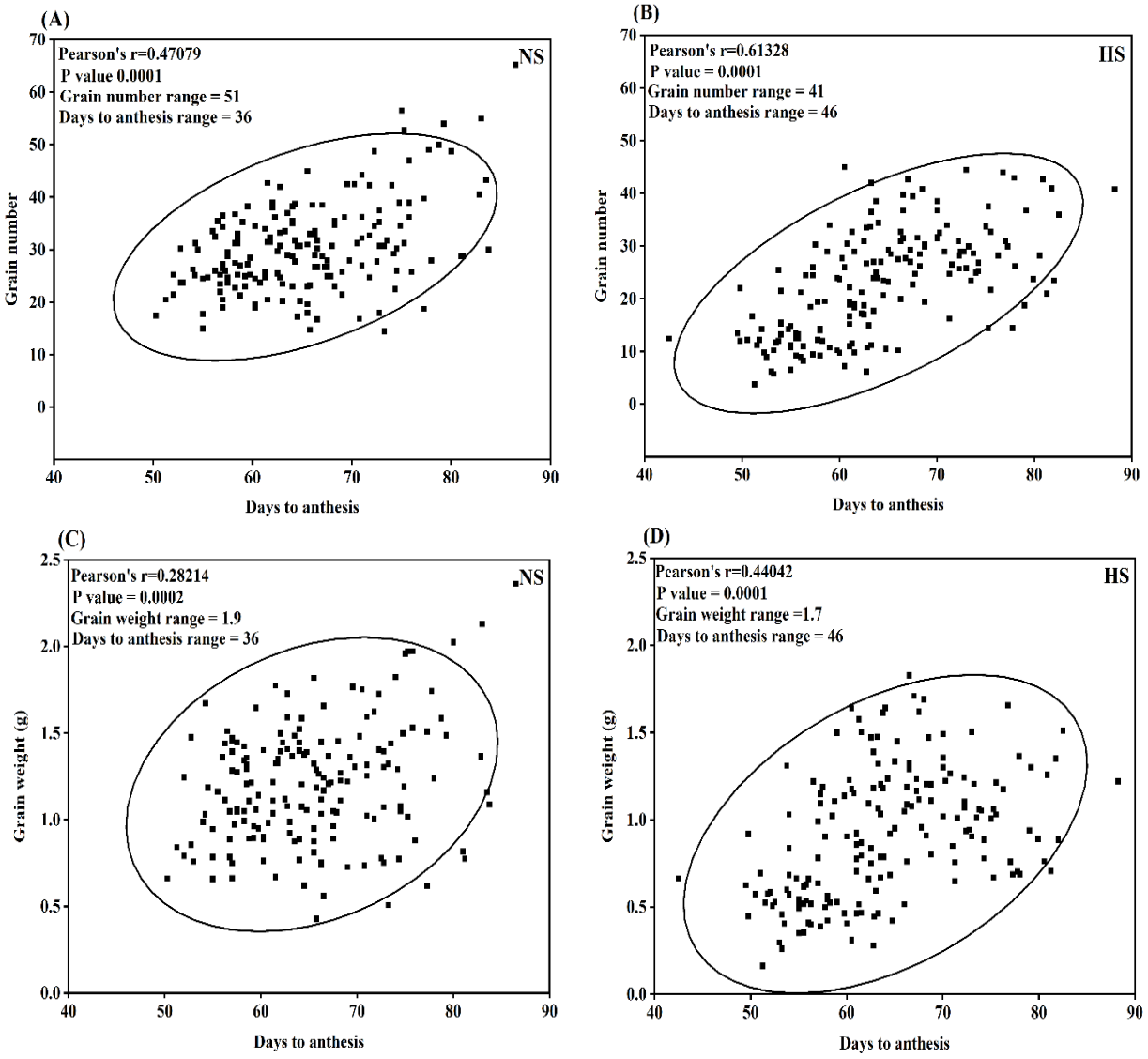

Figure 3.15. Scatter plot correlations of number of days to anthesis with the grain weight or the grain number of the main tiller spike of 'Attila' × 'CDC Go' RIL population grown under non-temperature stress (NS; A and C) and heat-stress (HS; B and D) conditions.

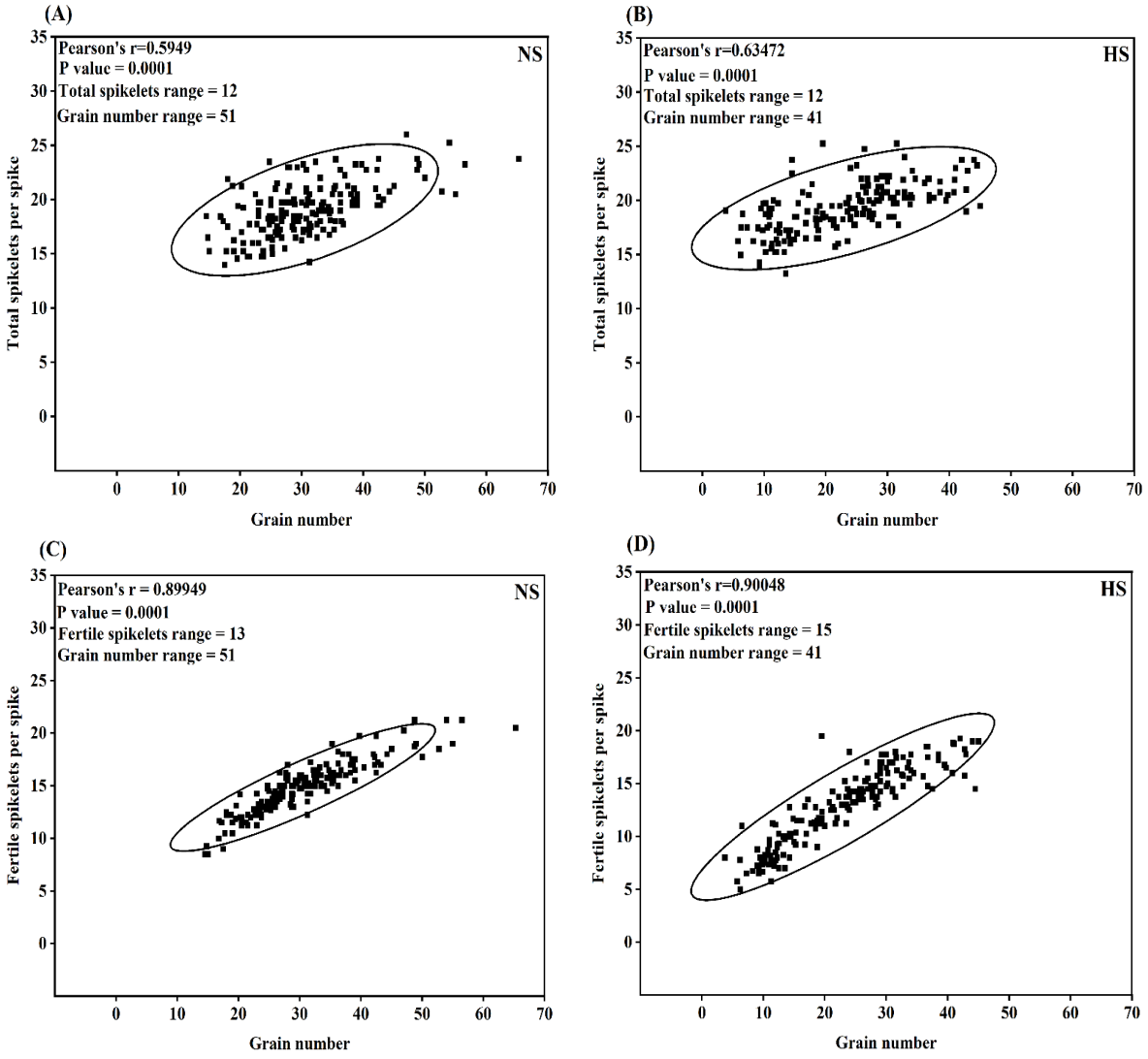

Figure 3.16. Scatter plot correlations of grain number with the number of total or fertile spikelets per spike on the main tiller spike of 'Attila' × 'CDC Go' RIL population grown under non-temperature stress (NS; A and C) and heat-stress (HS; B and D) conditions.

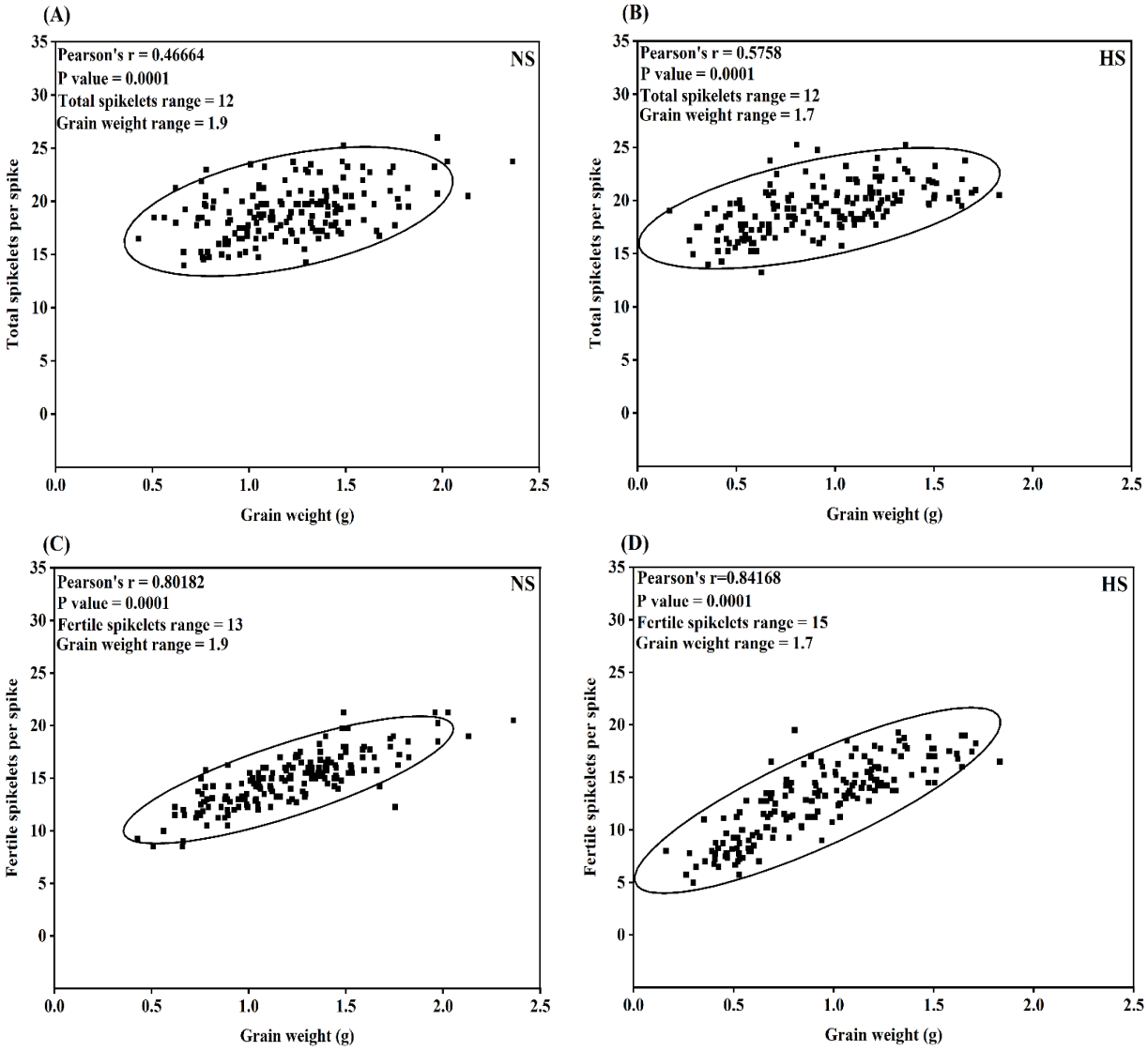

Figure 3.17. Scatter plot correlations of grain weight with the number of total or fertile spikelets per spike on the main tiller spike of 'Attila' × 'CDC Go' RIL population grown under non-temperature stress (NS; A and C) and heat-stress (HS; B and D) conditions.

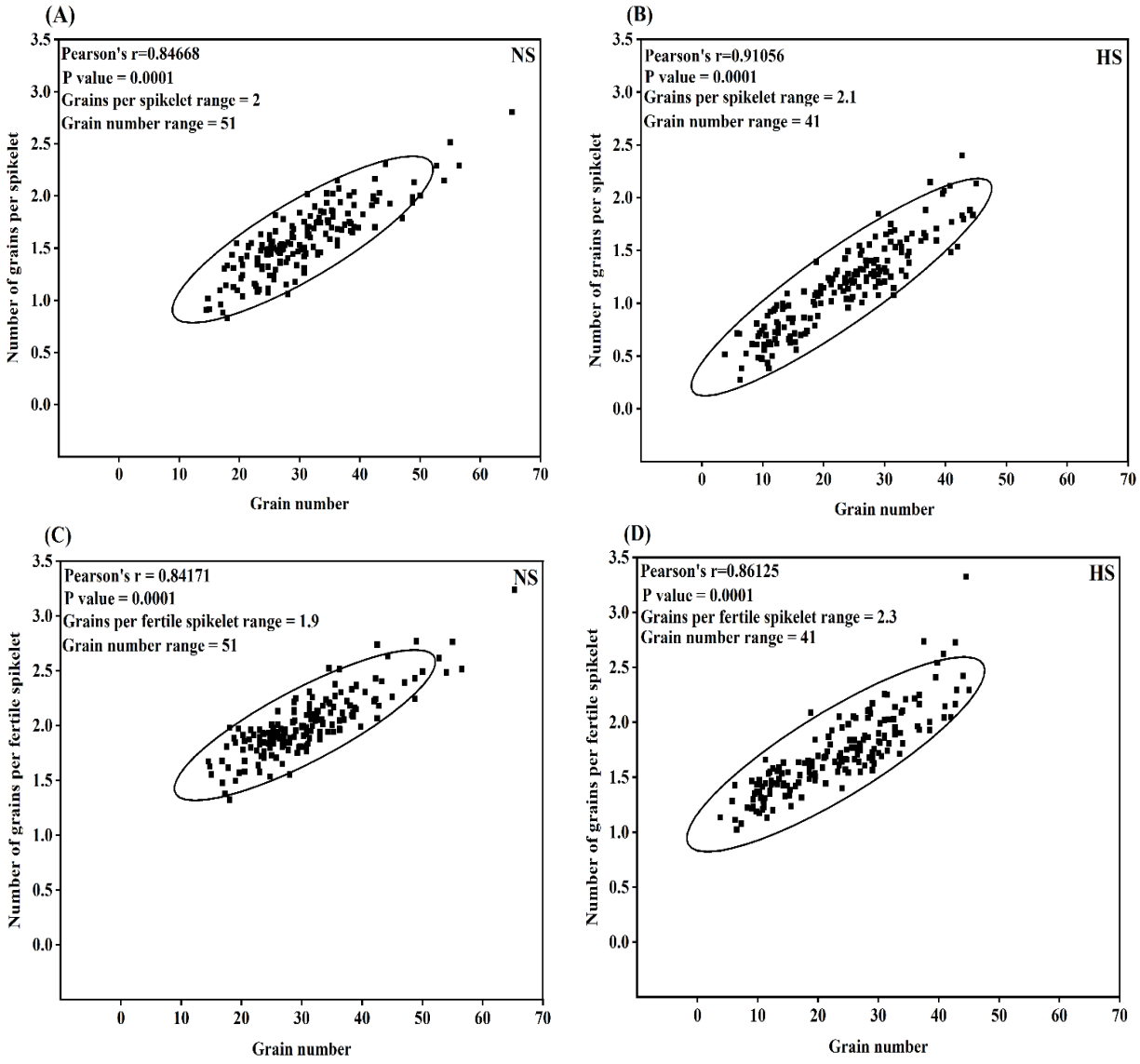

Figure 3.18. Scatter plot correlations of grain number with the number of grains per spikelet or per fertile spikelet on the main tiller spike of 'Attila' × 'CDC Go' RIL population grown under non-temperature stress (NS; A and C) and heat-stress (HS; B and D) conditions.

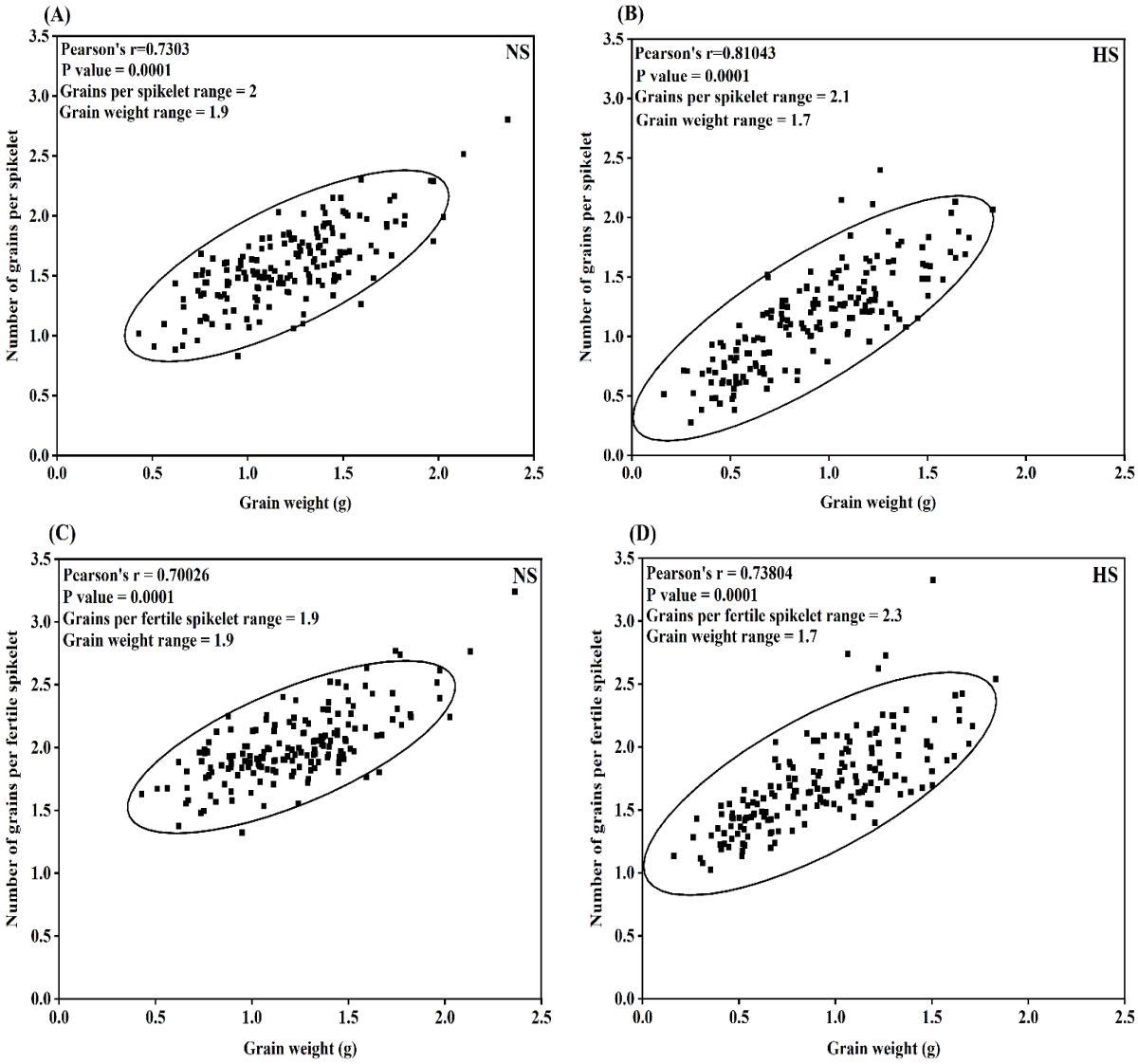

Figure 3.19. Scatter plot correlations of grain weight with the number of grains per spikelet or per fertile spikelet on the main tiller spike of 'Attila' × 'CDC Go' RIL population grown under non-temperature stress (NS; A and C) and heat-stress (HS; B and D) conditions.

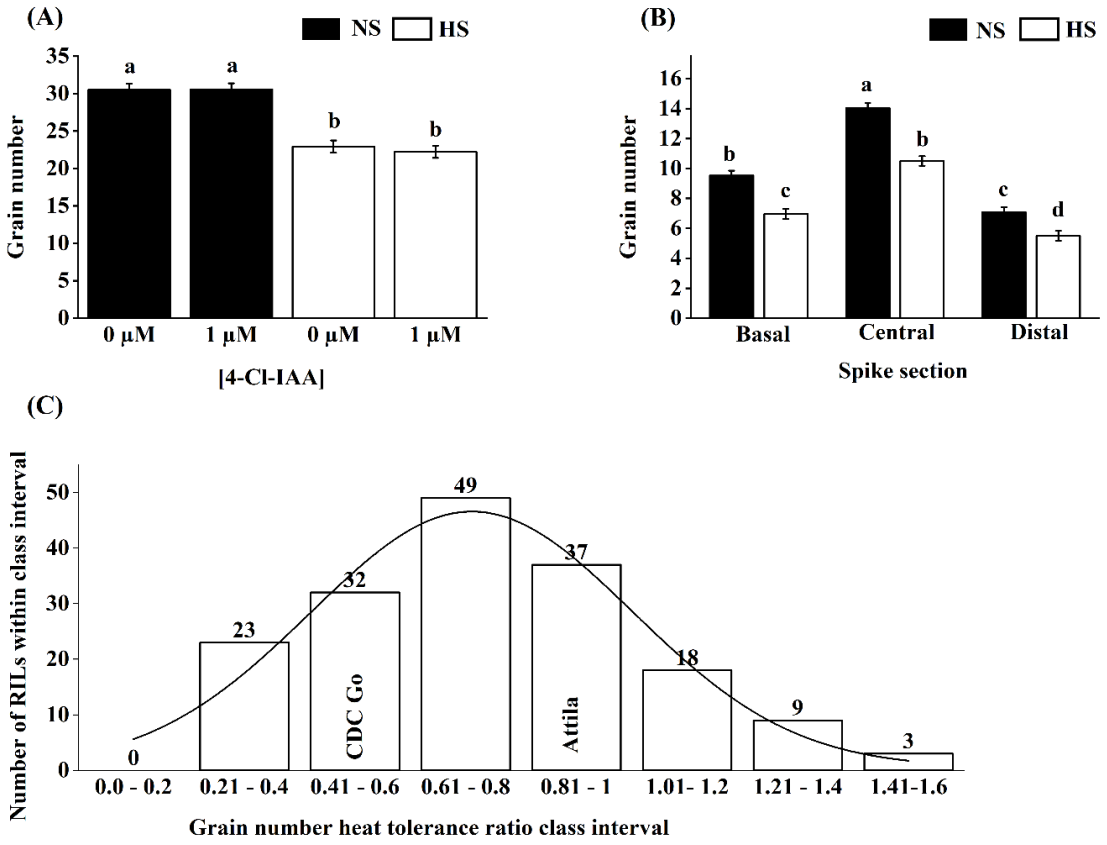

Figure 3.20. The effect of heat stress and 4-Cl-IAA application on the 'Attila' × 'CDC Go' RIL population main tiller grain number mean (A) and the means of the basal, central, and distal spike sections within the main tiller spike (B). Different letters indicate significantly different means among treatments by the LSD test, at $P \leq 0.05$ in A and B.

(C) Frequency distribution of RILs within specific heat tolerance ratio classes for grain number (heat tolerance ratio = mean grain number in the main tiller spike exposed to the heat stress treatment divided by that grown under non-temperature stress conditions). The heat tolerance ratio for grain number was 0.55 for ‘CDC Go’ and 0.93 for ‘Attila’.

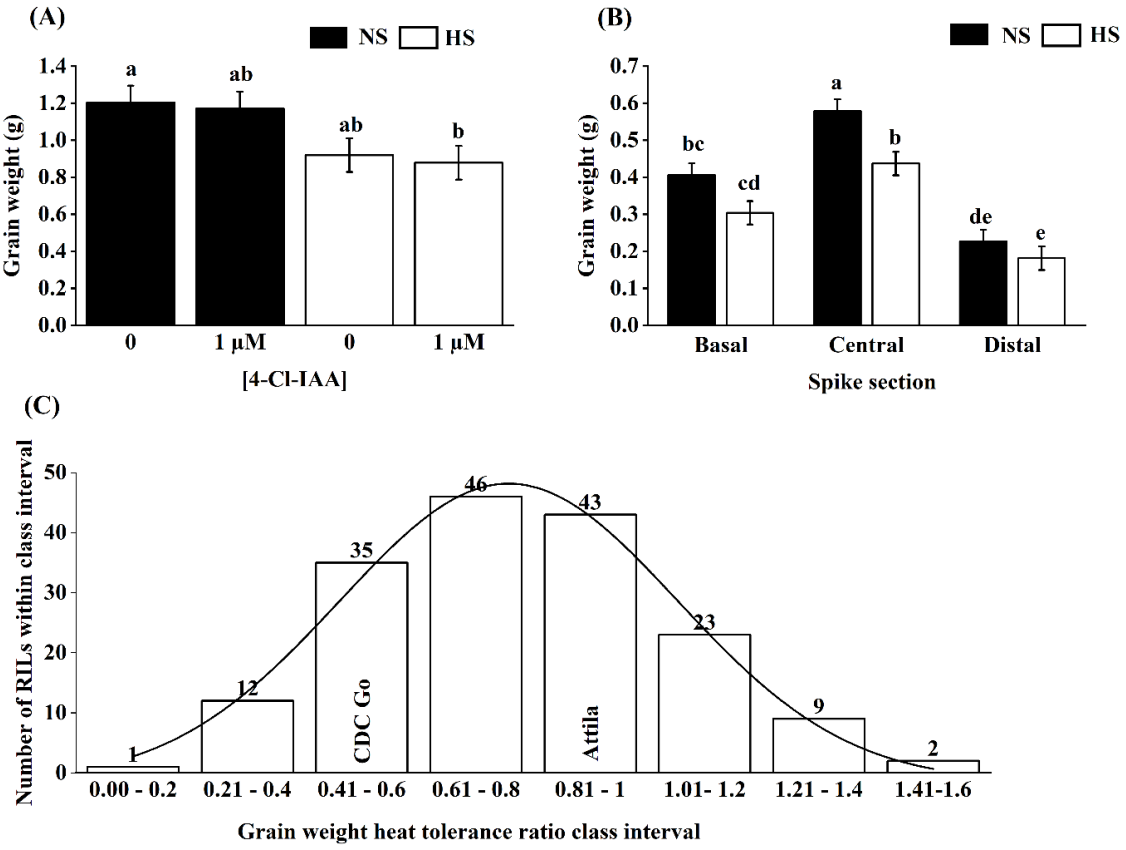

Figure 3.21. The effect of heat stress and 4-Cl-IAA application on the 'Attila' × 'CDC Go' RIL population main tiller grain weight mean (A) and the means of the basal, central, and distal spike sections within the main tiller spike (B). Different letters indicate significantly different means among treatments by the LSD test, at $P \leq 0.05$ in A and B.

(C) Frequency distribution of RILs within specific heat tolerance ratio classes for grain weight (heat tolerance ratio = mean grain weight in the main tiller spike exposed to the heat stress treatment divided by that grown under non-temperature stress conditions). The heat tolerance ratio for grain weight was 0.54 for 'CDC Go' and 0.96 for 'Attila'.

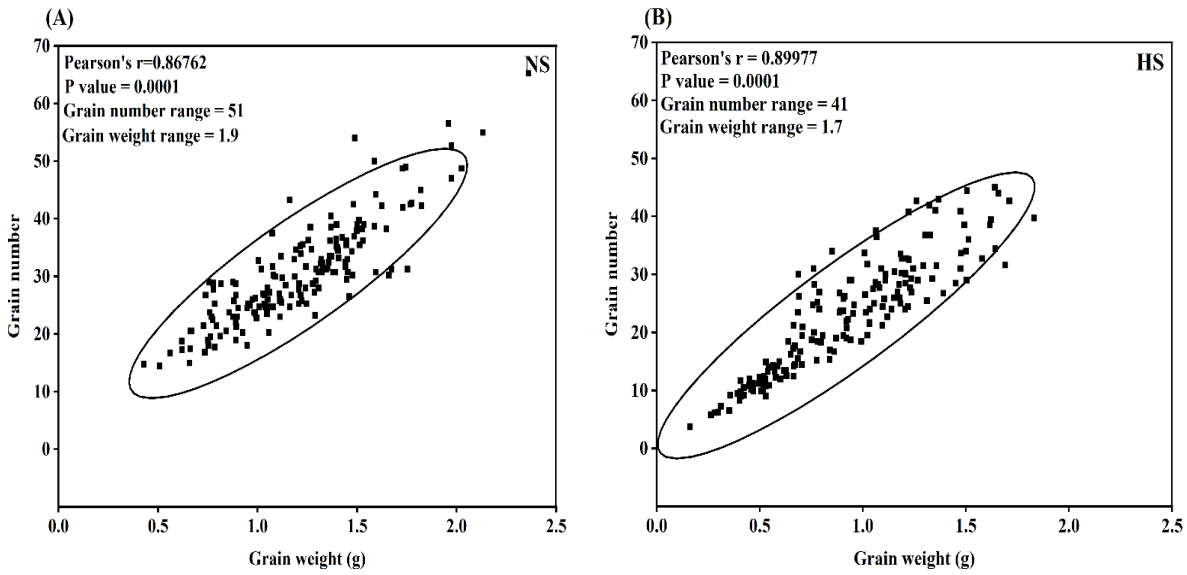

Figure 3.22. Scatter plot correlations of grain number with the grain weight on the main tiller spike of 'Attila' × 'CDC Go' RIL population grown under non-temperature stress (NS; A) and heat-stress (HS; B) conditions.

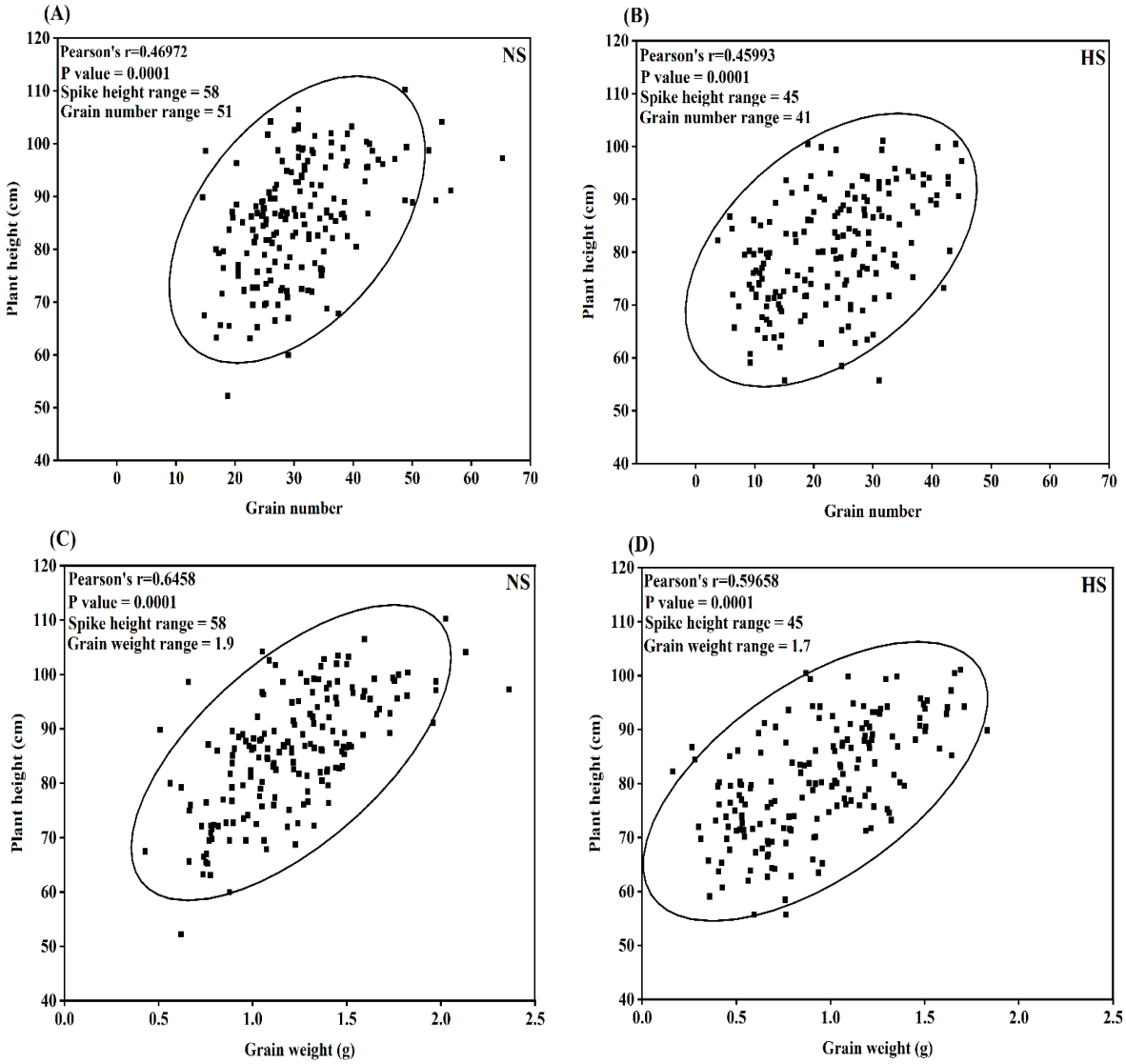

Figure 3.23. Scatter plot correlations of plant height with the grain number or the grain weight of 'Attila' × 'CDC Go' RIL population grown under non-temperature stress (NS; A and C) and heat-stress (HS; B and D) conditions.

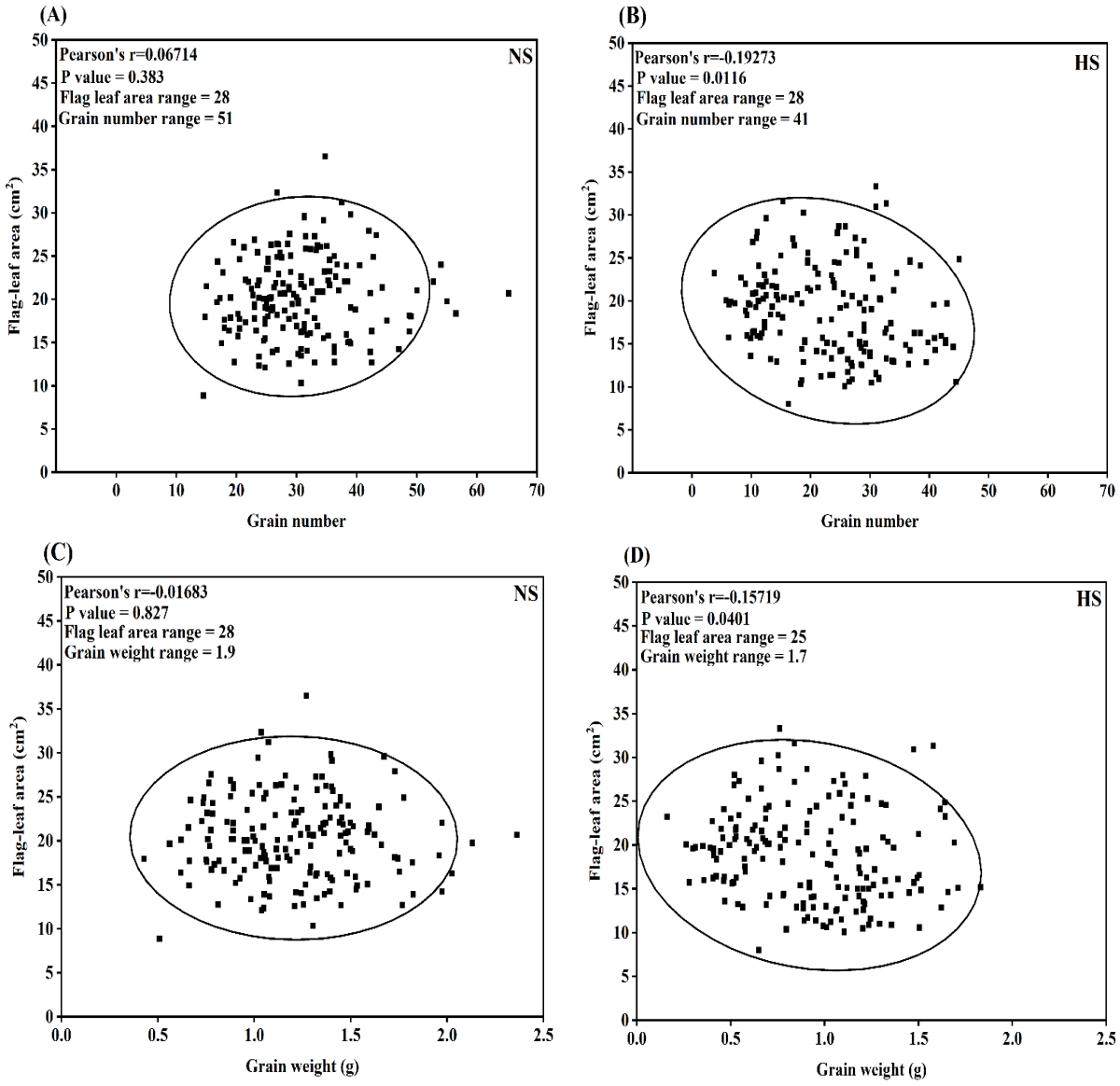

Figure 3.24. Scatter plot correlations of flag-leaf area with the grain number or the grain weight of 'Attila' × 'CDC Go' RIL population grown under non-temperature stress (NS; A and C) or heat-stress (HS; B and D) temperature conditions.

Chapter 4 – QTL associated with yield and yield component parameters in an 'Attila'× 'CDC Go' RIL population assessed under heat-stress and non-temperature stress conditions

4.1 Introduction

Genetic variation in quantitative traits like grain yield, plant height, and days to flowering or maturity is controlled by the collective effects of several genes located at different genomic regions known as Quantitative Trait Loci (QTL; Mackay et al., 2009). Location of QTL can be identified using linked genetic markers and that knowledge can be used as an indirect selection tool in plant breeding (Asins et al., 2009). With the onset of global warming, there is a growing interest to improve understanding of the genetic basis of yield and yield-related traits in wheat associated with heat tolerance and use that knowledge for marker-assisted selection (MAS) of heat-tolerant cultivars (Tahmasebi et al., 2017; Bhusal et al., 2017). The wheat cultivars CDC Go, and Attila showed variation in measured traits under non-temperature-stress and heat-stress conditions with respect to grain yield (see chapters 2 and 3). Variation of heat tolerance in the 'Attila' × 'CDC Go' RIL population was high with the RILs falling into three groups, heat-resistant (45%), moderately heat-susceptible (20.5 %), and highly heat-susceptible (7.6%), making the 'Attila' × 'CDC Go' RIL population useful for detection of QTL for heat tolerance. To this end, we used 'Attila' × 'CDC Go' RIL population to identify the genomic regions (QTL) associated with trait variation for heat tolerance with respect to grain yield in wheat.

The information from this study will help to identify 1) minor, moderate or major effect QTL associated with grain yield and yield component parameters specific to, or common with, non-temperature stress and/or heat-stress conditions at flowering 2) QTL clusters associated with two or more traits and their impact on phenotypes, and 3) temperature-based behavioral changes detected in QTL detected under both non-temperature-stress and heat-stress conditions at flowering.

4.2 Materials and methods

4.2.1 Plant materials

The analysis presented in this chapter is derived from the non-temperature stress and heat-stress treatments receiving the control treatment solution (aqueous 0.25% (v/v) Agral surfactant)

that are described in chapter 3. The analysis includes data from 167 RILs out of 171 RILs in the 'Attila' × 'CDC Go' RIL population (RIL numbers 14, 16, 135, and 158 were removed from the analyses due to the absence of genotypic data) which, were used to detect QTL under non-temperature stress and heat-stress conditions.

4.2.2 Plant growth conditions and phenotyping

In brief, seeds of the 'Attila' × 'CDC Go' F_6 RIL population were planted in root trainer blocks (see chapter 3 for more details) and grown under growth chamber conditions (19°C light/17°C dark) at the University of Alberta, Edmonton, Alberta with a 16 h photoperiod and an average photon flux density of 370 µmol $m^{-2}s^{-1}$ using 54 W/835/HO high fluorescent bulbs (Phillips, Holland). The two temperature treatments [non-temperature stress and heat-stress treatments receiving the control treatment solution (aqueous 0.25% (v/v) Agral surfactant)] used for chapter 4 analyses were arranged in a randomized complete block design and replicated 4 times (in one experimental replication each treatment consisted of 1 plant from each RIL).

For the heat-stress treatment, plants were moved to a growth chamber maintained at 35°C for 6 h per day for six days (see chapter 3 for more details) when the majority of plants were at the booting-flowering stage (BBCH 37 [flag-leaf initiation] – 49 [1st awns visible]); Lancashire et al., 1991). The photoperiod was 16 h light/8 h dark at an average photon flux density of 370 µmol $m^{-2}s^{-1}$ using 54 W/835/HO high fluorescent bulbs (Phillips, Holland). After six days, the heat stress-treated plants were returned to the original growth chamber and maintained under controlled temperature conditions until maturity. The non-temperature stress control plants remained in the original growth chamber for the entire length of the experiment.

Data were collected for the floral spike of the main tiller and subsequent tillers were removed as they developed (when tillers were 5-10 cm tall). Floral spikes harvest and drying was carried out as described in chapter two. The following plant growth parameters were taken on the main tiller spike as described in chapter two: number of days to anthesis, flag leaf length, flag-leaf area, plant height, grain weight and number per spike and per spike section, and the number of fertile spikelets per spike and per spike section.

4.2.3 DNA extraction, genotyping, and linkage map construction

DNA extraction, genotyping, and linkage map construction for the 'Attila' × 'CDC Go' RIL population was completed by the wheat breeding group, University of Alberta, Edmonton, Canada (Zou et al., 2017a and b). In brief, genomic DNA samples of 167 RILs were extracted from three weeks old seedlings using a modified Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle & Doyle, 1987). The DNA concentration was measured using a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, USA) and normalized to approximately 100 ng/µL. DNA samples were genotyped at the University of Saskatchewan, Wheat Genomics lab, Saskatoon, Canada, with the Wheat 90K Illumina iSelect SNP array that consisted of 81,587 SNPs (Wang et al., 2014). SNP alleles were identified with Illumina Genome Studio Polyploid Clustering V1.0 (Illumina, San Diego, USA) using default clustering parameters and filtered as described by Perez-Lara et al. (2016). In addition, the RILs and the two parents were also genotyped with gene specific markers of the photoperiod response (*Ppd-D1*; Beales et al., 2007), vernalization response (*Vrn-A1*; Chen et al., 2013) and height-reduction (*Rht-B1*; Ellis et al., 2002) genes at the Agricultural Genomics and Proteomics Lab, University of Alberta, Edmonton, Canada, as described by Perez-Lara et al. (2016).

For linkage map construction, all SNPs that were monomorphic between the two parents and those with >20% missing data were excluded, and the remaining SNPs and the three gene specific markers were used (Perez-Lara et al., 2016). Linkage groups were assigned to individual chromosomes based on existing high density SNP maps of wheat (Cavanagh et al., 2013; Maccaferri et al., 2014; Wang et al., 2014; by Perez-Lara et al., 2016).

4.2.4 Statistical and QTL analyses

Data analyses were performed using PROC UNIVARIATE, PROC MIXED and PROC IML procedures of SAS 9.3 (SAS Institute Inc. Cary, NC, USA, 2010). For each data parameter, normality and homogeneity of variance were tested using Shapiro-Wilk and Levene's tests, respectively. Statistical significance of the data for heat-stress treatments for the number of grains per spike, grain weight per spike, number of fertile spikelets per spike, days to anthesis, flag-leaf length, flag-leaf area, and plant height were determined using 2-factor factorial analyses of variance (ANOVA; 167 RILs × 2 temperature treatments).

The number of fertile spikelets, grain number and weight were further analyzed within three sections of the main tiller spike (basal, central, and distal) using a 3-factor ANOVA (167 RILs x 2 temperature treatments × 3 sections per spike). Least square means in each trait were tested for normality using PROC UNIVARIATE analyses. Heritability for each trait were estimated using PROC MIXED and PROC IML procedures (https://github.com/ncsumaize/Heritability_SAS). In all analyses, mean separations were conducted using the least significant difference (LSD) test and statistical significance was declared at $P \leq 0.05$.

For QTL detection, Inclusive Composite Interval Mapping (ICIM) was performed on the least squares means of heat stressed and non-temperature stressed traits separately using BIP function (QTL mapping in biparental populations) in QTL IciMapping v4.2 (Li et al., 2007; Meng et al., 2015). For QTL mapping parameters, missing data points were replaced by the trait mean values, and the following parameters were used: 1 cM walking distance, minimum logarithm of odds (LOD) score of 2.5, and the ICIM-ADD model for determining the additive effects at individual QTL. QTL names were designated following the International Rules of Genetic Nomenclature (http://wheat.pw.usda.gov/ggpages/wgc/98/Intro.htm), which consists of the letter 'Q' (symbol for QTL) followed by the trait designator, a period, a laboratory designator (jods; Jocelyn Ozga and Dean Spaner), a hyphen (-), the symbol for the chromosome in, which the QTL is located, and if necessary a period and an Arabic numerical to differentiate QTL identified for the same trait on the same chromosome. Adjacent QTL on the same chromosome for the same trait was considered as different when the confidence intervals between them were not overlapping as defined by Onyemaobi et al. (2018). Genomic region(s) on the same chromosome (same or overlapping confidence intervals) associated with two or more different traits were classified as QTL clusters and only the most important clusters were mentioned.

In this study, the QTL proportional contribution of phenotypic variance (PVE%) was defined as minor (<10%), moderate (10-20%), or major (>20%) as previously defined by Perez-Lara et al. (2016). QTL graphs were drawn using MapChart v2.1 (Voorrips, 2002). Mean differences of RILs carrying the 'Attila' type alleles and the 'CDC Go' type alleles in each QTL was tested using t-tests and statistical significance was declared at $P \leq 0.05$.

4.3 Results

4.3.1 Plant and yield traits under non-temperature stress and heat-stress conditions in the wheat cultivars 'Attila', 'CDC Go' and the 'Attila' × 'CDC Go' RIL population

'Attila' and 'CDC Go' data were presented in detail in chapter 3. In brief, for the main tiller spike, 'Attila' produced 33% higher grain weight, 46% higher grain number, 29% higher number of fertile spikelets, 6 cm shorter tillers, 3 cm shorter flag-leaves, similar flag-leaf area, and anthesis was 9 days later, as compared to 'CDC Go' under non-temperature stress conditions (Table 4.1). 'Attila' performed better with respect to grain yield than 'CDC Go' under heat stress. The main tiller spike of 'Atilla' produced 62% higher grain weight, 68% higher grain number, 53% higher number of fertile spikelets per spike, 8 cm shorter tillers; similar flag-leaf area, and reached anthesis 10 days later, compared to 'CDC Go' under heat stress (Table 4.1).

In the 'Attila' × 'CDC Go' RIL population, the population mean of grain weight, grain number, number of fertile spikelets, and days to anthesis were lower than 'Attila' and higher than 'CDC Go' under heat-stress and non-temperature stress conditions (Table 4.1). Population mean plant height was higher than both 'Attila' and 'CDC Go' under heat-stress and non-temperature stress conditions (Table 4.1). Population mean flag-leaf length and flag-leaf area were slightly different or similar either to 'Attila' or 'CDC Go' trait means under heat-stress and non-temperature stress conditions (Table 4.1). However, at the individual RILs level, all measured traits exhibited transgressive segregation (generation of phenotypes more extreme than parents) except in fertile spikelets at the spike section level (Table 4.1). Heat stress reduced the population means of measured traits except in the days to anthesis and flag-leaf area parameters (Table 4.1). Heat-stress-induced population mean grain weight reduction was observed in all three spike sections and population mean grain number reduction was observed mainly in the basal and central spike sections (Table 4.1). Under heat-stress conditions, the RIL population range of grain number (NS, 38; HS, 41), fertile spikelets per spike (NS,12; HS,15), sterile spikelets per spike (NS, 7; HS, 13), and days to anthesis (NS, 37; HS, 45) was increased, and the range of grain weight (NS, 1.9337; HS, 1.668), plant height (NS, 50; HS, 45), flag-leaf length (NS, 21; HS, 18), and flag-leaf area (NS, 28; HS; 24) was decreased (Table 4.1).

The phenotypic distribution of least square means for all traits measured under heat-stress and non-temperature stress conditions in the RIL population were normal under the Kolmogorov-Smirnov test (data not presented), except for number of spikelets per spike and within the spike

sections, so these data were removed from the QTL analyses. At the whole spike level, broad sense heritability (proportion of total phenotypic variation due to all genetic effects) varied from 0.33 for number of fertile spikelets per spike to 0.63 for days to anthesis under non-temperature stress conditions, and from 0.35 for number of fertile spikelets per spike to 0.7 for plant height under heat-stress conditions (Table 4.2). Within the spike, broad sense heritability varied from 0.23 for basal fertile spikelets to 0.38 for central fertile spikelets under non-temperature stress conditions, and from 0.23 for basal fertile spikelets to 0.47 for central grain number under heat-stress conditions (Table 4.2). Two factor factorial analyses for whole spike data and three factor factorial analyses for spike section data showed significant differences among the genotypes (167 RILs) for all the measured traits under heat-stress and non-temperature stress conditions (Table 4.1).

4.3.2 QTL associated with reproductive and vegetative traits in the 'Attila' × 'CDC Go' RIL population

Composite interval mapping (CIM) with 6 traits (days to anthesis, number of fertile spikelets, grain number, grain weight, plant height, and flag-leaf size) in the 'Attila' × 'CDC Go' RIL population at the whole spike and spike section levels under non-temperature stress (NS) and heat-stress (HS) conditions identified 73 QTL (NS, 37; HS, 36) on 14 of the 21 (1A, 1B, 2A, 2B, 2D, 3A, 4A, 4B, 5A, 5B, 6A, 6B, 7B, 7D) chromosomes (Table 4.3; Fig.4.1). These QTL individually explained 1.6 to 47.5% phenotypic variation with LOD values ranging from 2.5 to 25.8 (Table 4.3). Forty-nine QTL showed positive additive effects, indicating favorable alleles originated from the heat resistant and high yielding cultivar 'Attila' enhanced the corresponding trait values, while in 23 QTL alleles originated from the heat-sensitive cultivar 'CDC Go' (Table 4.3).

4.3.2.1 QTL associated with reproductive traits

Five major to moderate effect QTL associated with days to anthesis were detected under non-temperature stress and three under heat-stress conditions (Table 4.3). The five non-temperature stress days to anthesis QTL were located on chromosomes 1B (*QDa.jods-1B* at 91 cM), 2B (*QDa.jods-2B.2* at 174 cM), 4B (*QDa.jods-4B* at 16 cM), 5A (*QDa.jods-5A* at 296 cM), and 5B (*QDa.jods-5B* at 152 cM) and explained 49.6% of the total phenotypic variance (Tables 4.2 and 4.3). The major QTL, *QDa.jods-5A* was flanked by the *Vrn-A1* gene and explained 27.8%

individual phenotypic variance with 16.3 LOD score under non-temperature stress conditions (Table 4.3). RILs that had the 'Attila' type allele at the two flanking markers for this major QTL *QDa.jods-5A* flowered 3.9 days later than the RILs that had the 'CDC Go' type allele under non-temperature stress conditions (Table 4.4). The three QTL that were identified under heat-stress conditions for days to anthesis were located on chromosomes 2B (*QDa.jods-2B.1* at 184 cM), 4B (QDa.jods-4B at 15 cM), and 5A (*QDa.jods-5A* at 295 cM) and explained 48.4% of the total phenotypic variation (Tables 4.2 and 4.3). Major QTL, *QDa.jods-5A* was also flanked by the *Vrn-A1* gene and explained 32.2% individual phenotypic variance with 16.6 LOD score under heat-stress conditions (Table 4.3). RILs that had the 'Attila' type allele at the two flanking markers for this major QTL, *QDa.jods-5A* flowered 6 days later than the RILs that had the 'CDC Go' type allele under heat-stress conditions (Table 4.4). Days to anthesis QTL on chromosomes 4B and 5A were consistently detected under non-temperature stress and heat-stress conditions (Table C1). The days to anthesis QTL on chromosomes 2B, 5A, and 1B were carrying favorable allele from 'Attila' and chromosome 4B and 5B QTL were possessing 'CDC Go' type allele (Table 4.3).

Two minor and two major effect QTL associated with the number of fertile spikelets per spike were detected under non-temperature stress and heat-stress conditions respectively (Table 4.3). The two non-temperature stress associated QTL for the number of fertile spikelets per spike were located on chromosomes 2D (*QFsp.jods-2D* at 71 cM) and 5B (*QFsp.jods-5B* at 88 cM) and explained 24% of the total phenotypic variance (Tables 4.2 and 4.3). The two QTL associated with heat-stress conditions for the number of fertile spikelets per spike were located on chromosomes 2B (*QFsp.jods-2B* at 180 cM) and 5A (*QFsp.jods-5A* at 295 cM) and explained 22% of the total phenotypic variance (Tables 4.2 and 4.3). *QFsp.jods-5A* was flanked by the *Vrn-A1* gene and explained 12.2% individual phenotypic variance with 5.1 LOD score under heat-stress conditions (Table 4.3). RILs that had the 'Attila'-type allele at the two flanking markers for this major QTL *QFsp.jods-5A* produced 1.86 more fertile spikelets per spike than the RILs that had the 'CDC Go' type allele (Table 4.4). All the QTL associated with number of fertile spikelets per spike were carrying a favorable allele from 'Attila' (Table 4.3).

We detected nine QTL for number of fertile spikelets associated with the position of the spikelet within the spike under non-temperature stress and seven under heat-stress conditions. In the basal spike section, the two fertile spikelet QTL that explained 16.8% of the total phenotypic variance (Table 4.2) were detected on chromosomes 2D (*QFsp.jods-2D* at 74 cM) and 4B

(*QFsp.jods-4B.1* at 65 cM) under non-temperature stress conditions (Table 4.3). One fertile spikelet QTL that explained 7.6% of the total phenotypic variance (Table 4.2) was detected on chromosome 7B (*QFsp.jods-7B* at 239 cM) under heat-stress conditions (Table 4.3). In the central spike section, four fertile spikelet QTL that explained 26.5% of the total phenotypic variance (Table 4.2) were detected on chromosomes 1B (*QFsp.jods-1B* at 98 cM), 2D (*QFsp.jods-2D* at 70 cM), 4B (*QFsp.jods-4B.2* at 90 cM), and 7B (*QFsp.jods-7B* at 239 cM) under non-temperature stress conditions (Table 4.3). Two QTL that explained 19.8% of the total phenotypic variance (Table 4.2) were detected on chromosomes 5A (*QFsp.jods-5A* at 295 cM) and 7B (*QFsp.jods-7B* at 239 cM) under heat-stress conditions (Table 4.3). *QFsp.jods-4B.2* was flanked by the *Rht-B1* gene (explained 5.8% individual phenotypic variance), and *QFsp.jods-5A* was flanked by the *Vrn-A1* gene (explained 11% individual phenotypic variance; Table 4.3). In the distal spike section, three fertile spikelets QTL that explained 26.2% of the total phenotypic variance were detected on chromosomes 1B (*QFsp.jods-1B* at 108 cM), 2D (*QFsp.jods-2D* at 68 cM), and 6A (*QFsp.jods-6A* at 74 cM) under non-temperature stress conditions. Four fertile spikelets QTL that explained 29.3% of the total phenotypic variance were detected on chromosomes 2A (*QFsp.jods-2A.1* at 156 cM and *QFsp.jods-2A.2* at 160 cM), 4A (*QFsp.jods-4A* at 22 cM), and 5A (*QFsp.jods-5A* at 291 cM) under heat-stress conditions (Table 4.3). *QFsp.jods-2D* and *QFsp.jods-5A* were detected at both whole spike and spike section levels under non-temperature stress and heat-stress conditions, respectively (Table C1). *QFsp.jods-7B* was detected at the basal and central spike sections under heat-stress and/or non-temperature stress conditions (Table C1). Favorable allele for QTL from chromosomes 1B, *QFsp.jods-2A.1* on 2A, 2D, 4B, 5A, and 4A were originated from the cultivar 'Attila' and *QFsp.jods-2A.2* on 2A, 6A, and 7B were carrying allele from 'CDC Go' (Table 4.3).

At the whole spike level, three minor and three moderate to minor effect QTL associated with grain number were detected under non-temperature stress and heat-stress conditions, respectively (Table 4.3). The three non-temperature stress grain number QTL that explained 20% of the total phenotypic variance (Table 4.2) were located on chromosomes 1B (*QGn.jods-1B* at 108 cM), 2B (*QGn.jods-2B* at 177 cM), and 2D (*QGn.jods-2D* at 71 cM; Table 4.3). The three heat-stress grain number QTL that explained 32% of the total phenotypic variance (Table 4.2) were located on chromosomes 2B (*QGn.jods-2B* at 180 cM), 4A (*QGn.jods-4A* at 22 cM), and 5A (*QGn.jods-5A.1* at 295 cM; Table 4.3). Heat-stress QTL, *QGn.jods-5A.1* was flanked by *Vrn-A1* gene and explained 16.6% individual phenotypic variance with 8 LOD score (Table 4.3). RILs that

had the 'Attila' type allele at the two flanking markers for this grain number QTL *QGn.jods-5A.1*, produced 5.4 more grain number than the RILs that had the 'CDC Go' type allele (Table 4.4). Moreover, in the heat-stress grain number QTL *QGn.jods-4A*, the RILs that had the 'Attila' type allele at the two flanking markers produced 3.4 more grain number than the RILs had the 'CDC Go' type allele (Table 4.4). *QGn.jods-2B* was consistently detected under heat-stress and non-temperature stress conditions (Table C1). Favorable allele for all the grain number QTL detected under whole spike level were from the cultivar 'Attila' (Table 4.3).

When assessed with the position of the spikelet, five QTL associated with grain number were detected under non-temperature stress and nine QTL were detected under heat-stress conditions (Table 4.3). Their effects ranged from moderate to minor (Table 4.3). At the basal spike section, one QTL that explained 11.5% of the total phenotypic variance (Table 4.2) was detected on chromosome 4B (*QGn.jods-4B.2* at 62 cM) under non-temperature stress conditions (Table 4.3). Three QTL that explained 18.9% of the total phenotypic variance (Table 4.2) were detected on chromosomes 2A (*QGno.jods-2A* at 174 cM), 5B (*QGn.jods-5B* at 206 cM), and 6A (*QGn.jods-6A* at 6 cM) under heat-stress conditions (Table 4.3). At the central spike section, two QTL that explained 13% of the total phenotypic variance (Table 4.2) were detected on chromosomes 3A (*QGn.jods-3A* at 1 cM) and 4A (*QGn.jods-4A.3* at 0 cM) under non-temperature stress condition (Table 4.3). Two QTL that explained 21% of the total phenotypic variance (Table 4.2) were detected on chromosomes 5A (*QGn.jods-5A.1* at 295 cM) and 7B (*QGn.jods-7B* at 239 cM) under heat-stress conditions (Table 4.3). At the distal spike section, two QTL that explained 23% of the total phenotypic variance were detected on chromosome 2B (*QGn.jods-2B* at 176 cM) and 7D (*QGn.jods-7D* at 29 cM) under non-temperature stress condition (Table 4.2). Four QTL that explained 32% of the total phenotypic variance (Table 4.2) were detected on chromosomes 1A_LG2 (*QGn.jods-1A* at 18 cM), 1B (*QGn.jods-1B* at 108 cM), 2B (*QGn.jods-2B* at 172 cM), and 5A (*QGn.jods-5A.2* at 292 cM) under heat-stress conditions (Table 4.3). *QGn.jods-1B*, *QGn.jods-2B*, and *QGn.jods-5A.1* were detected at whole spike and spike section levels and *QGn.jods-5A.1* was specific to the heat-stress treated plants (Table 4.3 and Table C1). All the QTL associated with grain number in spike sections carrying favorable allele from 'Attila' except for QTL on chromosomes 1A_LG2, 7B, and 7D (Table 4.3).

At the whole spike level, one moderate effect grain weight QTL was detected from each temperature treatment (Table 4.3). Non-temperature stress QTL was detected on chromosome 4B

(*QGw.jods-4B.1* at 90 cM) and the heat-stress QTL was detected on chromosome 5A (*QGw.jods-5A* at 292 cM; Table 4.3). The non-temperature stress grain weight QTL, *QGw.jods-4B.1* was flanked by the *Rht-B1* gene and explained 11.5% individual phenotypic variance with 4.4 LOD score (Table 4.3). RILs that had the 'Attila' type allele at the two flanking markers of this *QGwt.jods-4B.1* produced 0.07g more grain weight than the RILs that had the 'CDC Go' type allele (Table 4.4). Grain weight QTL at the whole spike level were carrying favorable allele from 'Attila' (Table 4.3).

When assessed with the position of the spikelet, six QTL associated with grain weight were detected under non-temperature stress and two QTL were detected under heat-stress conditions (Table 4.3). At the basal spike section, one grain weight QTL that explained 11.4% of the total phenotypic variance (Table 4.2) was detected on chromosome 4B (*QGw.jods-4B.2* at 62 cM) under non-temperature stress conditions (Table 4.3). At the central spike section, two QTL that explained 14% of the total phenotypic variance (Table 4.2) were detected on chromosomes 4B (*QGw.jods-4B.1* at 90 cM) and 5A (*QGw.jods-5A* at 295 cM) under non-temperature stress conditions (Table 4.3); and one QTL that explained 20% of the total phenotypic variance (Table 4.2) was detected on chromosome 5A (*QGw.jods-5A* at 293 cM) under heat-stress conditions (Table 4.3). At the distal spike section, three QTL that explained 14% of the total phenotypic variance (Table 4.2) were detected on chromosomes 2D (*QGw.jods-2D* at 12 cM), 5A (*QGw.jods-5A* at 288 cM), and 6A (*QGw.jods-6A* at 78 cM) under non-temperature stress conditions; and one QTL that explained 15% of the total phenotypic variance (Table 4.2) was detected on chromosome 5A (*QGw.jods-5A* at 293 cM) under heat-stress conditions (Table 4.3). At both whole spike and spike section levels *QGw.jods-4B.1* was detected under non-temperature stress condition and *QGw.jods-5A* was detected under non-temperature stress and heat-stress conditions and favorable allele of these QTL were carrying from 'Attila' (Tables 4.3 and C1). Other grain weight QTL were identified only in specific spike sections with favorable alleles coming from 'CDC Go' (Table 4.3).

4.3.2.2 QTL associated with plant height and flag leaf size

Two moderate to minor QTL associated with plant height were detected for each temperature treatment (Table 4.3). Two non-temperature stress plant height QTL that explained 18.6% of total phenotypic variance (Table 4.2) were located on chromosomes 4B (*QPht.jods-4B*

at 85 cM), and 5A (*QPht.jods-5A.2* at 177 cM; Table 4.3). Two heat-stress plant height QTL were located at two positions on the chromosome 5A (*QPht.jods-5A.1* at 67 cM and *QPht.jods-5A.2* at 288 cM; Table 4.3) and explained 23.3% of the total phenotypic variance (Table 4.2). The plant height QTL *QPht.jods-5A.2* was consistently detected under non-temperature stress and heat-stress conditions (Table C1). RILs that had the 'Attila' type allele at the two flanking markers for non-temperature stress plant height QTL produced 4-4.5 cm shorter stem than the RILs that had the 'CDC Go' type allele (Table 4.4). RILs that had the 'Attila' type allele at the two flanking markers for *QPht.jods-5A.2* QTL produced 5.7 cm shorter stem than the RILs that had the 'CDC Go' type allele under heat-stress conditions (Table 4.4). All plant height QTL were carrying favorable allele from the cultivar 'Attila' except for *QPht.jods-5A.1* (Table 4.3).

Two non-temperature stress and two heat-stress QTL (one major and one minor effect QTL in each temperature condition) associated with flag-leaf length were detected on chromosomes 5A, 5B, and 6B (Table 4.3). Two non-temperature stress flag-leaf length QTL that explained 40% of the total phenotypic variance (Table 4.2) were located on chromosomes 5A (*QFll.jods-5A* at 298 cM) and 6B (*QFll.jods-6B* at 111 cM; Table 4.3). RILs that had the 'Attila' allele at the two flanking markers for these flag-leaf length QTL produced 2-3.5 cm shorter flag leaves than the RILs that had the 'CDC Go' allele under non-temperature stress conditions (Table 4.4). Two heat-stress flag-leaf length QTL that explained 53% of total phenotypic variance were located on chromosomes 5A (*QFll.jods-5A* at 296 cM) and 5B (*QFll.jods-5B* at 173 cM; Table 4.3). The heat-stress flag-leaf length QTL *QFll.jods-5A* was flanked by the *Vrn-A1* gene and explained 47.5% of the individual phenotypic variance with 25.8 LOD score (Table 4.3). RILs that had the 'Attila' allele at the two flanking markers for this flag-leaf length QTL *QFll.jods-5A*, produced 4.3 cm shorter flag leaves than the RILs that had the 'CDC Go' allele under heat-stress condition (Table 4.4). *QFll.jods-5A* was consistently detected under non-temperature stress and heat-stress conditions (Table C1). All flag-leaf length QTL were carrying favorable allele from the cultivar 'CDC Go' (Table 4.3).

Major and minor QTL associated with flag-leaf area were detected under non-temperature stress (2 QTL) and heat-stress (5 QTL) conditions (Table 4.3). Two non-temperature stress flag-leaf area QTL were located on chromosomes 1A (*QFla.jods-1A* at 51 cM) and 5A (*QFla.jods-5A.3* at 300 cM; Table 4.3). In the non-temperature stress flag-leaf area QTL *QFla.jods-5A.2*, RILs that had the 'Attila' allele at the two flanking markers produced 3.6 cm^2 smaller flag-leaf area than

the RILs that had the 'CDC Go' allele under non-temperature stress conditions (Table 4.4). Five heat-stress flag-leaf area QTL were located on chromosomes 2D (*QFla.jods-2D* at 12 cM), 4A (*QFla.jods-4A* at 0 CM), 5A (*QFla.jods-5A.1* at 92 cM, and *QFla.jods-5A.2* at 298 cM), and 5B (*QFla.jods-5B* at 172 cM; Table 4.3). The heat-stress flag-leaf area QTL *QFla.jods-2D* was flanked by the *Ppd-D1* gene and explained 7% of the individual phenotypic variance with 2.8 LOD score (Table 4.3). In the flag-leaf area QTL, *QFla.jods-5A.1* and *QFla.jods-5A.2*, RILs that had the 'Attila' allele at the two flanking markers produced 2.3-4.7 cm^2 smaller flag-leaf area than the RILs that had the 'CDC Go' allele under heat-stress conditions (Table 4.4). All flag-leaf area QTL were carrying favorable alleles from the cultivar 'CDC Go' except for the QTL *QFla.jods-4A* (Table 4.3).

4.3.2.3 QTL clusters detected in the 'Attila' × 'CDC Go' RIL population

Eight important QTL clusters in terms of enhancing yield potential were detected on chromosomes 5A, 4B,1B, 2B, and 2D (a cluster was defined as a genomic region associated with different traits; Table 4.5). QTL clusters 1 and 2 were detected on chromosome 5A between the confidence intervals 293.5-296.5 cM and 283.5-292.5 cM respectively. The cluster 1 was associated with five traits (days to anthesis, *QDa.jods-5A*; fertile spikelets, *QFsp.jods-5A*; grain number, *QGn.jods-5A*; grain weight, *QGw.jods-5A*; and flag-leaf length, *QFll.jods-5A*; Table 4.5). The QTL for days to anthesis, grain weight, and flag-leaf length were consistently detected under both non-temperature stress and heat-stress conditions while the other QTL in cluster 1 (*QGn.jods-5A* and *QFsp.jods-5A*) were detected only under heat-stress conditions (Table 4.3). QTL cluster 2 was associated with three traits (grain number, *QGn.jods-5A.2*; grain weight, *QGw.jods-5A*; and plant height, *QPht.jods-5A.2*; Table 4.5). *QGn.jods-5A.2* and *QGw.jods-5A* were detected in the distal spike section under heat-stress and non-temperature stress conditions, respectively (Table 4.3). *QPht.jods-5A.2* was detected both under heat-stress and non-temperature stress conditions (Table 4.3).

QTL clusters 3 and 4 were detected on chromosome 4B between the confidence intervals 57.5 to 65.5 cM and 80.5 to 100.5 cM, respectively (Table 4.5). QTL cluster 3 was associated with three traits, fertile spikelet number (*QFsp.jods-4B.1*), grain number (*QGn.jods-4B*), and grain weight (*QGw.jods-4B.2*; Table 4.5). QTL cluster 4 was also associated with three traits, fertile spikelet number (*QFsp.jods-4B.2),* grain weight (*QGw.jods-4B.1*) and plant height (*QPht.jods-4B*;

Table 4.5). The QTL associated with clusters 3 and 4 were only detected under non-temperature stress conditions (Table 4.3).

QTL cluster 5 was detected on chromosome 2B between the confidence interval 170.5-181.5 cM (Table 4.5). Three traits were associated with QTL cluster 5 (days to anthesis, *QDa.jods-2B.2*; fertile spikelet number, *QFsp.jods-2B*; and grain number, *QGn.jods-2B*; Table 4.5). The grain number QTL was detected under non-temperature stress and heat-stress conditions, the days to anthesis QTL under non-temperature stress conditions, and the fertile spikelet QTL under heat-stress conditions.

QTL cluster 6 was associated with two traits (fertile spikelets per spike, *QFsp.jods-1B* and grain number, *QGn.jods-1B*), and was detected on chromosome 1B between the confidence intervals 104.5-108 cM (Table 4.5). *QFsp.jods-1B* was detected only under non-temperature stress conditions, while *QGn.jods-1B* was detected at the whole spike level under non-temperature stress conditions, and in the distal spike section under heat-stress conditions (Table 4.3).

QTL clusters 7 and 8 were detected on chromosome 2D between the confidence intervals 63.5-74 cM and 0-25.5 cM (Table 4.5). Cluster 7 was associated with two traits, grain number (*QGn.jods-2D*) and fertile spikelets per spike (*QFsp.jods-2D*) under non-temperature stress conditions (Table 4.3). QTL cluster 8 was detected on chromosome 2D adjacent to the well-known photoperiod insensitive gene *Ppd-D1*. Cluster 8 was associated with the QTL for grain weight (*QGw.jods-2D*) and flag-leaf area (*QFla.jods-2D*). The grain weight QTL was detected under non-temperature stress, and the flag-leaf area QTL was detected under heat-stress conditions.

4.4 Discussion

In the context of climate change, every degree increase above the optimal growing temperature during the reproductive development in wheat has a severe impact on crop production (Carew et al., 2017). Therefore, understanding the impact of heat stress on different agronomic traits responsible for yield improvement and the genomic regions associated with those traits will help in designing strategies to overcome the yield loss. QTL (73) associated with six yield and yield component traits were identified under non-temperature stress and heat-stress conditions in an 'Attila' × 'CDC Go' RIL population (167 RILs) through ICIM mapping. Occurrence of the QTL was highest in the A genome (23 QTL), followed by the B genome (22 QTL), and then the D genome (5 QTL), after grouping of similar QTL within the same trait under non-temperature

stress and heat-stress conditions and/or whole spike and spike section levels. Continuous phenotypic variation and transgressive segregation was observed for all assessed traits in the RIL population, with one exception (transgressive segregation was absent in fertile spikelets at the spike section level). Furthermore, we observed two or more major to minor QTL associated with each measured trait (6 QTL for days to anthesis, 12 QTL for fertile spikelets per spike, 16 QTL for grain number, 6 QTL for grain weight, 3 QTL for plant height, 3 QTL for flag leaf length, and 7 QTL for flag-leaf area regardless of temperature treatment). These observations demonstrate the quantitative inheritance of these measured traits.

The parental cultivars Attila and CDC Go showed variation for measured traits, and both parents contributed beneficial alleles for measured traits in the RIL population, strengthening the usefulness of this population for more robust detection of QTL. Forty-nine of the 73 total QTL identified in this study showed a trait enhancing allele from the heat-resistant cultivar 'Attila' (Table 4.3), indicating that in general introgression of an allele from 'Attila' has a beneficial effect on improving most of the yield traits under non-temperature-stress or heat-stress conditions.

4.4.1 Important QTL and QTL clusters detected only under non-temperature stress conditions

Among the QTL clusters detected only under the non-temperature stress conditions, three were stable and interesting to mention. Even though the fertile spikelets per spike-related QTL on chromosome 2D (*QFsp.jods-2D;* 5.2 to 8.4 PVE%) was a minor QTL, it was detected at the whole spike (Fsp) as well as the basal (Fspb), central (Fspc), and the distal (Fspd) spike section levels (Table 4.3), indicating its stability. *QFsp.jods-2D* was co-located with the grain number-related QTL *QGn.jods-2D* (QTL cluster 7; 63.5-74 cM genomic region). The favorable alleles for both *QFsp.jods-2D* and *QGn.jods-2D* originated from the highest yielding parent cultivar Attila. Similarly, Zou et al. (2017b) reported the yield-related QTL *QYld.dms-2D.2*, the grain protein content-related QTL *QGpc.dms-2D,* and the maturity time-related QTL *QMat.dms-2D.2* in the same genomic region (62.5-74 cM) as QTL cluster 7 in the same RIL population grown under conventionally-managed field conditions. A grain yield-related QTL closer to the marker wsnp_Ex_c8303_14001708 as in QTL cluster 7 of our experiment was observed on chromosome 2D (Lopes et al., 2015). Common genomic regions for different traits can be the result of a linkage

between two genes or the pleiotropic effect of one gene (Pinto et al., 2010). Together these data stress the importance of the genomic region cluster 7 in affecting grain yield in wheat.

The grain weight-related QTL *QGw.jods-4B.1* (confidence interval 84.5-100.5 cM), which was flanked by the *Rht-B1* gene, was detected as a moderate effect (11.5 PVE %) QTL at the whole spike level (Gw) and as a minor effect QTL (8.5 PVE%) at the central spike section level (Gwc), indicating its stability (Table 4.3). *QGw.jods-4B.1* was also co-located with the fertile spikelet-related QTL *QFsp.jods-4B.2* (minor effect, 5.8 PVE%) and the plant height-related QTL, *QPht.jods-4B* (moderate effect, 11.9 PVE%) in QTL cluster 4 (80.5-100.5 cM genomic region). Favorable alleles for these QTL originated from 'Attila', which were associated with enhanced grain weight, higher fertile spikelet number, and shorter plant height. Targeting this genomic region in breeding could be valuable for simultaneously increasing grain yield, while reducing plant height. In the same RIL population three QTL were detected (plant height related QTL *QPht.dms-4B,* grain protein content-related QTL *QGpc.dms-4B,* and the flowering time-related QTL *QFlt.dms-4B*) in the same genomic region (79.5-113.5 cM) as cluster 4 under western Canadian field conditions (Zou et al., 2017b). These data highlight that the genes within this genomic region have a stable positive effect on grain yield and a negative effect on plant height under different environmental conditions. These data also provide further evidence for the pleiotropic architecture of the *Rht* locus, which is equally responsible for controlling plant height and other yield and yield component traits as mentioned in previous studies (Guan et al., 2018; Fan et al., 2019; Li et al., 2018). The genomic region around *Rht-B1* was reported to be a QTL hot spot harboring QTL associated with six traits (plant height, lodging score, tillering ability, grain protein content, grain yield and test weight; in the physical interval 29.4 and 33.1 Mb; chromosome 4B) containing a cluster of 37 candidate genes (Semagn et al., 2021). As this region contains a large number of candidate genes, the traits controlled by this genomic region are likely not solely associated with the *Rht-B1* gene. Therefore, linkage between several genes may be responsible for yield and yield component traits controlling ability in this region.

The grain number QTL *QGn.jods-4B* (confidence interval 57.5-64.5 cM; 13.1% PVE) was co-located with fertile spikelet-related QTL *QFsp.jods-4B.1* (9.6% PVE), and grain weight-related QTL, *QGw.jods-4B.2* (12.4% PVE) in cluster 3, and these QTL were only detected in the basal section of the spike (Gnb, Fspb, and Gwb, respectively; Table 4.3). Favorable alleles for these QTL originated from the highest yielding parental cultivar Attila, which were associated with

higher grain number and weight, and fertile spikelets in the basal spike section. These data highlight the importance of this genomic region in enhancing the yield potential in the basal spike section of wheat. Furthermore, the strong positive correlations observed between fertile spikelets, grain number and weight mentioned in chapter three may be at least partially associated with genes in cluster 3.

Other than the QTL clusters, one single QTL associated with flag leaf area was identified under non-temperature stress conditions. This QTL, *QFla.jods-5A.3,* was located on chromosome 5A (confidence interval 299.5-300.5 cM, LOD 10.4) and accounted for 21.8% PVE indicating its importance in affecting flag-leaf area. The favorable allele for this QTL originated from the cultivar CDC Go (Table 4.3). The RILs carrying a 'CDC Go' type allele in the *QFla.jods-5A.3* produced larger flag-leaves (3.6 cm^2, Table 4.4) compared to the RILs carrying an 'Attila' type allele indicating the importance of this QTL in improving flag leaf area in wheat. Chromosome 5A in wheat is known as a QTL-rich chromosome for flag leaf-related traits (Liu et al., 2018; Yang et al., 2016).

4.4.2 Important QTL and QTL clusters detected under non-temperature stress and heat-stress conditions

In general, a QTL that is consistent across environments is of great value for marker-assisted selection in breeding varieties adapted to various ecological environments (Assanga et al., 2017). Therefore, the QTL/QTL clusters detected under both non-temperature stress and heat-stress environments should be useful in the genetic improvement of wheat yield across environments.

An important genomic region (cluster 1) was detected on chromosome 5A with five stable QTL associated with days to anthesis, fertile spikelets, grain number, grain weight and flag-leaf length between 293.5 and 296.5 cM and flanked by *Vrn-A1*(Table 4.5). The grain number QTL *QGn.jods-5A* and fertile spikelets QTL *QFsp.jods-5A* were only detected under heat-stress conditions, while the days to anthesis QTL *QDa.jods-5A*, grain weight QTL *QGw.jods-5A*, and flag-leaf length QTL *QFll.jods-5A,* were consistently detected under non-temperature stress and heat-stress conditions. The genetic effects on the traits in *QDa.jods-5A*, *QGw.jods-5A*, and *QFll.jods-5A* were heat-stress driven, as the QTL peaked, and their effects progressed under heat stress (Table 4.3). Furthermore, the fertile spikelet number, grain number, and grain weight QTL

were detected at the whole spike and spike section levels (Fsp, Fspc and Fspd; Gn, Gnc and Gnd; Gw, Gwc and Gwd), and all five QTL were moderate to major effect QTL. These data stress the stability of these QTL. All the QTL related to reproductive traits were carrying favorable alleles from the higher yielding parent 'Attila', which increased the grain yield in genotypes carrying these QTL. The RILs carrying the 'CDC Go' alleles in the QTL *QFll.jods-5A* increase the flag leaf length (3.5-4.3 cm, Table 4.4) indicating the importance of this QTL in improving the flag leaf length in wheat (Table 4.4). The moderate to strong correlations between grain number and weight, fertile spikelet number, and days to anthesis, mentioned in chapter three may be at least partially associated with genes in cluster 1. In the same RIL population three QTL were detected (flowering time, *QFlt.dms.5A*; maturity time, *QMat.dms-5A-2*; and plant height, *QPht.dms-5A*) in the same genomic region (290.5-298.5 cM) as cluster 1 under western Canadian field conditions (Zou et al., 2017b). Other researchers also detected QTL associated with grain yield and yield component traits around the *Vrn-A1* in chromosome 5A (Kato et al., 2000; Gahlaut et al., 2017).

Another important QTL cluster (cluster 2) was detected adjacent to cluster 1 on chromosome 5A between the confidence intervals of 283.5-292.5 cM. The grain number-related QTL *QGn.jods-5A.2* (only under heat-stress conditions) and grain weight-related QTL *QGw.jods-5A* (only under non-temperature stress conditions) were detected in the distal spike section (Gnd and Gwd), indicating these QTL are important for grain yield in the distal spike section. These two QTL were also co-located with the plant height QTL *QPht.jods-5A.2*. *QPht.jods-5A.2* was detected under both heat-stress and non-temperature stress conditions and the effect peaked and the PVE% increased under heat-stress conditions (Table 4.3). Favorable alleles for the above mentioned three QTL originated from the parent cultivar Attila and they were associated with higher grain yield in the distal spike section while reducing the plant height.

QTL cluster 5 was detected between 170.5-181.5 cM on chromosome 2B. It was associated with days to anthesis, fertile spikelet number, and grain number QTL. The grain number QTL *QGn.jods-2B* was detected in whole spike (Gn) and distal spike sections (Gnd) under non-temperature-stress and heat-stress conditions stressing their stability. The PVE% and the LOD value were higher in the distal spike section regardless of the temperature conditions even though the QTL expression progressed under heat stress. These data indicate the importance of *QGn.jods-2B* in maintaining grain number in the distal spike section especially under heat-stress conditions. The fertile spikelet number QTL *QFsp.jods-2B* (10.3% PVE) was found only at the whole spike

level (Fsp) under heat-stress conditions, indicating that it is associated with maintaining grain yield under heat stress conditions, and its effect is not modified by spikelet position. The days to anthesis QTL *QDa.jods-2B.2* (8.2% PVE) was found only under non-temperature stress conditions, suggesting that it may be sensitive to heat stress. Favorable alleles for all the traits mentioned in cluster 5 originated from the cultivar Attila and they were responsible for increasing the trait values. The moderate to strong correlations observed between grain number and weight, fertile spikelets, and days to anthesis mentioned in chapter three may be at least partially associated with genes in cluster 5.

A genomic region (0-25.5 cM; cluster 8) with grain weight-related QTL, *QGw.jods-2D* and flag-leaf-area related QTL, *QFla.jods-2D* was flanked by the photoperiod insensitive gene *Ppd-D1*. *QGw.jods-2D* was detected only in the distal spike section (Gwd) under non-temperature stress conditions; however, it accounted for 11.4% PVE, indicating the importance of this QTL in affecting grain yield in the distal spike section. The favorable allele for *QGw.jods-2D* originated from the cultivar CDC Go, and 'CDC Go' and 'Attila' produced similar grain weight in the distal spike section under non-temperature stress conditions (Table 3.3; chapter 3). Similarly, there was no significant difference detected in the grain weight of the genotypes having 'CDC Go' allele and the 'Attila' allele in the *QGw.jods-2D* position. *QFla.jods-2D* was detected under heat-stress conditions and accounted for 6.7% PVE. The favorable allele originated from the cultivar CDC Go and there was no difference observed in the flag-leaf area of the genotypes carrying 'CDC Go' type allele and the 'Attila' type allele at the *QFla.jods-2D* position. In a study with the RIL population derived from a cross between Canadian western red spring wheat cultivars 'AC Barrie' and 'Cutler', three QTL were detected (flowering time-related QTL, *QFlt.dms-2D*; maturity-related QTL, *QMat.dms-2D*; and grain yield-related QTL, *QYld.dms-2D*) in an overlapping region (0-12 cM) to the position in this study, suggesting they are possibly the same QTL or a QTL-rich region with several QTL or genes (Perez-Lara et al., 2016). Furthermore, QTL related to spikelet number per spike, grain number in the basal spike section, thousand grain weight, and days to heading were identified in the *Ppd-D1* region, suggesting this region could be one of the hot spots for increasing grain yield-related traits in hexaploid wheat (Muzino et al., 2021).

Cluster 6 is also important to mention as it contained QTL related to fertile spikelets per spike and grain number. Cluster 6 was detected on chromosome 1B and associated with the fertile spikelet per spike-related QTL *QFsp.jods-1B* in the distal spike section (Fspd) under non-

temperature stress conditions. The grain number-related QTL *QGn.jods-1B* was detected at the whole spike level (Gn) under non-temperature stress conditions and in the distal spike section (Gnd) under heat-stress conditions (Table 4.3). Favorable alleles for these QTL originated from the higher yielding cultivar Attila. These observations suggest cluster 6 has the potential to increase the grain yield in wheat under different temperature conditions.

4.5 Conclusions

Within the 'Attila' × 'CDC Go' RIL population, 73 QTL (NS, 37; HS, 36) were detected on 14 of the 21 chromosomes (1A, 1B, 2A, 2B, 2D, 3A, 4A, 4B, 5A, 5B, 6A, 6B, 7B, 7D) that individually explained 1.6 to 47.5% phenotypic variation with Logarithm of Odds (LOD) values ranging from 2.5 to 25.8. Eight important QTL clusters that were associated with two or more important grain yield or yield-related traits were identified on chromosomes 5A, 4B, 2B, 2D, and 1B. These QTL clusters indicated the genetic interdependencies between measured yield and yield component traits. QTL clusters 3, 4, and 7 were detected only under non-temperature stress conditions, while clusters 1, 2, 5, 6, and 8 contained QTL related to heat-stress and/or non-temperature stress conditions. Some QTL like *QDa.jods-5A*, *QGw.jods-5A*, *QFll.jods-5A* (cluster 1), and *QPht.jods-5A.2* (cluster 2) were heat-stress driven, as heat stress increased the LOD score and PVE%. Furthermore, these QTL clusters are potential target regions for fine-mapping and marker-assisted selection in wheat breeding programs, as many of them have been previously detected in the wheat genome as QTL hot spots for wheat grain yield and spike architecture improvement.

4.6 Tables and figures

Table 4.1 Summary of least square means and F statistics of 167 recombinant inbred lines (RILs) derived from 'Attila' × 'CDC Go' evaluated under non-temperature stress and heat-stress conditions.

		Parents		RILs (descriptive and F statistics)						
Trait	Heat trt‡	'Attila'	'CDC Go'	Mean⁑	Min	Max	Range	SD	CV%	F value§
Days to anthesis	+	68& b¶	58 d	64 m	43	88	45	8.3	13	6.64
	-	70 a	61 c	65 m	50	87	37	7.7	11.8	7.56
Fertile spikelets per spike	+	17 a	8 c	13 n	5	20	15	3.6	28.3	3.12
	-	17 a	12 b	15 m	9	21	12	2.4	16.2	2.9
Fertile spikelets basal	+	5 a	3 b	2 m	1	3	2	0.5	26.9	2.62
	-	5 a	3 b	2 m	1	3	2	0.4	18.2	2.24
Fertile spikelets central	+	7 a	4 c	2 m	1	3	2	0.5	27.7	3.25
	-	7 a	5 b	2 m	1	3	2	0.4	15.9	3.4
Fertile spikelets distal	+	6 a	2 c	1 m	0	2	2	0.4	38.6	3.77
	-	6 a	4 b	1 m	1	2	1	0.3	18.7	2.57
Grain number per spike	+	38 a	12 c	24 n	4	45	41	9.6	40.7	4.43
	-	41 a	22 b	30 m	15	53	38	7.7	25.8	3.55
Grain number basal	+	12 a	4 a	7 n	0	16	16	3.2	47	2.7
	-	13 a	6 a	10 m	1	20	19	3.5	36.4	2.26
Grain number central	+	16 a	5 c	11 n	2	21	19	4.5	40.5	4.64
	-	17 a	10 b	14 m	7	21	14	3.1	22.5	3.56
Grain number distal	+	10 a	3 c	6 m	0	15	15	3.2	57.7	4.09
	-	11 a	6 b	7 m	2	13	11	2.3	34.2	3.46
Grain weight per spike (g)	+	1.385 a	0.531 c	0.936 n	0.162	1.83	1.668	0.4	39.5	3.59
	-	1.449 a	0.976 b	1.199 m	0.428	2.362	1.934	0.3	28.6	3.01
Grain weight basal (g)	+	0.499 a	0.180 b	0.294 n	0.014	0.646	0.632	0.1	46.7	2.72
	-	0.510 a	0.256 b	0.399 m	0.027	0.859	0.832	0.2	38.9	2.3
Grain weight central (g)	+	0.587 a	0.245 c	0.435 n	0.092	0.836	0.744	0.2	38.6	3.48
	-	0.637 a	0.460 b	0.576 m	0.229	1.015	0.786	0.1	25.6	3.11
Grain weight distal (g)	+	0.299 a	0.105 b	0.180 n	0.003	0.504	0.501	0.1	55.6	3.15
	-	0.302 a	0.260 a	0.216 m	0.032	0.478	0.447	0.1	38.5	2.66
Plant height (cm)	+	71 d	79 b	80 n	56	101	45	10	13	10
	-	76 c	82 a	86 m	60	110	50	10.6	12.4	6.41
Flag leaf length (cm)	+	23 b	23 b	21 n	12	30	18	4.6	21.5	6.05
	-	23 b	26 a	23 m	12	33	21	4.1	18	3.8
Flag leaf area (cm^2)	+	25 a	19 b	19 m	8	32	24	5.3	27.8	4.95
	-	24 a	23 a	20 m	9	37	28	4.7	23	3.01

‡ (+) Heat-stress treatment; (-) non-temperature stress-temperature treatment.
§ All F values were significant at $P < 0.05$.
& Parents data are means, n=40. ¶ means followed by different letters are significantly different among temperature treatments and cultivars (a, b, c, d), and within population means (m, n), within each parameter by the LSD test, with the significance level at $P \leq 0.05$.
⁑ RIL population means are means of n = 167 × 4 replications.

Table 4.2 Summary of QTL associated with six agronomic traits (days to anthesis, number of fertile spikelets, grain number, grain weight, plant height, and flag-leaf size) identified in 167 'Attila'× 'CDC Go' RILs evaluated under non-temperature stress and heat-stress conditions.

Trait	Heat trt‡	Heritability	Number of QTL identified	Total phenotypic variance explained by all additive QTL (%)
Days to anthesis	+	0.59	3	48.4
	-	0.63	5	49.6
Fertile spikelets per spike	+	0.35	2	21.5
	-	0.33	2	23.6
Fertile spikelets basal	+	0.23	1	7.6
	-	0.23	2	16.8
Fertile spikelets central	+	0.38	2	19.8
	-	0.38	4	26.5
Fertile spikelets distal	+	0.45	4	29.3
	-	0.32	3	26.2
Grain number per spike	+	0.44	3	31.8
	-	0.33	3	20.5
Grain number basal	+	0.27	3	18.9
	-	0.24	1	11.5
Grain number central	+	0.47	2	21.0
	-	0.32	2	13.3
Grain number distal	+	0.41	4	32.1
	-	0.26	2	22.6
Grain weight per spike (g)	+	0.39	1	10.1
	-	0.34	1	11.0
Grain weight basal (g)	+	0.27	0	0
	-	0.26	1	11.4
Grain weight central (g)	+	0.39	1	20.3
	-	0.35	2	14.0
Grain weight distal (g)	+	0.33	1	14.8
	-	0.25	3	14.2
Plant height (cm)	+	0.70	2	23.3
	-	0.59	2	18.6
Flag leaf length (cm)	+	0.56	2	53.1
	-	0.41	2	40.4
Flag leaf area (cm^2)	+	0.49	5	49.0
	-	0.34	2	31.1

‡ (+) Heat-stress treatment; (-) non-temperature stress treatment

Table 4.3 QTL associated with six traits based on 167 'Attila'× 'CDC Go' RILs evaluated under non-temperature stress and heat-stress conditions.

Trait*	Heat trt‡	QTL	Chrom. §	Position (cM)	Confidence interval (cM)	Left Marker	Right Marker	LOD	PVE ¶ (%)	Additive effect‡
Da	+	*QDa.jods-2B.1*	2B	184	183.5-189.5	Tdurum_contig54704_176	IACX1098	3	4.7	1.7
Da	+	*QDa.jods-4B*	4B	15	13.5-16.5	BS00073084_51	Kukri_rep_c78644_408	3.2	5.1	-1.79
Da	+	*QDa.jods-5A*	5A	295	294.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	16.6	32.2	4.49
Da	-	*QDa.jods-1B*	1B	91	90.5-92.5	BS00021697_51	Tdurum_contig45965_563	4.8	6.9	2.03
Da	-	*QDa.jods-2B.2*	2B	174	172.5-175.5	RAC875_c34516_70	RAC875_c26469_480	5.7	8.2	2.22
Da	-	*QDa.jods-4B*	4B	16	14.5-21.5	BS00073084_51	Kukri_rep_c78644_408	2.8	3.9	-1.54
Da	-	*QDa.jods-5A*	5A	296	294.5-296.5	Vrn-A1	Kukri_c12384_430	16.3	27.8	4.13
Da	-	*QDa.jods-5B*	5B	152	139.5-154.5	Tdurum_contig5017_993	RAC875_c30011_426	2.7	3.7	-1.57
Fsp	+	*QFsp.jods-2B*	2B	180	177.5-181.5	RAC875_c19690_358	BobWhite_c41535_52	4.2	10.3	1.12
Fsp	+	*QFsp.jods-5A*	5A	295	293.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	5.1	12.2	1.22
Fsp	-	*QFsp.jods-2D*	2D	71	68.5-74	wsnp_Ex_c8303_14001708	wsnp_Ex_rep_c66522_64795143	2.8	5.7	0.61
Fsp	-	*QFsp.jods-5B*	5B	88	86.5-92.5	Tdurum_contig45588_730	wsnp_Ra_c6374_11143280	3.2	6	0.63
Fspb	+	*QFsp.jods-7B*	7B	239	238.5-239.5	Kukri_c35975_593	BobWhite_rep_c50003_377	3	8.2	-0.13
Fspb	-	*QFsp.jods-2D*	2D	74	70.5-74	wsnp_Ex_c8303_14001708	wsnp_Ex_rep_c66522_64795143	3.2	8.4	0.11
Fspb	-	*QFsp.jods-4B.1*	4B	65	60.5-65.5	RAC875_c14455_1148	wsnp_CAP12_c1101_569783	3.6	9.6	0.12
Fspc	+	*QFsp.jods-5A*	5A	295	293.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	4.3	11.1	0.16
Fspc	+	*QFsp.jods-7B*	7B	239	238.5-239.5	Kukri_c35975_593	BobWhite_rep_c50003_377	3.9	9.9	-0.15
Fspc	-	*QFsp.jods-1B*	1B	98	91.5-104.5	BS00063537_51	Kukri_rep_c111174_132	5.9	13.8	0.14
Fspc	-	*QFsp.jods-2D*	2D	70	64.5-74	wsnp_Ex_c8303_14001708	wsnp_Ex_rep_c66522_64795143	2.6	5.4	0.08
Fspc	-	*QFsp.jods-4B.2*	4B	90	84.5-100.5	wsnp_Ra_c1146_2307483	Rht-B1	2.9	5.8	0.09
Fspc	-	*QFsp.jods-7B*	7B	239	238.5-240.5	Kukri_c35975_593	BobWhite_rep_c50003_377	2.5	5	-0.08
Fspd	+	*QFsp.jods-2A.1*	2A	156	153.5-158.5	wsnp_Ex_c22862_32074455	RFL_Contig3916_275	23.5	16.1	0.34
Fspd	+	*QFsp.jods-2A.2*	2A	160	159.5-161.5	wsnp_Ex_c4847_8646784	Excalibur_c66007_385	14.3	8.4	-0.25
Fspd	+	*QFsp.jods-4A*	4A_LG2	22	18.5-22	BobWhite_c14495_230	Excalibur_c13975_335	3	1.6	0.11
Fspd	+	*QFsp.jods-5A*	5A	291	290.5-293.5	Tdurum_contig10843_745	wsnp_Ex_rep_c101994_87256479	8	4.1	0.17

Trait*	Heat trt‡	QTL	Chrom. §	Position (cM)	Confidence interval (cM)	Left Marker	Right Marker	LOD	PVE ¶ (%)	Additive effect‡
Fspd	-	*QFsp.jods-1B*	1B	108	105.5-108	Kukri_rep_c111174_132	wsnp_BG274687B_Ta_2_1	8	15.7	0.11
Fspd	-	*QFsp.jods-2D*	2D	68	63.5-74	RAC875_rep_c73531_335	wsnp_Ex_c8303_14001708	3	5.2	0.06
Fspd	-	*QFsp.jods-6A*	6A	74	72.5-74.5	RAC875_rep_c70665_451	BS00074979_51	3.5	6.9	-0.07
Gn	+	*QGn.jods-2B*	2B	180	177.5-181.5	RAC875_c19690_358	BobWhite_c41535_52	2.7	5.5	2.17
Gn	+	*QGn.jods-4A.1*	4A_LG2	22	21.5-22	BobWhite_c14495_230	Excalibur_c13975_335	3.3	6.5	2.36
Gn	+	*QGn.jods-5A.1*	5A	295	293.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	8	16.6	3.78
Gn	-	*QGn.jods-1B*	1B	108	104.5-108	Kukri_rep_c111174_132	wsnp_BG274687B_Ta_2_1	4.4	9.8	2.4
Gn	-	*QGn.jods-2B*	2B	177	175.5-180.5	CAP12_rep_c3980_87	RAC875_c19690_358	2.6	5.8	1.8
Gn	-	*QGn.jods-2D*	2D	71	63.5-74	wsnp_Ex_c8303_14001708	wsnp_Ex_rep_c66522_64795143	3.1	7.5	2.1
Gnb	+	*QGn.jods-2A*	2A	174	168.5-180.5	RAC875_rep_c73925_276	JD_c3930_358	2.6	7.4	0.81
Gnb	+	*QGn.jods-5B*	5B	206	205.5-206.5	Kukri_c46932_65	wsnp_Ra_c27733_37249132	2.7	7.3	0.8
Gnb	+	*QGn.jods-6A*	6A	9	8.5-9.5	wsnp_Ex_c1050_2008598	JD_c12608_191	2.9	7.7	0.83
Gnb	-	*QGn.jods-4B*	4B	62	57.5-64.5	JD_c11606_1377	RAC875_c14455_1148	5.1	13.1	1.33
Gnc	+	*QGn.jods-5A.1*	5A	295	294.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	6.1	16.1	1.62
Gnc	+	*QGn.jods-7B*	7B	239	238.5-239.5	Kukri_c35975_593	BobWhite_rep_c50003_377	2.6	6.4	-1.03
Gnc	-	*QGn.jods-3A*	3A	1	0-1.5	BobWhite_c12428_371	wsnp_BG262734A_Ta_2_3	3.2	9.1	1.04
Gnc	-	*QGn.jods-4A.2*	4A	0	0-1.5	Excalibur_c82040_91	wsnp_Ra_rep_c70233_67968353	2.8	5.4	0.8
Gnd	+	*QGn.jods-1A*	1A_LG2	18	17.5-18	wsnp_Ex_c11939_19147602	RAC875_c11599_494	3.6	7.7	-0.83
Gnd	+	*QGn.jods-1B*	1B	108	104.5-108	Kukri_rep_c111174_132	wsnp_BG274687B_Ta_2_1	3.8	7.9	0.84
Gnd	+	*QGn.jods-2B*	2B	172	170.5-174.5	Excalibur_c36070_300	RAC875_c34516_70	5.7	12.1	1.03
Gnd	+	*QGn.jods-5A.2*	5A	292	288.5-292.5	Tdurum_contig10843_745	wsnp_Ex_rep_c101994_87256479	4.2	8.7	0.88
Gnd	-	*QGn.jods-2B*	2B	176	174.5-177.5	CAP12_rep_c3980_87	RAC875_c19690_358	3	6.7	0.57
Gnd	-	*QGn.jods-7D*	7D	59	53.5-62.5	wsnp_CAP11_c2839_1425826	Kukri_c23208_256	2.6	5.6	-0.53
Gw	+	*QGw.jods-5A*	5A	292	290.5-293.5	Tdurum_contig10843_745	wsnp_Ex_rep_c101994_87256479	4.1	11.5	0.12
Gw	-	*QGw.jods-4B.1*	4B	90	84.5-100.5	wsnp_Ra_c1146_2307483	Rht-B1	4.4	11.5	0.12
Gwb	-	*QGw.jods-4B.2*	4B	62	59.5-65.5	JD_c11606_1377	RAC875_c14455_1148	4.8	12.4	0.06
Gwc	+	*QGw.jods-5A*	5A	293	290.5-293.5	wsnp_Ex_rep_c101994_87256479	Excalibur_c26671_282	6	15.7	0.06
Gwc	-	*QGw.jods-4B.1*	4B	90	83.5-101.5	wsnp_Ra_c1146_2307483	Rht-B1	3	8.5	0.04

Trait*	Heat trt‡	QTL	Chrom. §	Position (cM)	Confidence interval (cM)	Left Marker	Right Marker	LOD	PVE ¶ (%)	Additive effect‡
Gwc	-	*QGw.jods-5A*	5A	295	293.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	2.7	7.2	0.04
Gwd	+	*QGw.jods-5A*	5A	293	290.5-293.5	wsnp_Ex_rep_c101994_87256479	Excalibur_c26671_282	3.8	9.9	0.03
Gwd	-	*QGw.jods-2D*	2D	12	0-22.5	Ppd-D1	GENE-0787_85	3.1	11.4	-0.03
Gwd	-	*QGw.jods-5A*	5A	288	284.5-290.5	IAAV108	Ra_c3966_2205	3.2	6.1	0.02
Gwd	-	*QGw.jods-6A*	6A	78	76.5-78.5	TA005366-0788	wsnp_Ku_rep_c112734_95776957	3	5.8	-0.02
Pht	+	*QPht.jods-5A.1*	5A	67	64.5-68.5	RAC875_c232_1895	wsnp_Ex_c7383_12655992	3.2	7.3	-2.79
Pht	+	*QPht.jods-5A.2*	5A	288	283.5-289.5	IAAV108	Ra_c3966_2205	7.4	17.5	4.33
Pht	-	*QPht.jods-4B*	4B	85	80.5-89.5	RAC875_c24550_1150	wsnp_Ra_c1146_2307483	4	11.9	3.57
Pht	-	*QPht.jods-5A.2*	5A	288	283.5-290.5	IAAV108	Ra_c3966_2205	3.6	9.8	3.11
Fll	+	*QFll.jods-5A*	5A	296	294.5-296.5	Vrn-A1	Kukri_c12384_430	25.8	47.5	-3.16
Fll	+	*QFll.jods-5B*	5B	173	172.5-175.5	RAC875_rep_c108940_113	RAC875_c36779_148	2.7	3.6	-0.89
Fll	-	*QFll.jods-5A*	5A	298	296.5-298.5	wsnp_Ex_c22727_31934296	wsnp_Ex_rep_c66689_65010988	16.2	33.2	-2.39
Fll	-	*QFll.jods-6B*	6B	111	108.5-111.5	wsnp_Ra_rep_c106754_90462550	Kukri_c33036_348	4.6	7.8	-1.15
Fla	+	*QFla.jods-2D*	2D	12	0-25.5	Ppd-D1	GENE-0787_85	2.8	6.7	-1.42
Fla	+	*QFla.jods-4A*	4A	0	0-1.5	Excalibur_c82040_91	wsnp_Ra_rep_c70233_67968353	3.9	4.9	1.22
Fla	+	*QFla.jods-5A.1*	5A	92	87.5-96.5	Kukri_c35659_250	GENE-3101_137	3	4	-1.09
Fla	+	*QFla.jods-5A.2*	5A	298	297.5-298.5	wsnp_Ex_c22727_31934296	wsnp_Ex_rep_c66689_65010988	22.4	37.2	-3.37
Fla	+	*QFla.jods-5B*	5B	172	171.5-172.5	IAAV2426	RAC875_rep_c108940_113	2.5	3.2	-1
Fla	-	*QFla.jods-1A*	1A	51	42.5-62.5	RAC875_c34888_65	BS00029346_51	3.6	10.4	-1.54
Fla	-	*QFla.jods-5A.3*	5A	300	299.5-300.5	wsnp_Ex_c7729_13177883	RAC875_rep_c113313_607	10.4	21.8	-2.24

* Da: days to anthesis; Fsp: fertile spikelets per spike; Fspb: fertile spikelets in the basal spike section; Fspc: fertile spikelets in the central spike section; Fspd: fertile spikelets in the distal spike section. Gn: grain number in spike one; Gnb: grain number in the basal spike section; Gnc: grain number in the central spike section; Gnd: grain number in the distal spike section; Gw: grain weight in spike one; Gwb: grain weight in the basal spike section; Gwc: grain weight in the central spike section; Gwd: grain weight in the distal spike section; Pht: plant height; Fll: flag leaf length; Fla: flag leaf area.

‡ (+) Heat-stress treatment; (-) non-temperature stress treatment. § Chromosome number.

¶ PVE= phenotypic variation explained by QTL at the current scanning position. ‡ Positive additive effects for Da, Fsp, Gn, Gw, Fll, and Fla indicate that favorable alleles originated from 'Attila', and negative additive effects indicate that alleles were originated from 'CDC Go' and for Pht it was the opposite.

Table 4.4 Comparison of RILs that had the 'Attila' or 'CDC Go' type alleles at the flanking markers of QTL associated with six traits in 'Attila'× 'CDC Go' RILs evaluated under non-temperature stress and heat-stress conditions.

Trait*	Heat trt‡	QTL	Chrom. §	Position (cM)	Confidence interval (cM)	Mean of RILs with 'Attila' type alleles	Mean of RILs with 'CDC Go' type alleles	Difference‡
Da	+	*QDa.jods-5A*	5A	295	294.5-296.5	67 a	61 b	6.07
Da	-	*QDa.jods-5A*	5A	296	294.5-296.5	67 a	63 b	3.91
Fsp	+	*QFsp.jods-5A*	5A	295	293.5-296.5	13.7 a	11.8 b	1.86
Fspb	+	*QFsp.jods-7B*	7B	239	238.5-239.5	1.6 b	1.8 a	-0.19
Fspc	+	*QFsp.jods-5A*	5A	295	293.5-296.5	1.9 a	1.7 b	0.21
Fspc	+	*QFsp.jods-7B*	7B	239	238.5-239.5	1.6 b	1.9 a	-0.26
Fspc	-	*QFsp.jods-7B*	7B	239	238.5-240.5	2.2 b	2.3 a	-0.17
Fspd	+	*QFsp.jods-4A*	4A_LG2	22	18.5-22	1.2 a	1.1 b	0.13
Fspd	+	*QFsp.jods-5A*	5A	291	290.5-293.5	1.2 a	1.1 b	0.13
Fspd	-	*QFsp.jods-2D*	2D	68	63.5-74	1.4 a	1.3 b	0.09
Gn	+	*QGn.jods-4A.1*	4A_LG2	22	21.5-22	25.5 a	22.1 b	3.43
Gn	+	*QGn.jods-5A*	5A	295	293.5-296.5	26.7 a	21.3 b	5.41
Gnb	+	*QGn.jods-2A*	2A	174	168.5-180.5	7.5 a	6.3 b	1.28
Gnb	+	*QGn.jods-5B*	5B	206	205.5-206.5	7.4 a	6.4 b	0.96
Gnb	+	*QGn.jods-6A*	6A	9	8.5-9.5	7.5	6.3	1.19
Gnc	+	*QGn.jods-5A*	5A	295	294.5-296.5	12.2 a	9.8 b	2.38
Gnc	+	*QGn.jods-7B*	7B	239	238.5-239.5	9.8 b	12.3 a	-2.51
Gnc	-	*QGn.jods-4A.2*	4A	0	0-1.5	14.3 a	13.1 b	1.28
Gnd	+	*QGn.jods-1A*	1A_LG2	18	17.5-18	5.1	6.02	-0.91
Gnd	+	*QGn.jods-2B*	2B	172	170.5-174.5	6.3 a	4.9 b	1.33
Gnd	-	*QGn.jods-2B*	2B	176	174.5-177.5	7.0 a	6.2 b	0.8
Gw	+	*QGw.jods-5A*	5A	292	290.5-293.5	1.0097 a	0.8803 b	0.13
Gwb	-	*QGw.jods-4B.2*	4B	62	59.5-65.5	0.4254 a	0.3605 b	0.06
Gwc	+	*QGw.jods-5A*	5A	293	290.5-293.5	0.4797 a	0.4009 b	0.08
Gwc	-	*QGw.jods-5A*	5A	295	293.5-296.5	0.6066 a	0.5522 b	0.05
Gwd	-	*QGw.jods-5A*	5A	288	284.5-290.5	0.2325 a	0.2021 b	0.03
Pht	+	*QPht.jods-5A.2*	5A	288	283.5-289.5	83 a	78 b	5.65
Pht	-	*QPht.jods-4B*	4B	85	80.5-89.5	87 a	82 b	4.55
Pht	-	*QPht.jods-5A.2*	5A	288	283.5-290.5	88 a	84 b	4.08
Fll	+	*QFll.jods-5A*	5A	296	294.5-296.5	18.8 b	23.1 a	-4.29
Fll	-	*QFll.jods-5A*	5A	298	296.5-298.5	20.6 b	24.1 a	-3.48
Fll	-	*QFll.jods-6B*	6B	111	108.5-111.5	21.4	23.58	-2.17
Fla	+	*QFla.jods-5A.1*	5A	92	87.5-96.5	17.88	20.15	-2.28
Fla	+	*QFla.jods-5A.2*	5A	298	297.5-298.5	16.4 b	21.1 a	-4.72
Fla	-	*QFla.jods-5A.3*	5A	300	299.5-300.5	18.3 b	21.9 a	-3.64

* Da: days to anthesis; Fsp: fertile spikelets per spike; Fspb: fertile spikelets in the basal spike section; Fspc: fertile spikelets in the central spike section; Fspd: fertile spikelets in the distal spike section. Gn: grain number in spike one; Gnb: grain number in the basal spike section; Gnc: grain number in the central spike section; Gnd: grain number in the distal spike section; Gw: grain weight in spike one; Gwb: grain weight in the basal spike section; Gwc: grain weight in the central spike section; Gwd: grain weight in the distal spike section; Pht: plant height; Fll: flag leaf length; Fla: flag leaf area.

‡ (+) Heat-stress treatment; (-) non-temperature stress treatment.

§ Chromosome number.

⁑ Difference between the mean of RILs with 'Attila' and 'CDC Go' type allele. Positive value indicates the mean of RILs with 'Attila' type allele is higher than the mean of RILs with 'CDC Go' type allele and vice versa.

Table 4.5 Chromosomal regions harboring QTL clusters for agronomic traits in the 'Attila' × 'CDC Go' RIL mapping population assessed under controlled environmental conditions.

QTL cluster	QTL within cluster	Trait	Chromosome	Position (cM)	Confidence interval (cM)
1	*QDa.jods-5A*	Days to anthesis	5A	295	294.5-296.5
	QFsp.jods-5A	Fertile spikelets	5A	295	293.5-296.5
	QGn.jods-5A	Grain number	5A	295	293.5-296.5
	QGw.jods-5A	Grain weight	5A	295	293.5-296.5
	QFll.jods-5A	Flag leaf length	5A	296	294.5-296.5
2	*QGn.jods-5A.2*	Grain number	5A	292	288.5-292.5
	QGw.jods-5A	Grain weight	5A	288	284.5-290.5
	QPht.jods-5A.2	Plant height	5A	288	283.5-290.5
3	*QFsp.jods-4B.1*	Fertile spikelets	4B	65	60.5-65.5
	QGn.jods-4B	Grain number	4B	62	57.5-64.5
	QGw.jods-4B.2	Grain weight	4B	62	59.5-65.5
4	*QFsp.jods-4B.2*	Fertile spikelets	4B	90	84.5-100.5
	QGw.jods-4B.1	Grain weight	4B	90	84.5-100.5
	QPht.jods-4B	Plant height	4B	85	80.5-89.5
5	*QDa.jods-2B.2*	Days to anthesis	2B	174	172.5-175.5
	QFsp.jods-2B	Fertile spikelets	2B	180	177.5-181.5
	QGn.jods-2B	Grain number	2B	172-180	170.5-181.5
6	*QFsp.jods-1B*	Fertile spikelets	1B	108	105.5-108
	QGn.jods-1B	Grain number	1B	108	104.5-108
7	*QGn.jods-2D*	Grain number	2D	71	63.5-74
	QFsp.jods-2D	Fertile spikelets	2D	68-74	63.5-74
8	*QGw.jods-2D*	Grain weight	2D	12	0-22.5
	QFla.jods-2D	Flag leaf area	2D	12	0-25.5

QTLs whole spike level
QTLs basal spike section
QTLs central spike section
QTLs distal spike section
Non-temperature stress Underlined
Heat stress No-underlined
1A
1A_LG2
1B
2A
2B
QFla.jods-1A
QGn.jods-1A
QGn.jods-1B
QFsp.jods-1B
QFsp.jods-1B
QDa.jods-1B
QGn.jods-2A
QFsp.jods-2A.2
QFsp.jods-2A.1
QGn.jods-2B
QGn.jods-2B
QGn.jods-2B
QFsp.jods-2B
QDa.jods-2B.2
QDa.jods-2B.1
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280 290 300

QPht.iods-4B
QGw.iods-4B.1
QGw.iods-4B.2
QGw.iods-4B.1
QGn.iods-4B
QFsp.iods-4B.2
QFsp.iods-4B.1
4B
QDa.iods-4B
QDa.iods-4B
QGn.iods-4A.1
4A_LG2
QFsp.iods-4A
QFla.iods-4A
4A
QGn.iods-4A.2
3A
QGn.iods-3A
QFla.iods-2D
QGw.iods-2D
2D
QGn.iods-2D
QFsp.iods-2D
QFsp.iods-2D
QFsp.iods-2D
QFsp.iods-2D
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
280
290
300

5A
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
280
290
300
310
320
QDa.jods-5A
QFsp.jods-5A
QFsp.jods-5A
QFsp.jods-5A
QGn.jods-5A.1
QGn.jods-5A.2
QGn.jods-5A.1
QGw.jods-5A
QGw.jods-5A
QGw.jods-5A
QGw.jods-5A
QPht.jods-5A.2
QPht.jods-5A.2
QFlt.jods-5A
QFla.jods-5A.2
QFla.jods-5A.3
QFla.jods-5A.1
QPht.jods-5A.1

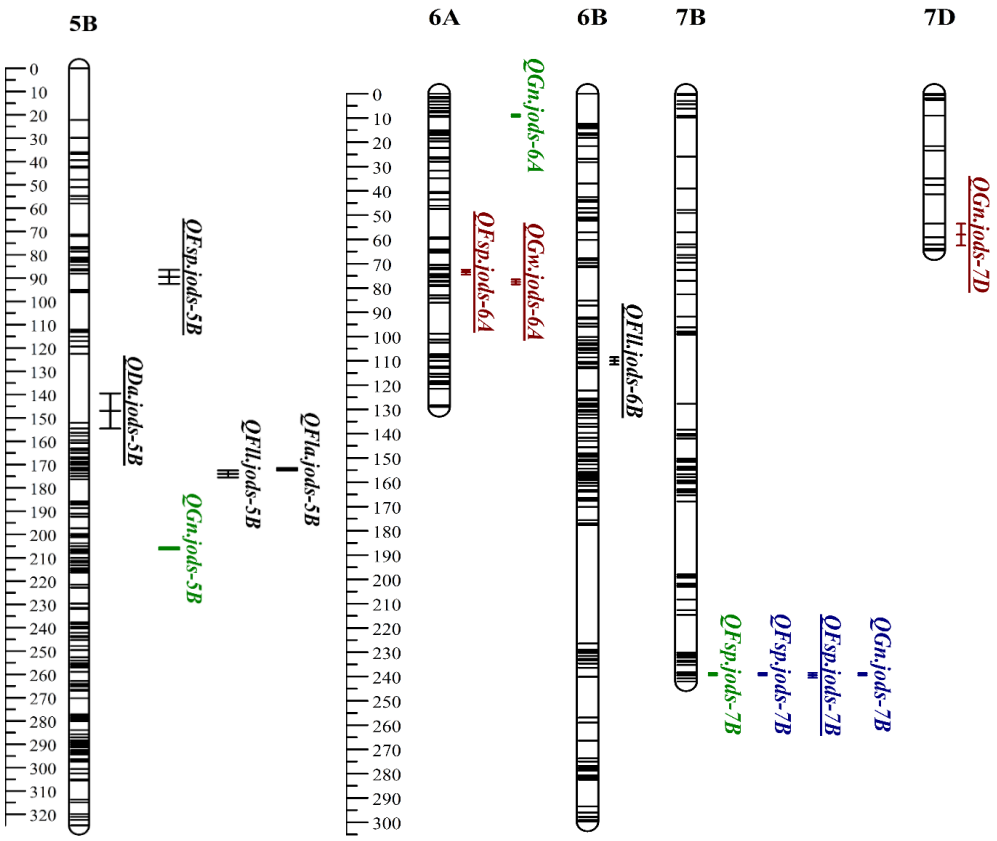

Figure 4.1. Linkage map of the 14 wheat chromosomes that have at least one QTL associated with days to anthesis, fertile spikelets, grain number, grain weight, plant height, and flag leaf size under non-temperature stress (underlined) and heat stress (not underlined) conditions in whole spike level (black) or spike section level [basal (green), central (blue), and distal (red)]. Each horizontal line inside the chromosomes represents a marker and map position is shown on the left side of the chromosomes in centiMorgans (cM). QTL are shown on the right side of each chromosome with bars indicating their confidence interval between the two flanking markers.

Chapter 5- General discussion, conclusions, and future research directions

The pre-anthesis late reproductive phase (stem elongation to anthesis) of wheat is highly sensitive to abiotic stresses, including heat stress, compared to the vegetative and pre-anthesis early reproductive phases (Fischer & Maurer, 1976; Kirby, 1988; Gol et al., 2017). Temperatures above the optimum level for wheat (17-23 °C) during this sensitive growth stage can extensively reduce the grain yield (Fischer & Maurer, 1976; Saini & Aspinall, 1982). Canada is experiencing more frequent and intensified heat stress events aligned with global warming (Sobie et al., 2021). Canada's annual average temperature has increased by 1.7 °C from 1948 to 2012 (Vincent et al., 2015) and is projected to increase by 4.4 °C (3.2 °C to 7.6 °C) by the 2080s (relative to 1971–2000; Sobie et al., 2021). In addition, temperature, and precipitation records from 1950 to 2010 showed an increase in warmer and drier conditions over the Canadian prairies (Singh et al., 2020). Selecting wheat cultivars to grow under heat-stressed environments in the Canadian prairies, where approximately 95% of the Canadian wheat production is occurs (USDA, 2019), will be a significant challenge for the future. Existing cultivars will require advanced agronomic management practices to stabilize the wheat grain yield under heat-stressed environments, as yield-related quantitative traits are often influenced by environmental factors and exhibit high genotype × environment interactions (Cooper et al., 2021). Furthermore, marker-assisted selection programs can be used to select heat-resistant wheat genotypes with respect to grain yield. QTL analyses play an important role in identifying genomic regions associated with improved grain yield and yield-related traits under heat-stressed environments as an initial step of marker-assisted selection. However, to the best of our knowledge, QTL analyses were minimally conducted on Canadian wheat genotypes to identify heat-resistance QTL associated with stable or higher grain yield under heat stress environments.

The experiments presented in this thesis focused on identifying the negative effects of flowering time heat stress on grain yield and yield component parameters in selected Canadian wheat cultivars and 171 RILs developed from a cross between Canadian wheat cultivar CDC Go and CIMMYT wheat cultivar Attila. In addition, the potential of 4-Cl-IAA (1 µM) application as an agronomic tool to ameliorate the negative effects of heat stress was also assessed. Finally, ICIM analyses were used to identify the stable QTL associated with six-grain yield-related agronomic

traits under heat-stress, and/or non-temperature stress conditions in the 'Attila' × 'CDC Go' RIL population.

5.1 Screening RIL parental cultivars for responses to heat-stress and auxin application on grain yield parameters

Study one evaluated the responses of the RIL parents ('Attila', 'CDC Go', and 'CDC Teal') to heat stress and one time foliar 4-Cl-IAA (1µM) application under non-temperature stress and heat-stress conditions at early flowering (pre-anthesis late reproductive growth stage). The RIL parent 'Attila' produced the highest grain number and weight under both non-temperature stress and heat-stress conditions compared to 'CDC Go' and 'CDC Teal', even after approximately 30% reduction under the heat-stress conditions (Table 2.4). These results indicate that the cultivar Attila is a potential cultivar to grow under warmer environments as suggested by the previous studies (Yang et al., 2002). Furthermore, 'CDC Go' and 'Attila' positively responded to the one time foliar 4-Cl-IAA (1µM) application and showed 4-Cl-IAA has the ability to ameliorate the negative effects of heat stress. In this study, the first three evaluated spikes of each cultivar responded similarly to the heat stress and 4-Cl-IAA. It indicates that growth stage variation observed between the spikes (Fig. A1) within the cultivar is not a factor that changes the treatment responses as far as the developing spikes remain in the pre-anthesis late reproductive phase. Results of this first study and the previous data from the wheat breeding group of University of Alberta (Asif et al., 2015; Zou et al., 2017 a and b), helped us to select the 'Attila' × 'CDC Go' RIL population as the best RIL population for an in-depth study on heat tolerance and the effect of 4-Cl-IAA (1µM) application on heat tolerance.

5.2 Effect of heat stress and auxin application on grain yield parameters of selected wheat cultivars and an 'Attila' x 'CDC Go' RIL population

The second study evaluated the effects of flowering time heat stress and one-time foliar 4-Cl-IAA (1µM) application on seven standard Canadian wheat cultivars and the 'Attila' × 'CDC Go' RIL population (171 RILs). Few important discissions were made while conducting and analyzing study two.

By considering the responses of first three spikes to heat stress and auxin treatments in study one, and the development of spikes withing the limited root medium (0.35-L) inside the root

trainer cells in study two, we decided to obtain data from the main tiller spike (spike 1) for study two. All the other tillers were removed as they developed (when tillers were 5-10 cm tall).

In this experiment heat stress and 4-Cl-IAA treatments were given when the majority of the plants were at BBCH 37-49 (a broad window between the flag leaf initiation to first awns visible). Number of days to anthesis under non-temperature stress treatment can give an indirect idea about the plant growth stage of each RIL during the treatment application. RIL population range for days to anthesis was 36 (maximum: 86; minimum: 50) under non-temperature stress conditions. However, the RILs with a lower number of days to anthesis (higher growth rate; 50-55 days to anthesis; 9% of the RILs) and a higher number of days to anthesis (slower growth rate; 80-86 days to anthesis; 5% of the RILs) were also ended up in different heat tolerance categories (heat resistant, moderately heat susceptible and heat susceptible) regardless of their growth stage during the treatment application. These results indicate that even the RILs at the extreme ends of tested growth stages have not escaped from the heat stress treatments. Therefore, the treatment application window (BBCH 37-49) was appropriate for identifying the heat stress effects on evaluated cultivars and the RIL population.

The available space inside the growth chamber allowed us only to use one plant from each RIL and from each standard wheat cultivar per treatment [4 treatments (2 temperatures x 2 auxin treatment solutions) and a total of 200 plants per treatment; 171 RILs + 11 Attila + 11 CDC Go + 7 standard cultivar plants]. Therefore, we repeated the experiment 4 times. However, statistical analyses of 'Attila' and 'CDC Go' with 4 replicates (n=4; Table 3.1) and 40 replicates (n=40; Table 3.2) showed the importance of having more replicates. The number of replications (n=4) used in this experiment was only sufficient to differentiate larger differences. For example, in the standard wheat cultivars, heat stress reduced the number of days to anthesis in 'Cutler' by 15 days, and this difference was large enough to statistically differentiate with a lower number of replications (Table 3.1). However, a 2-day difference in number of days to anthesis under non-temperature stress and heat-stress conditions was not large enough to statistically differentiate when analyzed with a lower number of replicates (n=4; 'AC Andrew'; Table 3.1), but it was large enough when analyzed with a greater number of replicates (n=40; 'Attila'; Table 3.2). Therefore, we did not compare standard wheat cultivars with respect to heat-stress or 4-Cl-IAA treatment responses. Results and discussion related to the standard wheat cultivars were arranged with a focus on RIL parents 'Attila' and 'CDC Go'.

‘Attila’ showed a higher heat tolerance ratio (0.93-0.96) with respect to grain yield (grain number and weight) compared to ‘CDC Go’ (0.54-0.55; Figs. 3.20C and 3.21C) under the root trainer cell conditions. These results confirmed the findings of study one: that the RIL parents ‘Atilla’ and ‘CDC Go’ vary in heat tolerance, and ‘Attila’ produced more grain yield under heat stress. The results suggest that the root trainer system is appropriate to measure heat-tolerance related phenotypes.

5.2.1 Results summary

Study 2 identified the most relevant yield components responsible for wheat grain yield improvement under non-temperature stress and heat-stress conditions. Strong to weak positive relationships were observed between the grain yield (grain number and weight) and the number of days to anthesis in the standard wheat cultivars and the RIL population (Figs. 3.2 and 3.15). These results indicate that a longer flowering time is beneficial for increasing wheat grain yield unless the growing season length is short. Furthermore, Pearson’s correlations showed traits related to the spike architecture (grain number per spike, total or fertile spikelets per spike, grain number per spikelet or per fertile spikelet) strongly or moderate strongly positively correlated with the grain weight under non-temperature stress and heat-stress conditions in the standard wheat cultivars and the RIL population (Figs. 3.4, 3.6, 3.7, 3.17, 3.19, and 3.22). These results showed that the spike architecture-related traits are important to consider in wheat breeding programs to enhance the grain yield, as mentioned by previous researchers (Shearman et al., 2005; Slafer et al., 2014; Guo et al., 2018; Wolde et al., 2019). The strength or the significance level of a few relationships between measured traits were modified by the heat stress treatment (relationships between total or fertile spikelets per spike and the days to anthesis in standard wheat cultivars, Fig. 3.1; total or fertile spikelets per spike and the grain weight in standard wheat cultivars, Fig. 3.4; total spikelets per spike and the days to anthesis in the RIL population, Fig. 3.14) stressing the importance of cultivar trait evaluation under environments where the cultivar will be grown, as grain yield parameters are highly sensitive to the growing environment (Cooper et al., 2021).

Heat stress reduced the population mean grain weight and number with a substantial reduction in fertile spikelets per spike and grain number per spikelet or per fertile spikelet (Table 3.4). Heat stress-induced grain number reduction was reflected by the lower grain number in all three spike sections of the main tiller spike (Table 3.5). Grain weight reduction was more

pronounced in the central spike section (Table 3.5). Each RIL responded differently to the heat stress treatment. The heat tolerance ratio of grain weight was used to categorize RILs into three heat tolerance groups: heat resistant (45% of the RILs, 77 RILs), moderately heat susceptible (20.5% of the RILs, 35 RILs), and highly heat susceptible (7.6% RILs, 13 RILs; Table B4). Significant differences observed in most of the measured traits under non-temperature stress and heat stress conditions, and the range of heat tolerance observed among the RILs, made the 'Attila' × 'CDC Go' RIL population suitable for in-depth genotypic analyses.

With respect to 4-Cl-IAA effect, a lower number of replications only allowed us to identify the most pronounced 4-Cl-IAA treatment responses. However, the results showed 4-Cl-IAA can negate the negative effects of heat stress on grain yield in some genotypes but not all (RILs 46, 80, and 145; Table 3.6). 4-Cl-IAA induced grain yield increase was reflected in the increased number of grains per spikelet or per fertile spikelet and the number of fertile spikelets per spike (Table 3.6). Furthermore, results showed 4-Cl-IAA could increase the grain yield under non-temperature stress conditions (RILs 70 and 18; Table 3.6). These results confirm our previous findings (Abeysingha et al., 2021) and indicate that auxin application promoted photoassimilate partitioning into the spikes, improving grain set and weight as suggested by Bangerth et al. (1985) and Darussalam et al. (1998).

5.3 QTL associated with yield and yield component parameters in an 'Attila'× 'CDC Go' RIL population assessed under heat-stress and non-temperature stress conditions

Study 3 was conducted to identify the QTL associated with grain yield and yield component parameters specific to, or common with, non-temperature stress and/or heat-stress conditions at flowering. ICIM identified 73 QTL associated with six traits (days to anthesis, number of fertile spikelets, grain number, grain weight, plant height, and flag-leaf size) in the 'Attila' × 'CDC Go' RIL population (167 RILs) at the whole spike and spike section levels under non-temperature stress (NS) and heat-stress (HS) conditions. Two or more major to minor QTL were identified for each trait, demonstrating the quantitative nature of those traits. Among the detected QTL, 49 were obtained trait enhancing alleles from the highest yielding parental cultivar 'Attila', stressing the importance of those QTL in increasing grain yield in wheat. This study identified eight important QTL clusters associated with two or more grain yield or yield-related traits on chromosomes 5A, 4B, 2B, 2D, and 1B. Among them, three QTL clusters were specific to

non-temperature stress conditions (63.5-74 cM genomic region on chromosome 2D; 80.5-100.5 and 57.5-65.5 cM genomic regions on chromosome 4B) and five were common to non-temperature stress and heat-stress conditions (293.5-296.5 and 283.5-292.5 cM genomic regions on chromosome 5A; 170.5-181.5 cM genomic region on chromosome 2B; 0-25.5 cM genomic region on chromosome 2D; and 104.5-108 cM genomic region on chromosome 1B). Other than the QTL clusters, single important QTL associated with flag leaf area was identified in the 299.5-300.5 cM genomic region on chromosome 5A under non-temperature stress conditions. Most of the genomic regions identified as important in this study were previously identified in the wheat genome (Kato et al., 2000; Gahlaut et al., 2017; Zou et al., 2017b; Liu et al., 2018), especially 63.5-74 cM genomic region on chromosome 2D, 84.5-100.5 cM genomic region on chromosome 4B, and 293.5-296.5 cM genomic region on chromosome 5A was previously detected in the same RIL population under western Canadian field conditions (Zou et al., 2017b). It indicates the experimental design used in this study was appropriate to precisely identify the QTL associated with grain yield and yield-related components under non-temperature stress and heat-stress conditions.

5.4 Future research directions

Studies 1 and 2 indicated that flowering time heat stress negatively affects wheat grain yield and yield component parameters, and the magnitude of the effect depends on the genotype. Therefore, multi-environment trials (including environments that represent future predicted heat-stress conditions) to select superior Canadian wheat cultivars based on their performance will be advantageous. In addition, heat stress in the field often occurs with drought stress, which may affect the expression of the tolerance. Therefore, controlled environment experiments involving both heat and water stress conditions would help understand stress-related plant responses in a realistic direction.

Fine mapping of the important genomic regions identified in this study for days to anthesis, number of fertile spikelets, grain number, grain weight, plant height, and flag-leaf size that are specific to or common under non-temperature stress and heat-stress conditions will be helpful in marker assisted breeding.

Further, testing of RILs with more replications to identify the ability of 4-Cl-IAA to increase grain yield in wheat and to detect QTL related to 4-Cl-IAA responses will be beneficial for broadening our understanding of how auxins can be used to increase wheat grain yield.

References

Abeysingha, D. N., Ozga, J. A., Strydhorst, S., Doyle, P., Iqbal, M., Yang, R. C., & Reinecke, D. M. (2021). The effect of auxins on amelioration of heat stress-induced wheat (*Triticum aestivum* L.) grain loss. *Journal of Agronomy & Crop Science*, *207*, 970-983.

Agriculture and Agri-food Canada (2021). Outlook for principal field crops. https://agriculture.canada.ca/en/canadas-agriculture-sectors/crops/reports-and-statistics-data-canadian-principal-field-crops/canada-outlook-principal-field-crops-2021-08-20 Accessed, December 2021.

Akter, N., & Islam R. M. (2017). Heat stress effects and management in wheat. A review. *Agronomy for Sustainable Development. 37*, 37.

Aloni, R., Aloni, E., Langhans, M., & Ullrich, C. I. (2006). Role of auxin in regulating *Arabidopsis* flower development. *Planta*, *223*, 315-328.

Asif, M. (2014). Quantitative trait loci (QTL) mapping and doubled haploid technology for spring wheat improvement. Ph.D. thesis, University of Alberta.

Asif, M., Yang, R. C., Navabi, A., Iqbal, M., Kamran, A., Lara, E. P., Randhawa, H., Pozniak, C. & Spaner, D. (2015). Mapping QTL, selection differentials, and the effect of under organic and conventionally managed systems in the 'Attila' × 'CDC Go' spring wheat mapping population. *Crop Science*, *55*, 1129-1142.

Asins, M. J., Bernet, G. P., Villalta, I., & Carbonell, E. A. (2010). QTL analysis in plant breeding. Jain M. S. & Brar D. S. (eds). *Molecular Techniques in Crop Improvement*. *Springer, Dordrecht*.

Assanga, S. O., Fuentealba, M., Zhang, G., Tan, C., Dhakal, S., Rudd, J. C., Ibrahim, A., Xue, Q., Haley, S., Chen, J., Chao, S., Baker, J., Jessup, K., & Liu, S. (2017). Mapping of quantitative trait loci for grain yield and its components in a US popular winter wheat TAM 111 using 90 K SNPs. *PLOS ONE*, *12*, e0189669.

Asseng, S., Ewert, F., Martre, P., Rötter, R. P., Lobell, D. B., Cammarano, D., Kimball, B. A., Ottman, M. J., Wall, G. W., White, J. W., & Reynolds, M. P. (2015). Rising temperatures reduce global wheat production. *Nature Climate Change*, *5*, 143-147.

Asseng, S., Foster, I. & Turner, N. C. (2011). The impact of temperature variability on wheat yields. *Global Change Biology*, *17*, 997-1012.

Båga, M., Fowler, D. B., & Chibbar, R. N. (2009). Identification of genomic regions determining the phenological development leading to floral transition in wheat (*Triticum aestivum* L.). *Journal of Experimental Botany*, *60*, 3575-3585.

Bangerth, F., Aufhammer, W., & Baum, O. (1985). IAA level and dry matter accumulation at different positions within a wheat ear. *Physiologia Plantarum*, *63*, 121-125.

Beales, J., Turner, A., Griffiths, S., Snape, J. W., & Laurie, D. A. (2007). A pseudo-response regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (*Triticum aestivum* L.). *Theoretical & Applied Genetics. 115*, 721-733.

Bheemanahalli, R., Sunoj, V. S. J., Saripalli, G., Prasad, P. V. V., Balyan, H. S., Gupta, P. K., Grant, N., Gill, K. S., & Jagadish, S. V. K. (2019). Quantifying the impact of heat stress on pollen germination, seed set, and grain filling in spring wheat. *Journal of Crop Science*, *59*, 684-696.

Bhusal, N., Sarial, A. K., Sharma, P., & Sareen, S. (2017). Mapping QTL for grain yield components in wheat under heat stress. *PLOS ONE*, *12*, e0189594.

Botstein, D., White, R. L., Skolnick, M., & Davis, R. W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics*, *32*, 314-331.

Bowden, P., Edwards, J., Ferguson, N., McNee, T., Manning, B., Roberts, K., Schipp, A., Schulze, K., & Wilkins, J. (2008). Wheat growth and development. In Edwards, J., Roberts, K., eds, *Reproductive Development*. *New South Wales Department of Primary industries*, 51-58.

Brenchley, R., Spannagl, M., Pfeifer, M., Barker, G. L., D'Amore, R., Allen, A. M., McKenzie, N., Kramer, M., Kerhornou, A., Bolser, D., Kay, S., Waite, D., Trick, M., et al. (2012). Analysis of the bread wheat genome using whole-genome shotgun sequencing. *Nature*, *491*, 705-710.

Brown, T. A., Jones, M. K., Powell, W., & Allaby, R. G. (2009). The complex origins of domesticated crops in the Fertile Crescent. *Trends in Ecology & Evolution*, *24*, 103-109.

Browne, R. G., Iacuone, S., Li, S. F., Dolferus, R., & Parish, R. W. (2018). Anther morphological development and stage determination in *Triticum aestivum*. *Frontiers in Plant Science*, *9*, 228.

Calderini, D. F. & Reynolds, M. P. (2000). Changes in grain weight as a consequence of de-graining treatments at pre- and post-anthesis in synthetic hexaploid lines of wheat (*Triticum durum* × T. *tauschii*). *Australian Journal of Plant Physiology*, *27*, 183-191.

Canadian Food Inspection Agency (2019). Crop reports. Available at http://www.inspection.gc.ca/english/plaveg/pbrpov/cropreport/whe/app00007650e.shtml Accessed, January 2019.

Canadian Grain Commission (2021). Canadian wheat classes. https://grainscanada.gc.ca/en/grain-quality/grain-grading/wheat-classes.html Accessed, December 2021.

Cao, Y. Y., & Zhao, H. (2008). Protective roles of brassinolide on rice seedlings under high temperature stress. *Rice Science*, *15*, 63-68.

Carew, R., Meng, T., Florkowski, W. J., Smith, R., & Blair, D. H. (2017). Climate change impacts on hard red spring wheat yield and production risk: evidence from Manitoba, Canada. *Canadian Journal of Plant Science*, *98*, 782-795.

Cavanagh, C. R., Chao, S., Wang, S., Huang, B. E., Stephen, S., Kiani, S., Forrest, K. et al. (2013). Genome-wide comparative diversity uncovers multiple targets of selection for improvement in hexaploid wheat landraces and cultivars. *Proceedings of the National Academy of Sciences of United States of America*, *110*, 8057-8062.

Cecchetti, V., Altamura, M. M., Falasca, G., Costantino, P., & Cardarelli, M. (2008). Auxin regulates *Arabidopsis* anther dehiscence, pollen maturation, and filament elongation. *The Plant Cell, 20*, 1760-1774.

Chen, F., Gao, M., Zhang, J., Zuo, A., Shang, X., & Cui, D. (2013). Molecular characterization of vernalization and response genes in bread wheat from the Yellow and Huai Valley of China. *BMC Plant Biology*, *13*, 199.

Chen, H., Iqbal, M., Perez-Lara, E., Yang, R. C., Pozniak, C. & Spaner, D. (2015). Earliness *per se* quantitative trait loci and their interaction with *Vrn-B1* locus in a spring wheat population. *Molecular Breeding*, *35*, 182.

Chen, S., Gao, R., Wang, H., Wen, M., Xiao, J., Bian, N., Zhang, R., Hu, W., Cheng, S., Bie, T., & Wang, X. (2015). Characterization of a novel reduced height gene (*Rht23*) regulating panicle morphology and plant architecture in bread wheat. *Euphytica*, *203*, 583-594.

Cheng, Y., Dai, X., & Zhao, Y. (2006). Auxin biosynthesis by the YUCCA flavin monooxygenases controls the formation of floral organs and vascular tissues in *Arabidopsis*. *Genes & Development*, *20*, 1790-1799.

Chowdhury, A. R., Ghosh, M., Lal, M., Pal, A., Hazra, K. K., Acharya, S., Chaurasiya, A., & Pathak, S. K. (2020). Foliar spray of synthetic osmolytes alleviates terminal heat stress in late-sown wheat. *International Journal of Plant Production*. *14*, 321-333.

Cole, D. M. A., & Patrick, J. W. (1998). Auxin control of photoassimilate transport to and within developing grains of wheat. *Australian Journal of Plant Physiology*, *25*, 69-77.

Cooper, M., Voss-Fels, K. P., Messina, C. D., Tang, T., & Hammer, G. L. (2021). Tackling G × E × M interactions to close on-farm yield-gaps: Creating novel pathways for crop improvement by predicting contributions of genetics and management to crop productivity. *Theoretical & Applied Genetics*, *134*, 1625-1644.

Darussalam, M., Cole, M. A., & Patrick, J. W. (1998). Auxin control of photoassimilate transport to and within developing grains of wheat. *Australian Journal of Plant Physiology, 25*, 69-77.

De Vries, A. P. (1971). Flowering biology of wheat, particularly in view of hybrid seed production-A review. *Euphytica*, *20*, 152-170.

Deng, S. M., Wu, X. R., Wu, Y. Y., Zhou, R. H., Wang, H. G., Jia, J., & Liu, S. (2011). Characterization and precise mapping of a QTL increasing spike number with pleiotropic effects in wheat. *Theoretical & Applied Genetics*, *122*, 281-289.

DePauw, R., & O'Brien, L. (2016). Wheat breeding: Exploiting and fixing genetic variation by selection and evaluation. *Encyclopedia of Food Grains*, *4*, 279-286.

Djanaguiraman, M., Perumal, R., Jagadish, S. V. K., Ciampitti, I. A., Welti, R., & Prasad, P. V. V. (2018). Sensitivity of sorghum pollen and pistil to high-temperature stress. *Plant, Cell & Environment*, *41*, 1065-1082.

Doebley, J. F., Gaut, B. S. & Smith, B. D. (2006). The molecular genetics of crop domestication. *Cell*, *127*, 1309-1321.

Doyle, J. J. & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, *19*, 11-15.

Dwivedi, S. K., Basu, S., Kumar, S., Kumar, G., Prakash, V., Kumar, S., Mishra, J. S., Bhatt, B. P., Malviya, N., Singh, G. P., & Arora, S. A. (2017). Heat stress induced impairment of

starch mobilization regulates pollen viability and grain yield in wheat: study in Eastern Indo-Gangetic Plains. *Field Crops Research*, *206*, 106-11.

Dwivedi, S. K., Basu, S., Kumar, S., Kumari, S., Kumar, A., Jha, S., Mishra, J. S., Bhatt, B. P. & (2019). Enhanced antioxidant enzyme activities in developing anther contributes to heat stress alleviation and sustains grain yield in wheat. *Functional Plant Biology, 46*, 1090-1102.

Ellis, H., Spielmeyer, W., Gale, R., Rebetzke, J., & Richards, A. (2002). "Perfect" markers for the Rht-B1b and Rht-D1b dwarfing genes in wheat. *Theoretical & Applied Genetics, 105*, 1038-1042.

El-Saeid, H. M., Abou-Hussein, S. D., & El-Tohamy, W. A. (2010). Growth characters, yield, and endogenous hormones of cowpea plants in response to IAA application. *Research Journal of Agriculture & Biological Sciences*, *6*, 27-31.

Estelle, M. (1996). Plant tropisms: The ins and outs of auxin. *Current Biology*, *6*, 1589-1591.

Fábián, A., Sáfrán, E., Szabó-Eitel, G., Barnabás, B., & Jäger, K. (2019). Stigma functionality and fertility are reduced by heat and drought co-stress in wheat. *Frontiers in Plant Science*, *10*, 244.

Fan, X., Cui, F., Ji, J., Zhang, W., Zhao, X., Liu, J., Meng, D., Tong, Y., Wang, T., & Li, J. (2019). Dissection of pleiotropic QTL regions controlling wheat spike characteristics under different nitrogen treatments using traditional and conditional QTL mapping. *Frontiers in Plant Science*, *10*, 187.

Fan, X., Cui, F., Zhao, C., Zhang, W., & Yang, L. (2015). QTL for flag leaf size and their influence on yield-related traits in wheat (*Triticum aestivum* L.). *Molecular Breeding*, *35*, 24.

Farooq, M., Bramley, H., Palta, J. A. & Siddique, K. H. M. (2011). Heat stress in wheat during reproductive and grain-filling phases. *Critical Reviews in Plant Sciences, 30*, 491-507.

Fischer, R. (1985). Number of kernels in wheat crops and the influence of solar radiation and temperature. *Journal of Agricultural Science*, *105*, 447-461.

Fischer, R. A. & Maurer, O. R. (1976). Crop temperature modification and yield potential in a dwarf spring wheat. *Crop Science*, *16*, 855-859.

Foulkes, M. J., Slafer, G. A., Davies, W. J., Berry, P. M., Sylvester-Bradley, R., Martre, P., Calderini, D. F., Griffiths, S., & Reynolds, M. P. (2011). Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance, *Journal of Experimental Botany*, *62*, 469-486.

Foyer, C. H., Lopez-Delgardo, H., Dat, J. F., & Scott, I. M. (1997). Hydrogen peroxide and glutathione associated mechanisms of acclimatory stress tolerance and signaling. *Physiologia Plantarum*, *100*, 241-254.

Francki, M. G., Walker, E., Crawford, A. C., Broughton, S., Ohm, H. W., Barclay, I., Wilson, R. E., & McLean, R. (2009). Comparison of genetic and cytogenetic maps of hexaploid wheat (*Triticum aestivum* L.) using SSR and DArT markers. *Molecular Genetics & Genomics*, *281*, 181-191.

Gahlaut, V., Jaiswal, V., Tyagi, B. S., Singh, G., Sareen, S., Balyan, H. S., & Gupta, P. K. (2017). QTL mapping for nine drought-responsive agronomic traits in bread wheat under irrigated and rain-fed environments. *PLOS ONE*, *12*, e0182857.

Giraldo, P., Benavente, E., Manzano-Agugliaro, F. & Gimenez, E. (2019). Worldwide research trends on wheat and barley: A bibliometric comparative analysis. *Agronomy*, *9*, 352.

Gol, L., Tomé, F., & Von Korff, M., (2017). Floral transitions in wheat and barley: interactions between photoperiod, abiotic stresses, and nutrient status. *Journal of Experimental Botany, 68*, 1399-1410.

González, F. G., Miralles, D. J., & Slafer, G. A. (2011). Wheat floret survival as related to pre-anthesis spike growth. *Journal of Experimental Botany, 62*, 4889-4901.

González, F. G., Slafer, G. A., & Miralles, D. J. (2003). Grain and floret number in response to photoperiod during stem elongation in fully and slightly vernalized wheats. *Field Crops Research*, *81*, 17-27.

Guan, P., Lu, L., Jia, L., Kabir, M. R., Zhang, J., Lan, T., Zhao, Y., Xin, M., Hu, Z., Yao, Y., Ni, Z., Sun, Q., & Peng, H. (2018). Global QTL analysis identifies genomic regions on chromosomes 4A and 4B harboring stable loci for yield-related traits across different environments in wheat (*Triticum aestivum* L.). *Frontiers in Plant Science*, *9*, 529.

Guo, Z., Zhao, Y., Röder, M. S., Reif, J. C., Ganal, M. W., Chen, D., & Schnurbusch, T. (2018). Manipulation and prediction of spike morphology traits for the improvement of grain yield in wheat. *Scientific Reports*, *8*, 14435.

Hanaa, H. A. & Safaa, A. M. (2019). Foliar application of IAA at different growth stages and their influence on growth and productivity of bread wheat (*Triticum aestivum* L.). *Journal of Physics: Conference Series, 1294*, 092029.

Huang, X. Q., Kempf, H., Ganal, M. W., & Röder, M. S. (2004). Advanced backcross QTL analysis in progenies derived from a cross between a German elite winter wheat variety and a synthetic wheat (*Triticum aestivum* L.). *Theoretical & Applied Genetics*, *109*, 933-943.

Hughes, G. R. & Hucl, P. (1993). CDC Teal hard red spring wheat. *Canadian Journal of Plant Science*, *73*, 193-197.

Hyles, J., Bloomfield, M., Hunt, J., Trethowan, R., & Trevaskis, B. (2020). Phenology and related traits for wheat adaptation. *Heredity*, *125*, 1-14.

Iqbal, M., Navabi, A., Salmon, D. F., Yang, R-C, Murdoch, B. M., Moore, S. S., & Spaner, D. (2007a). Genetic analysis of flowering and maturity time in high latitude spring wheat: Genetic analysis of earliness in spring wheat. *Euphytica*, *154*, 207-218.

Iqbal, M., Navabi, A., Yang, R. C., Salmon, D. F. & Spaner, D. (2007b). The effect of vernalization genes on earliness and related agronomic traits of spring wheat in northern growing regions. *Crop Science*, *47*, 1031-1039.

Iqbal, M., Shahzad, A. & Ahmed, I. (2011). Allelic variation at the *Vrn-A1, Vrn-B1, Vrn-D1, Vrn-B3* and Ppd-D1a loci of Pakistani spring wheat cultivars. *Electronic Journal of Biotechnology*, *14*, 1-8.

Jaccoud, D., Peng, K., Feinstein, D., & Kilian, A. (2001). Diversity arrays: a solid-state technology for sequence information independent genotyping. *Nucleic Acids Research*, *29*, e25.

Jansen, R. C. (1993). Interval mapping of multiple quantitative trait loci. *Genetics*, *135*, 205-211.

Jansen, R. C., & Stam, P. (1994). High resolution of quantitative traits into multiple loci via interval mapping. *Genetics*, *136*, 1447-1455.

Jia, H., Wan, H., Yang, S., Zhang, Z., Kong, Z., Xue, S., Zhang, L., & Ma, Z. (2013). Genetic dissection of yield related traits in a recombinant inbred line population created using a key breeding parent in China's wheat breeding. *Theoretical & Applied Genetics*, *126*, 2123-2139.

Jiang, G. L. (2013). "Molecular markers and marker-assisted breeding in plants" in plant breeding from laboratories to fields, *IntechOpen*, *Croatia*, 45-83.

Jobson, E. M., Johnston, R. E., Oiestad, A. J., Martin, J. M., & Giroux, M. J. (2019). The impact of the wheat *Rht-B1b* semi-dwarfing allele on photosynthesis and seed development under field conditions. *Frontiers in Plant Science*, *10*, 51.

Jordan, S. A., & Humphries, P. (1994). Single nucleotide polymorphism in exon 2 of the BCP gene on 7q31 - q35. *Human Molecular Genetics*, *3*, 1915.

Kamran, A., Randhawa, H. S., Pozniak, C. & Spaner, D. (2013). Phenotypic effects of the flowering gene complex in Canadian spring wheat germplasm. *Crop Science*, *53*, 84-94.

Kato, K., Miura, H., & Sawada, S. (2000). Mapping QTL controlling grain yield and its components on chromosome 5A of wheat. *Theoretical & Applied Genetics*, *101*, 1114-1121.

Kaur, G., Tak, Y., & Asthir, B. (2022). Salicylic acid: a key signal molecule ameliorating plant stresses. *Cereal Research Communications*. https://doi-org.login.ezproxy.library.ualberta.ca/10.1007/s42976-021-00236-z

Khan, S. (2015). QTL Mapping: A tool for improvement in crop plants. *Research Journal of Recent Sciences*, *4*, 7-12.

Kirby, E. J. M. (1974). Ear development in spring wheat. *The journal of Agricultural Science*, *82*, 437-447.

Kirby, E. J. M. (1988). Analysis of leaf, stem, and ear growth in wheat from terminal spikelet stage to anthesis. *Field Crops Research, 18*, 127-140.

Knott, S. A. (2005). Regression-based quantitative trait loci mapping: robust, efficient, and effective. *Philosophical transactions of the Royal Society of London. Series B, Biological Sciences*, *360*, 1435-1442.

Koppolu, R., Jiang, G., Milner, S. G., Muqaddasi, Q. H., Rutten, T., Himmelbach, A., Guo, Y., Stein, N., Mascher, M., & Schnurbusch, T. (2022). The barley mutant multiflorus2.b reveals quantitative genetic variation for new spikelet architecture. *Theoretical & Applied Genetics*, *135*, 571-590.

Kuchel, H., Hollamby, G., Langridge, P., Williams, K., & Jeferies, S. P. (2006). Identification of genetic loci associated with ear-emergence in bread wheat. *Theoretical & Applied Genetics*, *13*, 1103-1112.

Kumar, R. R., Goswami, S., Gadpayle, K. A., Singh, K., Sharma, S. K., Singh, G. P., Pathak, H., & Rai, R. D. (2013). Ascorbic acid at pre-anthesis modulate the thermotolerance level of wheat (*Triticum aestivum* L.) pollen under heat stress. *Journal of Plant Biochemistry & Biotechnology, 23*, 293-306.

Kumarapeli, K. A. D. V. (2021). Effect of heat stress on reproductive development of wheat (*Triticum aestivum* L.). M. Sc. Thesis, University of Alberta.

Kumari, A., & Hemantaranjan, A. (2019). Mitigating effects of 24-epibrassinolide on heat stress damage by shifting biochemical and antioxidant defense mechanisms in wheat (*Triticum aestivum* L.) at pre-flowering stage and post-flowering stage. *Journal of Pharmacognocy & Phytochemistry*, *8*, 1157-1161.

Lancashire, P. D., Bleiholder, H., van den Bloom, T., Langeluddeke, P., Stauss R., Weber, E., & Witzenberger, A. (1991). A uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology*, *119*, 561-601.

Lander, E. S., & Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, *121*, 185-199.

Li, F., Wen, W., He, Z., Liu, J., Jin, H., Cao, S., Geng, H., Yan, J., Zhang, P., Wan, Y., & Xia, X. (2018). Genome-wide linkage mapping of yield-related traits in three Chinese bread wheat populations using high-density SNP markers. *Theoretical & Applied Genetics*, *131*, 1903-1924.

Li, H., Ye, G., & Wang, J. (2007). A modified algorithm for the improvement of composite interval mapping. *Genetics*, *175*, 361-374.

Li, X., Lawas, L. M., Malo, R., Glaubitz, U., Erban, A., Mauleon, R., Heuer, S., Zuther, E., Kopka, J., Hincha, D. K., & Jagadish, K. S. (2015). Metabolic and transcriptomic signatures of rice floral organs reveal sugar starvation as a factor in reproductive failure under heat and drought stress. *Plant, Cell & Environment*, *38*, 2171-2192.

Li, Y., Fu, X., Zhao, M., Zhang, W., Li, B., An, D., Li, J., Zhang, A., Liu, R., & Liu, X. (2018). A genome-wide view of transcriptome dynamics during early spike development in bread wheat. *Scientific Reports*, *8*, 15338.

Liu, B., Liu, L., Asseng, S., Zhang, D., Ma, W., Tang, L., Cao, W., & Zhu, Y. (2020). Modelling the effects of post-heading heat stress on biomass partitioning, and grain number and weight of wheat. *Journal of Experimental Botany*, *71*, 6015-6031.

Liu, B., Liu, L., Asseng, S., Zou, X., Li, J., Cao, W., & Zhu, Y. (2016). Modelling the effects of heat stress on post-heading durations in wheat: A comparison of temperature response routines. *Agricultural & Forest Meteorology*, *222*, 45-58.

Liu, K., Xu, H., Liu, G., Guan, P., Zhou, X., Peng, H., Yao, Y., Ni, Z., Sun, Q., & Du, J. (2018). QTL mapping of flag leaf-related traits in wheat (*Triticum aestivum* L.). *Theoretical & Applied Genetics*, *131*, 839-849.

Lopes, M. S., Dreisigacker, S., Peña, R. J., Sukumaran, S., & Reynolds, M. P. (2015). Genetic characterization of the wheat association mapping initiative (WAMI) panel for dissection of complex traits in spring wheat. *Theoretical & Applied Genetics*, *128*, 453-464.

Maccaferri, M., Cane', M. A., Sanguineti, M. C., Salvi, S., Colalongo, M. C., Massi, A., Clarke, F., Knox, R., Pozniak, C. J., Clarke, J. M., Fahima, T., Dubcovsky, J., Xu, S., Ammar, K., Karsai, I., Vida, G., & Tuberosa, R. (2014). A consensus framework map of durum wheat (*Triticum durum* Desf.) suitable for linkage disequilibrium analysis and genome-wide association mapping. *BMC Genomics*, *15*, 873.

Mackay, T., Stone, E., & Ayroles, J. (2009). The genetics of quantitative traits: challenges and prospects. *Nature Reviews Genetics,10*, 565-577.

Martinez-Perez, E., Shaw, P., Aragon-Alcaide, L. & Moore, G. (2003). Chromosomes form into seven groups in hexaploid and tetraploid wheat as a prelude to meiosis. *The Plant Journal for Cell & Molecular Biology*, *36*, 21-29.

Martinez-Perez, E., Shaw, P., Reader, S., Aragon-Alcaide, L., Miller, T., & Moore, G. (1999). Homologous chromosome pairing in wheat. *Journal of Cell Science*, *112*, 1761-1769.

Mason, R. E., Mondal, S., Beecher, F. W., Pascheo, A., Jampla, B., Ibrahim, A. M. H., & Hays, D. B. (2010). QTL associated with heat susceptibility index in wheat (*Triticum aestivum* L.) under short-term reproductive stage heat stress. *Euphytica*, *174*, 423-436.

Masson-Delmotte, Zhai, V. P., Pörtner, H. O. et al. (2018). IPCC: Summary for policymakers in global warming of 1.5°C. An IPCC special report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty. *World Meteorological Organization, Geneva, Switzerland*, 32.

Masuda, Y. (1990). Auxin induced cell elongation and cell wall changes. *The Botanical Magazine Tokyo*, *103*, 345-370.

Matthes, M. S., Best, N. B., Robil, J. M., Malcomber, S., Gallavotti, A., & McSteen, P. (2019). Auxin EvoDevo: Conservation and diversification of genes regulating auxin biosynthesis, transport, and signaling. *Molecular Plant*, *12*, 298-320.

McMaster G. S. (1997). Phenology, development, and growth of the wheat (*Triticum aestivum* L.) shoot apex, a review. *Advances in Agronomy*, *59*, 63-118.

Meng, L., Li, H., Zhang, L., & Wang, J. (2015). QTL IciMapping: Integrated software for genetic linkage map construction and quantitative trait locus mapping in biparental populations. *Crop Journal, 3*, 269-283.

Miles, C. & Wayne, M. (2008). Quantitative trait locus (QTL) analysis. *Nature Education*, *1*, 208.

Milner, S. G., Maccaferri, M., Huang, B. E., Mantovani, P., Massi, A., Frascaroli, E., Tuberosa, R., & Salvi, S. (2016). A multi-parental cross population for mapping QTL for agronomic traits in durum wheat. *Plant Biotechnology Journal*, *14*, 735-748.

Miralles, D. J., Richards, R. A., & Slafer, G. A. (2000). Duration of the stem elongation period influences the number of fertile florets in wheat and barley. *Functional Plant Biology*, *27*, 931-940.

Mizuno, N., Ishikawa, G., Kojima, H., Tougou, M., Kiribuchi-Otobe, C., Fujita, M., & Nakamura, K. (2021). Genetic mechanisms determining grain number distribution along the spike and their effect on yield components in wheat. *Molecular Breeding*, *41*, 62.

Mohammadi, V., Abolfazl, Z., & Mohammad, B. (2008). Mapping QTL for heat stress tolerance in wheat, *Journal of Agricultural Science & Technology*, *10*, 261-267.

Nazir, M., Bano, A., Ullah, F., Khan, S., & Khan, N. (2021). Physiological evaluation of wheat (*Triticum aestivum* L.) genotypes at pre-anthesis stage under heat stress conditions. *American Journal of Plant Sciences*, *12*, 1780-1790.

Neill, E. M., Byrd, M. C. R., Billman, T., Brandizzi, F., & Stapleton, A. E. (2019). Plant growth regulators interact with elevated temperature to alter heat stress signaling via the unfolded protein response in maize. *Scientific Reports, 9*, 10392.

Ochagavía, H., Prieto, P., Savin, R., Griffiths, S., & Slafer, G. A. (2018). Dynamics of leaf and spikelet primordia initiation in wheat as affected by Ppd-1a alleles under field conditions. *Journal of Experimental Botany*, *69*, 2621-2631.

Onyemaobi, I., Ayalew, H., Liu, H., Siddique, K. H. M., & Yan, G. (2018). Identification and validation of a major chromosome region for high grain number per spike under meiotic stage water stress in wheat (*Triticum aestivum* L.). *PLOS ONE*, *13*, e0194075.

Peng, Z. S., Li, X., Yang, Z. J., & Liao, M. L. (2011). A new reduced height gene found in the tetraploid semi-dwarf wheat landrace Aiganfanmai. *Genetics & Molecular Research, 10*, 2349-2357.

Pérez-Gianmarco, T. I., Slafer, G. A., & González, F. G. (2019). Photoperiod-sensitivity genes shape floret development in wheat. *Journal of Experimental Botany*, *70*, 1339-1348.

Perez-Lara, E., Semagn, K., Chen, H., Iqbal, M., N'Diaye, A., Kamran, A., Navabi, A., Pozniak, C., & Spaner, D. (2016). QTL associated with agronomic traits in the 'Cutler' × 'AC Barrie' spring wheat mapping population using single nucleotide polymorphic markers. *PLOS ONE*, *11*, e0160623.

Philipp, N., Weichert, H., Bohra, U., Weschke, W., Schulthess, A. W., & Weber, H. (2018). Grain number and grain yield distribution along the spike remain stable despite breeding for high yield in winter wheat. *PLOS ONE*, *13*, e0205452.

Pinto, R. S., Reynolds, M. P., Mathews, K. L., McIntyre, C. L., Olivares-Villegas J. J., & Chapman, S. C. (2010). Heat and drought adaptive QTL in a wheat population designed to minimize confounding agronomic effects. *Theoretical & Applied Genetics, 121*, 1001-1021.

Porter, J. R. & Gawith, M. (1999). Temperatures and the growth and development of wheat: a review. *European Journal of Agronomy*, *10*, 23-36.

Preece, C., Clamp, N. F., Warham, G., Charles, M., Rees, M., Jones, G., & Osborne, C. P. (2018). Cereal progenitors differ in stand harvest characteristics from related wild grasses. *The Journal of Ecology*, *106*, 1286-1297.

Quarrie, S. A. & Jones, H. G. (1979). Genotypic variation in leaf water potential, stomatal conductance and abscisic acid concentration in spring wheat subjected to artificial drought stress. *Annals of Botany, 44*, 323-327.

Rawson, H. M. & Bagga, A. K. (1979). Influence of temperature between floral initiation and flag leaf emergence on grain number in wheat. *Functional Plant Biology, 6*, 391-400.

Rehman, A., Habib, I., Ahmad, N., Hussain, M., Khan M. A., Farooq, J., & Ali, M. A. (2009). Screening wheat germplasm for heat tolerance at terminal growth stage. *Plant Omics Journal*, *2*, 9-19.

Reid, T. A., Yang, R. C., Salmon, D. F., Navabi, A., & Spaner, D. (2011). Realized gains from selection for spring wheat grain yield are different in conventional and organically managed systems. *Euphytica*, *177*, 253-266.

Reif, J. C., Zhang, P., Dreisigacker, S., Warburton, M. L., van Ginkel, M., Hoisington, D., Bohn, M., & Melchinger, A. E. (2005). Wheat genetic diversity trends during domestication and breeding. *Theoretical & Applied Genetics, 110*, 859-864.

Rieu, I., Twell, D., & Firon, N. (2017). Pollen development at high temperature: From acclimation to collapse. *Plant Physiology*, *173*, 1967-1976.

Rosewarne, G. M., Singh, R. P., Huerta-Espino, J., & Rebetzke, G. J. (2008). Quantitative trait loci for slow-rusting resistance in wheat to leaf rust and stripe rust identified with multi-environment analysis. *Theoretical & Applied Genetics*, *116*, 1027-1034.

Saini, H. S., & Aspinall, D. (1981). Effect of water deficit on sporogenesis in wheat (*Triticum aestivum* L.). *Annals of Botany*, *48*, 623-633.

Saini, H. S., & Aspinall, D. (1982). Abnormal sporogenesis in wheat (*Triticum aestivum* L.) induced by short periods of high temperature. *Annals of Botany*, *49*, 835-846.

Saini, H. S., Sedgley, M., & Aspinall, D. (1983). Effect of heat stress during floral development on pollen tube growth and ovary anatomy in wheat (*Triticum aestivum* L.). *Australian Journal of Plant Physiology*, *10*, 137-144.

Saini, H. S., Sedgley, M., & Aspinall. D. (1984). Developmental anatomy in wheat of male sterility induced by heat stress, water deficit or abscisic acid. *Australian Journal of Plant Physiology*, *11*, 243-253.

Sakata, T., Oshino, T., Miura, S., Tomabechi, M., Tsunaga, Y., Higashitani, N., Miyazawa, Y., Takahashi, H., Watanabe, N., & Higashitani, A. (2010). Auxins reverse plant male sterility caused by high temperatures. *Proceedings of the National Academy of Sciences of the United States of America*, *107*, 8569-8574.

Sakuma, S., Golan, G., Guo, Z., Ogawa, T., Tagiri, A., Sugimoto, K., Bernhardt, N., Brassac, J., Mascher, M., Hensel, G., Ohnishi, S., Jinno, H., Yamashita, Y., Ayalon, I., Peleg, Z., Schnurbusch, T., & Komatsuda, T. (2019). Unleashing floret fertility in wheat through the mutation of a homeobox gene. *Proceedings of the National Academy of Sciences of the United States of America*, *116*, 5182-5187.

Sangwan, S., Munjal, R., Ram, K., & Kumar, N. (2019). QTL mapping for morphological and physiological traits in RILs of spring wheat population of WH1021 × WH711. *Journal of Environmental Biology*, *40*, 674-682.

SasK Seed Guide (2019). https://saskseed.ca/seed-guides/ Accessed, March 2019.

Semagn, K., Bjørnstad, A., & Ndjiondjop, M. (2006). An overview of molecular marker methods for plants. *African Journal of Biotechnology*, *525*, 2540-2568.

Semagn, K., Iqbal, M., Chen, H., Perez-Lara, E., Bemister, D. H., Xiang, R., Zou, J., Asif, M., Kamran, A., N'Diaye, A., Randhawa, H., Beres, B. L., Pozniak, C., & Spaner, D. (2021). Physical mapping of QTL associated with agronomic and end-use quality traits in spring wheat under conventional and organic management systems. *Theoretical & Applied Genetics,134*, 3699-3719.

Sharma, L., Dalal, M., Verma, R. K., Kumar, V. V. S., Yadav, S. K., Pushkar, S., Kushwaha, S. R., Bhowmik, A., & Chinnusamy, V. (2018). Auxin protects spikelet fertility and grain yield under drought and heat stresses in rice. *Environmental & Experimental Botany*, *150*, 9-24.

Shearman, V. J., Sylvester-Bradley, R., Scott, R. K., & Foulkes, M. J. (2005). Physiological processes associated with wheat yield progress in the UK. *Crop Science*, *45*, 175-85.

Shi, H., Chen, L., Ye, T., Liu, X., Ding, K., & Chan, Z. (2014). Modulation of auxin content in *Arabidopsis* confers improved drought stress resistance. *Plant Physiology & Biochemistry*, *82*, 209-21.

Shi, W., Li, X., Schmidt, R. C., Struik, P. C., Yin, X., & Jagadish, S. V. K. (2018). Pollen germination and in vivo fertilization in response to high temperature during flowering in hybrid and inbred rice. *Plant, Cell & Environment*, *41*, 1287-1297.

Shiferaw, B., Smale, M., Braun, H. J., Duveiller, E., Reynolds, M., & Muricho, G. (2013). Crops that feed the world. Past successes and future challenges to the role played by wheat in global food security. *Food Security*, *5*, 291-317.

Shirley, N. J., Aubert, M. K., Wilkinson, L. G., Bird, D. C., Lora, J., Yang, X., & Tucker, M. R. (2019). Translating auxin responses into ovules, seeds, and yield: Insight from *Arabidopsis* and the cereals. *Journal of Integrative Plant Biology*, *61*, 310-336.

Siddique, K. H. M., Kirby, E. J. M., & Perry, M. W. (1989). Ear: stem ratio in old and modern wheat varieties; relationship with improvement in number of grains per ear and yield. *Field Crops Research*, *21*, 59-78.

Singh, H., Pirani, F. J., & Najafi, M. R. (2020). Characterizing the temperature and precipitation covariability over Canada. *Theoretical & Applied Climatol*ogy, *139*, 1543-1558.

Slafer, G. A. & Rawson, H. M. (1994). Sensitivity of wheat phasic development to major environmental factors: A re-examination of some assumptions made by physiologist and modelers. *Australian Journal of Plant Physiology*, *21*, 393-426.

Slafer, G. A., Abeledo, L. G., Miralles, D. J., González, F. G., & Whitechurch, E. M. (2001). Photoperiod sensitivity during stem elongation phase as an avenue to rise potential yield in wheat. *Euphytica*, *119*, 191-197.

Slafer, G. A., Savin, R., & Sadras, V. O. (2014). Coarse and fine regulation of wheat yield components in response to genotype and environment. *Field Crops Research, 157*, 71-83.

Smýkal, P., Nelson, M. N., Berger, J. D., & Von Wettberg, E. J. B. (2018). The impact of genetic changes during crop domestication. *Agronomy*, *8*, 119.

Sobie, S., Zwiers, F., & Curry, C. (2021). Climate model projections for Canada: A comparison of CMIP5 and CMIP6. *Atmosphere-ocean*, *59*, 269-284.

Sobol, S., Chayut, N., Nave, N., Kafle, D., Hegele, M., Kaminetsky, R., Wunsche, J. N., & Samach, A. (2014). Genetic variation in yield under hot ambient temperatures spotlights a role for cytokinin in protection of developing floral primordia. *Plant, Cell & Environment*, *37*, 643-657.

Song, Q. J., Shi, J. R., Singh, S., Fickus, E. W., Costa, J. M., Lewis, J., Gill, B. S., Ward, R., & Cregan, P. B. (2005). Development and mapping of microsatellite (SSR) markers in wheat. *Theoretical & Applied Genetics*, *110*, 550-560.

Spiertz, J. H. J, & Ewert, F. (2009). Crop production and resource use to meet the growing demand for food, feed, and fuel: opportunities and constraints. *Journal of Life Sciences*, *56*, 281-300.

Statista (2022). Global wheat production. https://www.statista.com/statistics/267268/production-of-wheat-worldwide-since-1990/ Accessed, January 2022.

Stelmakh, A. F. (1993). Genetic effect of *Vrn* genes on heading date and agronomic traits in bread wheat. *Euphytica*, *65*, 53-60.

Storme, N. & Geelen, D. (2013). The impact of environmental stress on male reproductive development in plants: Biological processes and molecular mechanisms. *Plant, Cell & Environment*, *37*, 1-18.

Studnicki, M., Wijata, M., Sobczyński, G., Samborski, S., Gozdowski, D., & Rozbicki, J. (2016). Effect of genotype, environment and crop management on yield and quality traits in spring wheat. *Journal of Cereal Science*, *72*, 30-37.

Sun, C., Dong, Z., Zhao, L., Ren, Y., Zhang, N., & Chen, F. (2020). The wheat 660K SNP array demonstrates great potential for marker-assisted selection in polyploid wheat. *Plant Biotechnology Journal*, *18*, 1354-1360.

Sun, Q. X., & Quick, J. S. (1991). Chromosomal locations of genes for heat tolerance in tetraploid wheat. *Cereal Research Communication*, *19*, 431-437.

Tahmasebi, S., Heidari, B., Pakniyat, H., & McIntyre, C. L. (2017). Mapping QTL associated with agronomic and physiological traits under terminal drought and heat stress conditions in wheat (*Triticum aestivum* L.). *Genome*, *60*, 26-45.

Telfer, P., Edwards, J., Bennett, D., Ganesalingam, D., Able, J., & Kuchel, H. (2018). A field and controlled environment evaluation of wheat (*Triticum aestivum* L.) adaptation to heat stress. *Field Crops Research*, *229*, 55-65.

Thimann, K. V. (1938). Hormones and the analysis of growth. *Plant Physiology*, *13*, 437-449.

Thomas, J. O. (2020). Mass selection and the basic plant breeding algorithm. *Horticultural Plant Breeding, Academic Press*, 85-95.

USDA (2019). Wheat outlook. https://www.ers.usda.gov/webdocs/outlooks/93715/whs-19h.pdf?v=5766.9 Accessed, April 2020.

Venske, E., Dos Santos, R. S., Busanello, C., Gustafson, P., & De Oliveira, A. C. (2019). Bread wheat: a role model for plant domestication and breeding. *Hereditas*, *156*, 16.

Vincent, L. A., Zhang, X., Brown, R. D., Feng, Y., Mekis, E., Milewska, E. J., Wan, H. & Wang, X. L. (2015). Observed trends in Canada's climate and influence of low-frequency variability modes; *Journal of Climate*, *28*, 4545-4560.

Voorrips, R. E. (2002). MapChart: Software for the graphical presentation of linkage maps and QTL. *The Journal of Heredity*, *93*, 77-78.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., & Kuiper, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research*, *23*, 4407-4414.

Wahid, A., Farooq, M., Hussain, I., Rasheed, R., & Galani, S. (2012). Responses and management of heat stress in plants. In: Ahmad, P., & Prasad, M. (eds). Environmental adaptations and stress tolerance of plants in the era of climate change. *Springer, New York, NY.*

Wang, B., Liu, C., Zhang, D., He, C., Zhang, J., & Li, Z. (2019). Effects of maize organ-specific drought stress response on yields from transcriptome analysis. *BMC Plant Biology*, *19*, 335.

Wang, J., Li, H., Zhang, L., & Meng, L. (2019). Users' Manual of QTL IciMapping, integrated software for linkage analysis and genetic mapping in biparental populations. Quantitative

genetics group, Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, Beijing 100081, China. Version 4.2, released July 2019.

Wang, P., Zhou, G., Yu, H., & Yu, S. (2011). Fine mapping a major QTL for flag leaf size and yield-related traits in rice. *Theoretical & Applied Genetics*, *123*, 1319-1330.

Wang, S., Wong, D., Forrest, K., Allen, A., Chao, S., Huang, B. E., et al. (2014). Characterization of polyploid wheat genomic diversity using a high-density 90,000 single nucleotide polymorphism array. *Plant Biotechnology Journal*, *12*, 787-796.

Went, F. W. (1942). Growth, auxin, and tropisms in decapitated *Avena* coleoptiles. *Plant Physiology*, *17*, 236-49.

Wenzl, P., Carling, J., Kudrna, D., Jaccoud, D., Huttner, E., Kleinhofs, A., & Kilian, A. (2004). Diversity Arrays Technology (DArT) for whole-genome profiling of barley. *Proceedings of the National Academy of Sciences of the United States of America*, *101*, 9915-9920.

Wenzl, P., Suchánková, P., Jason, C., Šimková, H., Huttner, E., Kubaláková, M., Sourdille, P., Paul, E., Feuillet, C., Kilian, A., & Dolezel, J. (2010). Isolated chromosomes as a new and efficient source of DArT markers for the saturation of genetic maps. *Theoretical & Applied Genetics*, *121*, 465-474.

Whitford, R., Fleury, D., Reif, J. C., Garcia, M., Okada, T., Korzun, V., & Langridge, P. (2013). Hybrid breeding in wheat: technologies to improve hybrid wheat seed production. *Journal of Experimental Botany*, *64*, 5411-5428.

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic Acids Research*, *18*, 6531-6535.

Winfield, M. O., Allen, A. M., Burridge, A. J., Barker, G. L., Benbow, H. R., Wilkinson, P. A., Coghill, J., Waterfall, C., Davassi, A., Scopes, G., Pirani, A., Webster, T., Brew, F., Bloor, C., King, J., West, C., Griffiths, S., King, I., Bentley, A. R., & Edwards, K. J. (2016). High-density SNP genotyping array for hexaploid wheat and its secondary and tertiary gene pool. *Plant Biotechnology Journal*, *14*, 1195-1206.

Wolde, G. M., Mascher, M., & Schnurbusch, T. (2019). Genetic modification of spikelet arrangement in wheat increases grain number without significantly affecting grain weight. *Molecular Genetics & Genomics*, *294*, 457-468.

Wu, Q., Chen, Y., Fu, L., Zhou, S., Chen, J., Zhao, X., Zhang, D., Ouyang, S., Wang, Z., Li, D., Wang, G., Zhang, D., Yuan, C., Wang, L., You, M., Han, J., & Liu, Z. (2016). QTL mapping of flag leaf traits in common wheat using an integrated high-density SSR and SNP genetic linkage map. *Euphytica*, *208*, 337-351.

Xu, X., Bai, G., Carver, B. F., & Shaner, G. E. (2005). A QTL for early heading in wheat cultivar Suwon 92. *Euphytica*, *146*, 233-237.

Xue, S., Xu, F., Li, G., Zhou, Y., Lin, M., Zhongxia, G., Xiuhong, S., Xiaowu, X., Ge, J., Shuang, Z., Haiyan, J., Zhongxin, K., Lixia, Z., & Zhengqiang, M. (2013). Fine mapping *TaFLW1*, a major QTL controlling flag leaf width in bread wheat (*Triticum aestivum* L.). *Theoretical & Applied Genetics*, *126*, 1941-1949.

Yang, D., Liu, Y., Cheng, H., Chang, L., Chen, J., Chai, S., & Li, M. (2016). Genetic dissection of flag leaf morphology in wheat (*Triticum aestivum* L.) under diverse water regimes. *BMC Genetics*, *17*, 94.

Yang, J., Sears, R. G., Gill, B. S., & Paulsen, G. M. (2002). Genotypic differences in utilization of assimilate sources during maturation of wheat under chronic heat and heat shock stresses. *Euphytica*, *125*, 179-188.

Zajączkowska, U., Denisow, B., Łotocka, B., Dołkin-Lewko, A., & Rakoczy-Trojanowska, M. (2021). Spikelet movements, anther extrusion and pollen production in wheat cultivars with contrasting tendencies to cleistogamy. *BMC Plant Biology*, *21*, 136.

Zhang, C., Feng, B., Chen, T., Zhang, X., Tao, L. & Fu, G. (2017). Sugars, antioxidant enzymes and IAA mediate salicylic acid to prevent rice spikelet degeneration caused by heat stress. *Plant Growth Regulation*, *83*, 313-323.

Zhang, J., Dell, B., Biddulph, B., Drake-Brockman, F., Walker, E., Khan, N. Wong, D., Hayden, M., & Appels, R. (2013). Wild-type alleles of *Rht-B1* and *Rht-D1* as independent determinants of thousand-grain weight and kernel number per spike in wheat. *Molecular Breeding*, *32*, 771-783.

Zhao, Y. (2012). Auxin biosynthesis: a simple two-step pathway converts tryptophan to indole-3-acetic acid in plants. *Molecular Plant*, *5*, 334-338.

Zhao, Y. (2014). Auxin biosynthesis. *The Arabidopsis Book*, *12*, e0173.

Zou, J., Semagn, K., Iqbal, M., Chen, H., Asif, M., N'Diaye, A., Navabi, A., Perez-Lara, E., Pozniak, C., Yang, R. C., Randhawa, H., & Spaner, D. (2017b). QTL associated with

agronomic traits in the ‘Attila’ × ‘CDC Go’ spring wheat population evaluated under conventional management. *PLOS ONE*, *12*, e0171528.

Zou, J., Semagn, K., Iqbal, M., N'Diaye, A., Chen, H., Asif, M., Navabi, A., Perez-Lara, E., Pozniak, C., Yang, R.-C., Randhawa, & H. & Spaner, D. (2017a). Mapping QTL controlling agronomic traits in the ‘Attila’ × ‘CDC Go’ spring wheat population under organic management using 90K SNP array. *Crop Science, 57*, 365-377.

Zulfiqar, F., Ashraf, M., & Siddique, K. H. M. (2022). Role of glycine betaine in the thermotolerance of plants. *Agronomy*, *12*, 276.

Appendix

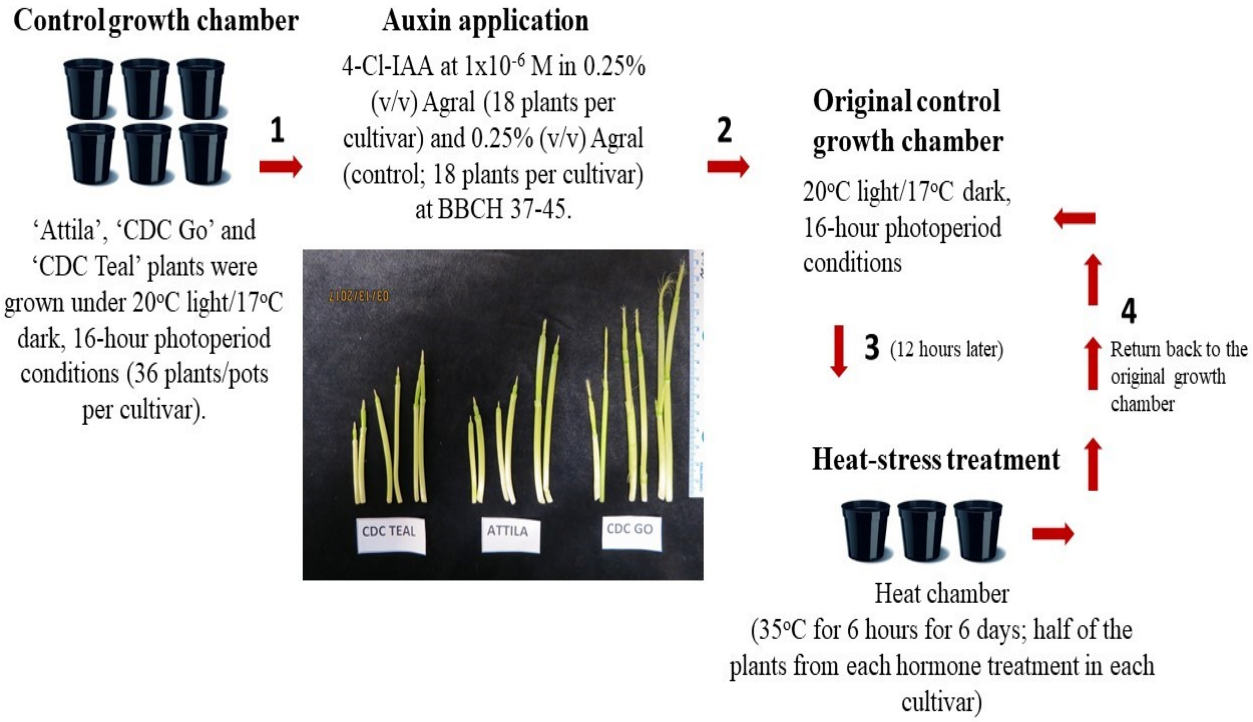

Figure A1. Diagram of experiment procedure and floral spike progression of spike one, two and three of RIL parental wheat cultivars Attila, CDC Go and CDC Teal.

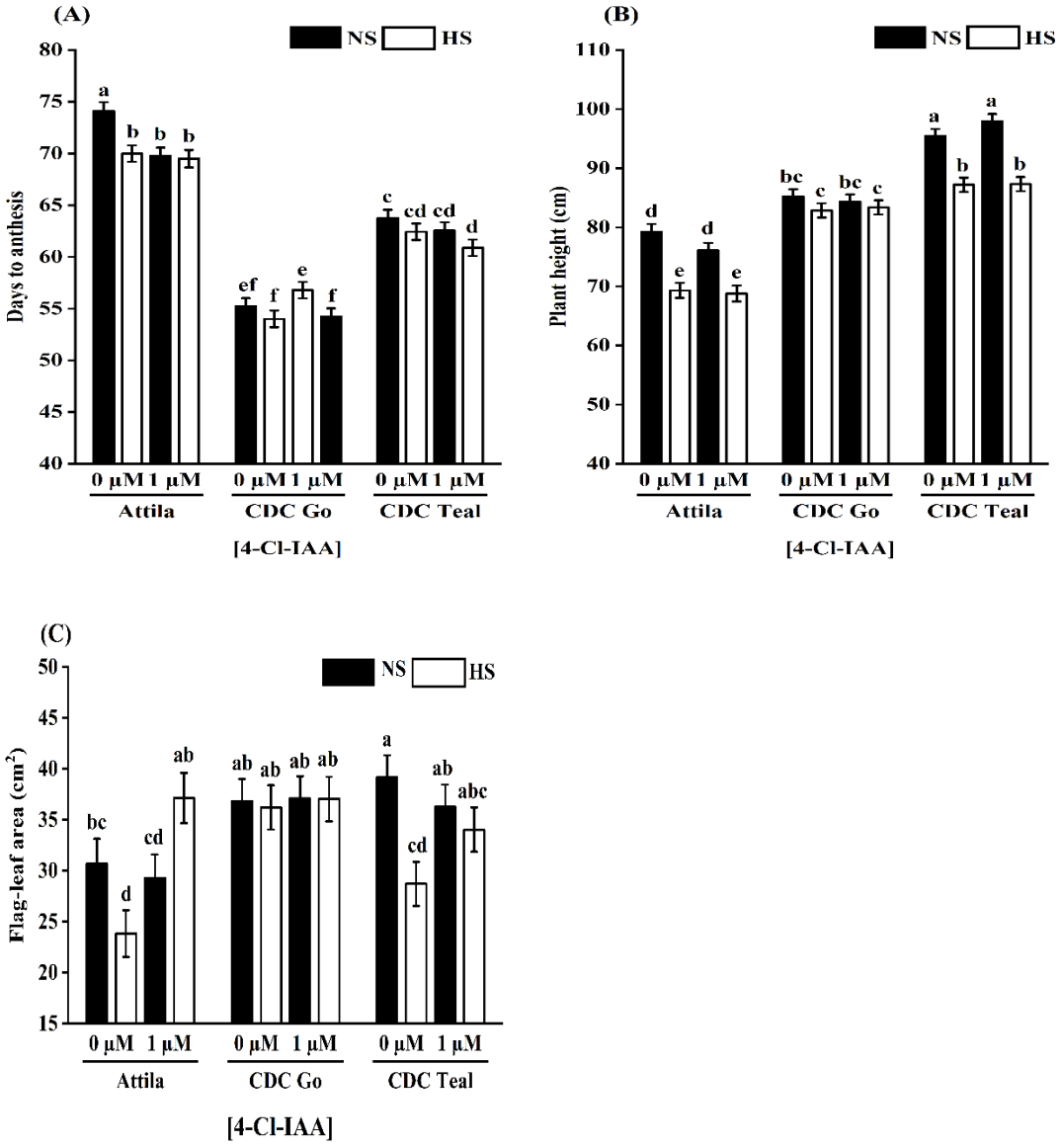

Figure A2. Effect of 4-Cl-IAA and heat stress on (A) days to anthesis, (B) plant (tiller) height, and (C) flag-leaf area of the main tiller (spike 1) of cultivars Attila, CDC Go, and CDC Teal. Data are means ± SE; n = 9 (one plant is a replicate). NS = non-stress temperature treatment (control), HS = heat-stress treatment. Means followed by different letters are significantly different among 4-Cl-IAA treatments, temperature treatments, and cultivars, within each parameter, at $P \leq 0.05$.

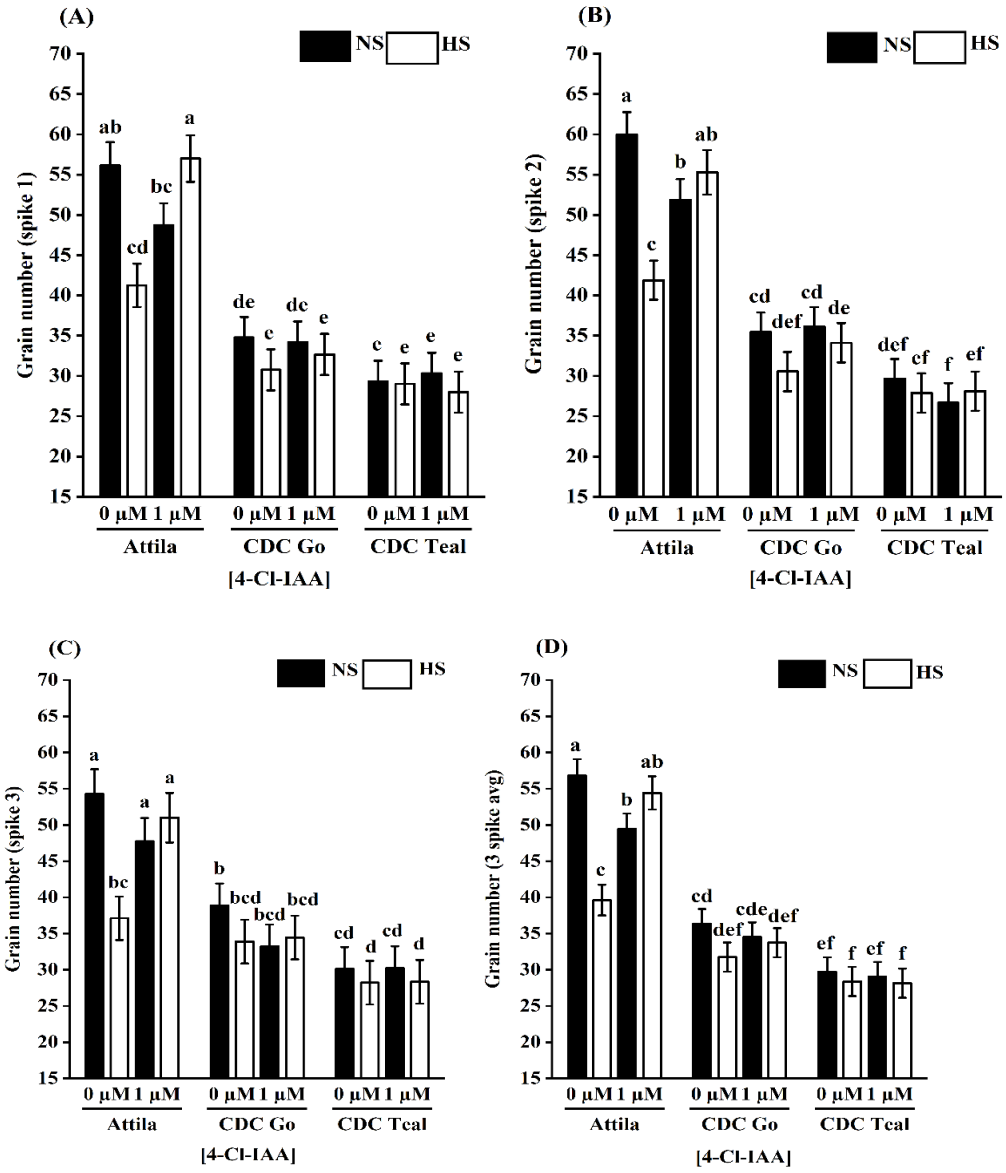

Figure A3. Effect of 4-Cl-IAA and heat stress on grain number of (A) spike 1 (B) spike 2, (C) spike 3, and (D) average of spikes 1, 2 and 3 of cultivars Attila, CDC Go, and CDC Teal. Data are means ± SE; n = 9 (one plant is a replicate). NS = non-stress temperature treatment (control), HS = heat-stress treatment. Means followed by different letters are significantly different among 4-Cl-IAA treatments, temperature treatments, and cultivars, within each parameter, at $P \leq 0.05$.

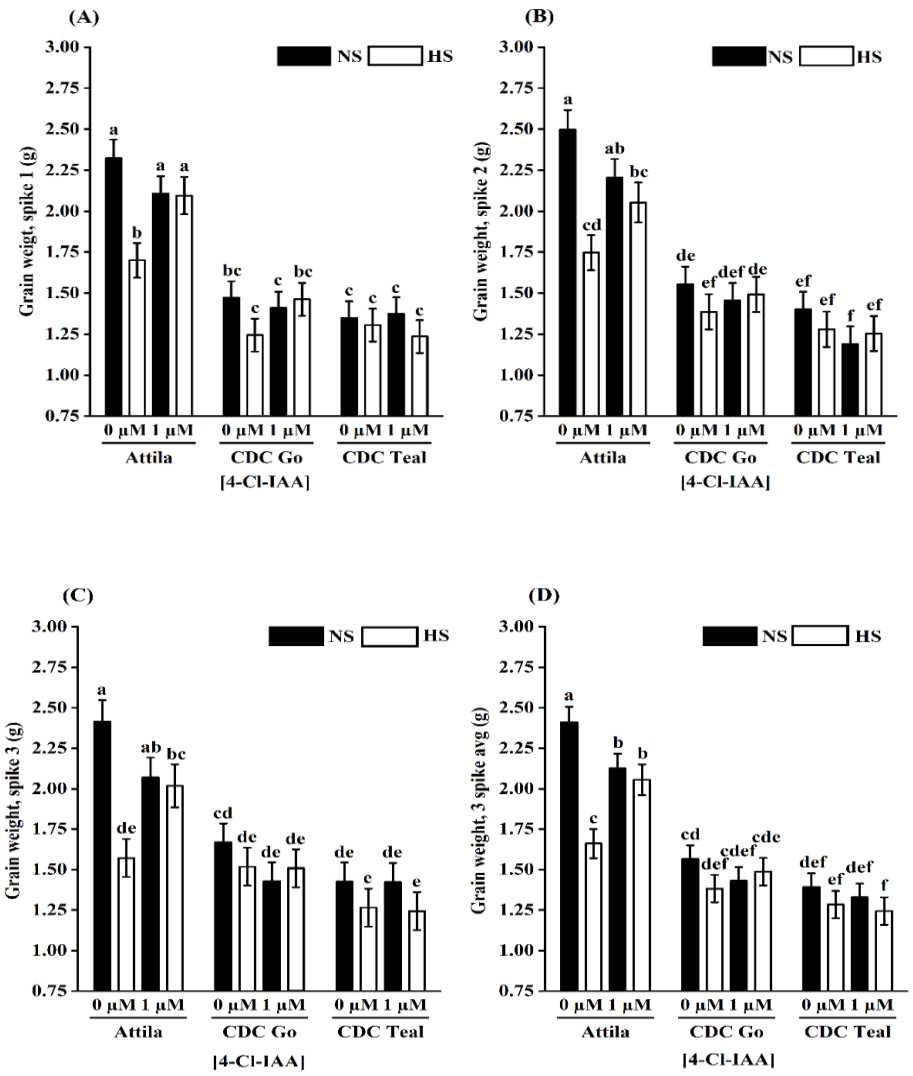

Figure A4. Effect of 4-Cl-IAA and heat stress on grain weight of (A) spike 1 (B) spike 2, (C) spike 3, and (D) average of spikes 1, 2 and 3 of cultivars Attila, CDC Go, and CDC Teal. Data are means ± SE; n = 9 (one plant is a replicate). NS = non-stress temperature treatment (control), HS = heat-stress treatment. Means followed by different letters are significantly different among 4-Cl-IAA treatments, temperature treatments, and cultivars, within each parameter, at $P \leq 0.05$.

	1	2	3	4
1	RIL 23	AC Splender	RIL 45	CDC Go
2	RIL60	RIL 99	RIL 34	RIL 12
3	RIL 78	RIL 55	Attila	RIL100
4	RIL 59	RIL 108	RIL 116	RIL 62
5	RIL 111	RIL 84	RIL 134	RIL 93
6	RIL 47	CDC GO	RIL 125	RIL 155
7	RIL 36	RIL 174	RIL 20	RIL 144
8	RIL 18	RIL 167	RIL 159	RIL 48
9	RIL 138	RIL 102	RIL 29	RIL 88
10	RIL 176	Attila	RIL 180	RIL 113

Figure B1. A representative root-trainer block containing 40 cells planted with one plant per cell. One experimental replication consisted of 5 root-trainer blocks per treatment planted at the same time (4 treatments x 5 blocks = total of 20 root-trainer blocks per replication). Each replication consisted of 171 RILs from 'Attila' × 'CDC Go' RIL population, 7 standard cultivars, and 11 plants of each RIL parent (Attila and CDC Go) planted randomly in five 40-cell root-trainer blocks (a total of 200 plants per replication). The experiment consisted of 4 replications.

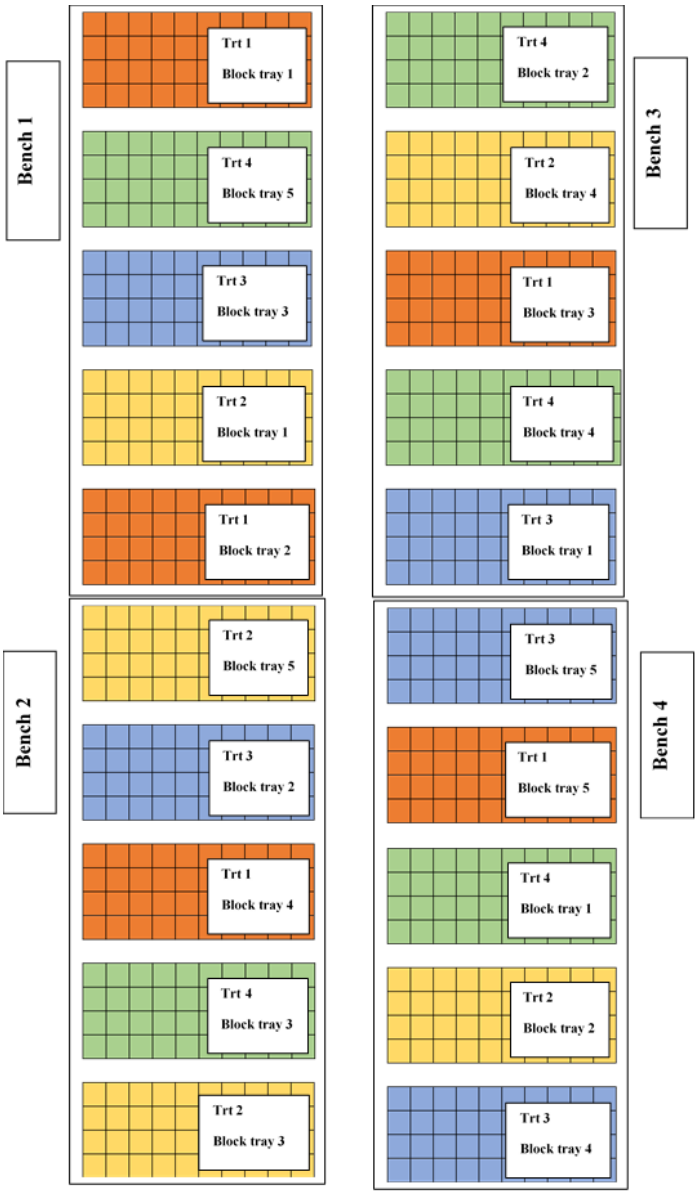

Figure B2. A representative root trainer block arrangement on four benches inside the growth chamber. One experimental replication consisted of 5 root-trainer blocks per treatment planted at the same time (4 treatments x 5 blocks = total of 20 root-trainer blocks per replication). 20 root-trainer blocks were randomized inside the growth chamber at two weeks intervals. The experiment consisted of 4 replications.

Table B1 The cultivar group mean, minimum, maximum, and median and standard deviation of plant and yield parameters of nine wheat cultivars grown under heat-stress and non-stress temperature conditions.

	Mean	SD	Minimum	Maximum	Median
Non-heat stress					
Grain weight (g)	1.433	0.5	1.039	2.347	1.219
Grain number	36	14	20	62	29
Days to anthesis	65	10	53	76	62
Spike height (cm)	90	10	80	111	85
Flag leaf area (cm^2)	23	5	17	30	24
Total spklts* per spike	20	4	15	26	18
Fertile spklts per spike	16	3	10	20	15
Number of grains per spklt	1.8	0.5	1.3	2.9	1.8
Number of grains per fertile spklt	2.2	0.4	1.8	3.1	2
Heat stress					
Grain weight (g)	1.153	0.4	0.674	1.726	1.088
Grain number	27	9	14	43	23
Days to anthesis	64	9	53	79	61
Spike height (cm)	82	4	77	88	82
Flag leaf area (cm^2)	22	5	14	29	21
Total spklts per spike	18	3	14	23	18
Fertile spklts per spike	14	3	10	19	13
Number of grains per spklt	1.5	0.4	0.8	2.4	1.5
Number of grains per fertile spklt	2	0.5	1.5	3.2	1.8

*spklts = spikelets

Table B2 Population mean, minimum, maximum, median, and standard deviation of plant and yield parameters of 171 'Attila' × 'CDC Go' RILs grown under heat-stress and non-stress temperature conditions.

	Mean	SD	Minimum	Maximum	Median
Non-heat stress					
Grain weight (g)	1.204	0.3	0.428	2.361	1.207
Grain number	31	9	15	65	30
Days to anthesis	65	8	50	87	65
Spike height (cm)	86	11	52	110	87
Flag leaf area (cm2)	20	5	9	37	20
Total spklts* per spike	19	2.5	14	26	19
Fertile spklts per spike	15	2	9	21	15
Number of grains per spklt	1.6	0.3	0.8	2.8	1.5
Number of grains per fertile spklt	2	0.3	1.3	2	3.2
Heat stress					
Grain weight (g)	0.920	0.4	0.162	1.830	0.910
Grain number	23	10	4	45	24
Days to anthesis	64	8	43	88	63
Spike height (cm)	80	10	56	101	80
Flag leaf area (cm2)	19	5	8	33	19
Total spklts per spike	19	2	13	25	19
Fertile spklts per spike	13	4	5	20	13
Number of grains per spklt	1.2	0.4	0.28	2.4	1.2
Number of grains per fertile spklt	1.7	0.4	1	3.3	1.7

*Spklts = spikelets

Table B3 Heat tolerance ratios calculated for 171 RILs based on grain number in the main tiller spike.

Grain number heat tolerance ratio class intervals*	**RIL number**
0.00 - 0.20	0 (Total 0)
0.21 - 0.40	13,23,26,29,40,44,46,52,57,59,68,110,114,116,128,131,140,145,153,154,160, 173,178 (Total 23)
0.41 - 0.60	11,12,17,19,32,34,38,39,41,45,48,49,55,66,67,71,74,80,91,92,102,113,115,12 4,127,132,138,141,161,165,168,179 (Total 32)
0.61 - 0.80	14,15,20,22,24,25,35,36,37,43,51,53,54,56,58,60,64,65,73,75,83,85,86,88,89, 90,94,95,96,97,99,103,105,107,111,112,119,120,126,136,139,152,163,167,16 9,171,175,177,180 (Total 49)
0.81 –1.00	10,16,30,33,42,47,50,61,63,72,76,77,78,82,84,98,101,104,108,109,121,123,1 29,130,135,137,143,144,147,149,159,162,164,166,172,174,176 (Total 37)
1.01 - 1.20	21,31,62,69,79,81,87,100,117,118,133,142,146,150,151,155,157,158 (Total 18)
1.21 - 1.40	18,27,28,93,122,125,134,148,170 (9)
1.41 - 1.60	70,106,156 (Total 3)

* Heat tolerance ratio = mean grain number in the main tiller spike exposed to the heat-stress treatment divided by that grown under non-stress conditions.

Table B4 Heat tolerance ratios calculated for 171 RILs based on grain weight in the main tiller spike.

Grain weight heat tolerance ratio class intervals*	**RIL Number**
0.0 - 0.20†	40 (Total 01)
0.21 - 0.40†	12,23,26,46,68,110,114,116,128,140,160,178 (Total 12)
0.41 - 0.60‡	11,13,17,25,29,32,34,38,39,44,48,52,54,57,59,64,66,71, 74,91,92,102,113,120,124,127,131,138,141,145,153,154, 168,173,179 (Total 35)
0.61 - 0.80	15,19,22,35,36,37,41,43,45,47,49,53,56,58,65,67,72,75,77, 78,80,82,84,85,86,88,96,97,99,103,105,107,109,111,115, 119,132,152,161,163,165,167,169,171,175,177 (Total 46)
0.81 – 1.00¶	10,14,16,20,24,30,33,42,50,51,54,60,61,63,69,73,76,83,89, 90,94,95,98,101,112,117,123,126,129,130,135,136,137,139, 143,146,147,162,166,172,174,176,180 (Total 43)
1.01- 1.20¶	21,27,28,31,79,81,87,93,104,108,118,121,133,134,144,149, 150,151,157,158,159,164,170 (Total 23)
1.21 - 1.40¶	18,62,70,100,106,122,142,148,155 (Total 09)
1.41-1.60¶	125,156 (Total 02)

* Heat tolerance ratio = mean grain number in the main tiller spike exposed to the heat-stress treatment divided by that grown under non-stress conditions.
† Highly heat sensitive RILs
‡ Moderately heat sensitive RILs
¶ Heat resistant RILs

Table C1 QTL that consistently detected for same trait under non-temperature stress and heat-stress conditions and/or whole spike and spike section levels in the 'Attila' × 'CDC Go' RIL mapping population assessed under controlled environmental conditions.

Trait*	Heat trt‡	QTL	Chrom. §	Position (cM)	Confidence interval (cM)	Left Marker	Right Marker	LOD	PVE¶ (%)	Additive effect
Da	+	*QDa.jods-4B*	4B	15	13.5-16.5	BS00073084_51	Kukri_rep_c78644_408	3.2	5.1	-1.79
Da	-	*QDa.jods-4B*	4B	16	14.5-21.5	BS00073084_51	Kukri_rep_c78644_408	2.8	3.9	-1.54
Da	+	*QDa.jods-5A*	5A	295	294.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	16.6	32.2	4.49
Da	-	*QDa.jods-5A*	5A	296	294.5-296.5	Vrn-A1	Kukri_c12384_430	16.3	27.8	4.13
Fsp	+	*QFsp.jods-5A*	5A	295	293.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	5.1	12.2	1.22
Fspc	+	*QFsp.jods-5A*	5A	295	293.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	4.3	11.1	0.16
Fspd	+	*QFsp.jods-5A*	5A	291	290.5-293.5	Tdurum_contig10843_745	wsnp_Ex_rep_c101994_87256479	8	4.1	0.17
Fsp	-	*QFsp.jods-2D*	2D	71	68.5-74	wsnp_Ex_c8303_14001708	wsnp_Ex_rep_c66522_64795143	2.8	5.7	0.61
Fspb	-	*QFsp.jods-2D*	2D	74	70.5-74	wsnp_Ex_c8303_14001708	wsnp_Ex_rep_c66522_64795143	3.2	8.4	0.11
Fspc	-	*QFsp.jods-2D*	2D	70	64.5-74	wsnp_Ex_c8303_14001708	wsnp_Ex_rep_c66522_64795143	2.6	5.4	0.08
Fspd	-	*QFsp.jods-2D*	2D	68	63.5-74	RAC875_rep_c73531_335	wsnp_Ex_c8303_14001708	3	5.2	0.06
Fspb	+	*QFsp.jods-7B*	7B	239	238.5-239.5	Kukri_c35975_593	BobWhite_rep_c50003_377	3	8.2	-0.13
Fspc	+	*QFsp.jods-7B*	7B	239	238.5-239.5	Kukri_c35975_593	BobWhite_rep_c50003_377	3.9	9.9	-0.15
Fspc	-	*QFsp.jods-7B*	7B	239	238.5-240.5	Kukri_c35975_593	BobWhite_rep_c50003_377	2.5	5	-0.08
Gn	+	*QGn.jods-2B*	2B	180	177.5-181.5	RAC875_c19690_358	BobWhite_c41535_52	2.7	5.5	2.17
Gn	-	*QGn.jods-2B*	2B	177	175.5-180.5	CAP12_rep_c3980_87	RAC875_c19690_358	2.6	5.8	1.8
Gnd	+	*QGn.jods-2B*	2B	172	170.5-174.5	Excalibur_c36070_300	RAC875_c34516_70	5.7	12.1	1.03
Gnd	-	*QGn.jods-2B*	2B	176	174.5-177.5	CAP12_rep_c3980_87	RAC875_c19690_358	3	6.7	0.57
Gn	+	*QGn.jods-5A*	5A	295	293.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	8	16.6	3.78
Gnc	+	*QGn.jods-5A*	5A	295	294.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	6.1	16.1	1.62
Gnd	+	*QGn.jods-5A*	5A	292	288.5-292.5	Tdurum_contig10843_745	wsnp_Ex_rep_c101994_87256479	4.2	8.7	0.88
Gn	-	*QGn.jods-1B*	1B	108	104.5-108	Kukri_rep_c111174_132	wsnp_BG274687B_Ta_2_1	4.4	9.8	2.4
Gnd	+	*QGn.jods-1B*	1B	108	104.5-108	Kukri_rep_c111174_132	wsnp_BG274687B_Ta_2_1	3.8	7.9	0.84
Gw	+	*QGw.jods-5A*	5A	292	290.5-293.5	Tdurum_contig10843_745	wsnp_Ex_rep_c101994_87256479	4.1	11.5	0.12
Gwc	+	*QGw.jods-5A*	5A	293	290.5-293.5	wsnp_Ex_rep_c101994_87256479	Excalibur_c26671_282	6	15.7	0.06
Gwc	-	*QGw.jods-5A*	5A	295	293.5-296.5	wsnp_Ex_c621_1230852	Vrn-A1	2.7	7.2	0.04
Gwd	+	*QGw.jods-5A*	5A	293	290.5-293.5	wsnp_Ex_rep_c101994_87256479	Excalibur_c26671_282	3.8	9.9	0.03

Trait*	Heat trt‡	QTL	Chrom. §	Position (cM)	Confidence interval (cM)	Left Marker	Right Marker	LOD	PVE¶ (%)	Additive effect
Gwd	-	*QGw.jods-5A*	5A	288	284.5-290.5	IAAV108	Ra_c3966_2205	3.2	6.1	0.02
Gw	-	*QGw.jods-4B.1*	4B	90	84.5-100.5	wsnp_Ra_c1146_2307483	Rht-B1	4.4	11.5	0.12
Gwc	-	*QGw.jods-4B.1*	4B	90	83.5-101.5	wsnp_Ra_c1146_2307483	Rht-B1	3	8.5	0.04
Pht	+	*QPht.jods-5A.2*	5A	288	283.5-289.5	IAAV108	Ra_c3966_2205	7.4	17.5	4.33
Pht	-	*QPht.jods-5A.2*	5A	288	283.5-290.5	IAAV108	Ra_c3966_2205	3.6	9.8	3.11
Fll	+	*QFll.jods-5A*	5A	296	294.5-296.5	Vrn-A1	Kukri_c12384_430	25.8	47.5	-3.16
Fll	-	*QFll.jods-5A*	5A	298	296.5-298.5	wsnp_Ex_c22727_31934296	wsnp_Ex_rep_c66689_65010988	16.2	33.2	-2.39

* Da: days to anthesis; Fsp: fertile spikelets per spike; Fspb: fertile spikelets in the basal spike section; Fspc: fertile spikelets in the central spike section; Fspd: fertile spikelets in the distal spike section. Gn: grain number in spike one; Gnc: grain number in the central spike section; Gnd: grain number in the distal spike section; Gw: grain weight in spike one; Gwc: grain weight in the central spike section; Gwd: grain weight in the distal spike section; Pht: plant height; Fll: flag leaf length.

‡ (+) Heat-stress treatment; (-) non-temperature stress treatment.

§ Chromosome number.

¶ PVE= phenotypic variation explained by QTL at the current scanning position.

‡ Positive additive effects indicate that favorable alleles originated from 'Attila' enhance corresponding trait values

, and negative additive effects indicate that favorable alleles originated from 'CDC Go' enhance the corresponding trait values.

Appendix D

Table D.1 ANOVA F-values and probabilities for the main effect means of cultivar, 4-Cl-IAA and temperature, and their interactions, for the reproductive parameters of spike 1 of wheat cultivars Attila, CDC Go, and CDC Teal presented in Table 2.1.

Treatment	Grain weight (g)		Grain number		Total spklts* per spike		Fertile spklts per spike		Grains per spklt		Grains per fertile spklt	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Cultivar	58	<.0001	72.04	<.0001	119.1	<.0001	20.43	<.0001	31.85	<.0001	57.49	<.0001
4-Cl-IAA	0.66	0.4194	1.13	0.2913	0.01	0.9394	0	0.984	1.02	0.3161	1.7	0.1954
Temperature	7.7	0.0067	2.65	0.1069	2	0.1608	0.02	0.9009	5.47	0.0216	9.23	0.0031
Cultivar × 4-Cl-IAA	0.36	0.6965	0.68	0.5071	1.7	0.1884	0.13	0.8798	1.16	0.3192	0.54	0.587
Cultivar × temperature	1.53	0.2221	0.15	0.8587	0.21	0.8102	0.68	0.5079	0.26	0.7691	0.36	0.6984
4-Cl-IAA × temperature	4.98	0.0281	6.68	0.0114	3.65	0.0591	2.77	0.0993	2.31	0.1323	1.52	0.2211
Cultivar × 4-Cl-IAA × temperature	2.85	0.0633	6.11	0.0032	1.74	0.1807	1.62	0.2031	2.69	0.0735	3.43	0.0365

* spklts = spikelets

Table D.2 ANOVA F-values and probabilities for the main effect means of cultivar, 4-Cl-IAA and temperature, and their interactions, for the reproductive parameters of spike 2 of wheat cultivars Attila, CDC Go, and CDC Teal presented in Table 2.2.

Treatment	Grain weight (g)		Grain number		Total spklts per spike		Fertile spklts per spike		Grains per spklt		Grains per fertile spklt	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Cultivar	61.28	<.0001	96.21	<.0001	256.21	<.0001	31.84	<.0001	49.9	<.0001	59.82	<.0001
4-Cl-IAA	0.33	0.5698	0.6	0.4416	0.44	0.5081	0.01	0.932	0.76	0.3855	0.8	0.3727
Temperature	8.09	0.0055	6.36	0.0134	1.25	0.2666	0.33	0.5676	4.98	0.0281	8.03	0.0057
Cultivar × 4-Cl-IAA	0.43	0.6529	0.77	0.4638	1.12	0.3311	0.99	0.3763	0.58	0.5643	0.16	0.8508
Cultivar × temperature	4.26	0.0171	2	0.1419	1.25	0.291	1.4	0.253	1.35	0.2639	0.92	0.4015
4-Cl-IAA × temperature	6.7	0.0112	10.11	0.002	0.01	0.9167	4.51	0.0364	10.69	0.0015	3.98	0.0491
Cultivar × 4-Cl-IAA × temperature	1.06	0.3511	4.28	0.0167	0.54	0.5824	1.79	0.1734	2.84	0.0634	0.83	0.4379

* spklts = spikelets

Table D.3 ANOVA F-values and probabilities for the main effect means of cultivar, 4-Cl-IAA and temperature, and their interactions, for the reproductive parameters of spike 3 of wheat cultivars Attila, CDC Go, and CDC Teal presented in Table 2.3.

Treatment	Grain weight (g)		Grain number		Total spklts per spike		Fertile spklts per spike		Grains per spklt		Grains per fertile spklt	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Cultivar	32.18	<.0001	34.91	<.0001	174.68	<.0001	14.53	<.0001	22.63	<.0001	22.31	<.0001
4-Cl-IAA	0.17	0.6804	0.05	0.819	0.72	0.3977	0.45	0.5032	0.17	0.6813	0.2	0.6565
Temperature	9.74	0.0024	4	0.0484	0.3	0.5851	0.3	0.5861	4.27	0.0416	5.06	0.0269
Cultivar × 4-Cl-IAA	0.54	0.5869	0.99	0.3773	0.02	0.9802	1.08	0.3431	1.67	0.1941	0.28	0.7531
Cultivar × temperature	2.9	0.0603	0.85	0.4321	0.83	0.4388	0.89	0.4153	0.3	0.7441	0.16	0.8562
4-Cl-IAA × temperature	5.77	0.0183	6.16	0.0149	0.75	0.3877	1.92	0.1693	5.39	0.0225	3.17	0.0786
Cultivar × 4-Cl-IAA × temperature	2.83	0.0643	2.73	0.0704	0.49	0.6166	1.11	0.3356	1.78	0.1749	0.78	0.4617

* spklts = spikelets

Table D.4 ANOVA F-values and probabilities for the main effect means of cultivar, 4-Cl-IAA and temperature, and their interactions, for the reproductive parameters of wheat cultivars Attila, CDC Go, and CDC Teal (average of the first three spikes) presented in Table 2.4.

Treatment	Grain weight (g)		Grain number		Total spklts per spike		Fertile spklts per spike		Grains per spklt		Grains per fertile spklt	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Cultivar	78.17	<.0001	107.25	<.0001	283	<.0001	34.83	<.0001	64.87	<.0001	79.14	<.0001
4-Cl-IAA	0.01	0.9436	0.88	0.3516	0.2	0.6554	0.15	0.704	1.22	0.2715	0.53	0.4704
Temperature	14.24	0.0003	7.61	0.007	0.01	0.9287	0.22	0.6437	10.09	0.002	13.26	0.0005
Cultivar × 4-Cl-IAA	0.37	0.6945	1.12	0.3316	0.76	0.4699	0.55	0.5763	1.81	0.1702	0.51	0.6023
Cultivar × temperature	4.56	0.013	1.42	0.2462	1.13	0.3278	1.5	0.2296	0.95	0.3899	0.42	0.6584
4-Cl-IAA × temperature	9.74	0.0024	13.43	0.0004	2.25	0.1375	5.43	0.022	11.26	0.0012	5.18	0.0253
Cultivar × 4-Cl-IAA × temperature	3.49	0.0346	7.44	0.001	1.57	0.2127	2.34	0.1019	4.85	0.01	2.57	0.0823

* spklts = spikelets

Table D.5 ANOVA F-values and probabilities for the main effect means of cultivar, 4-Cl-IAA, temperature, and spike section and their interactions, on reproductive parameters at the basal, central, and the distal sections of spikes 1, 2, and 3 of wheat cultivars Attila, CDC Go, and CDC Teal presented in Table 2.5.

Treatment	Grain weight (g)		Grain number		Total spklts per spike		Fertile spklts per spike	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Spike 1								
Cultivar	78.69	<.0001	101.45	<.0001	166.31	<.0001	23.92	<.0001
4-Cl-IAA	0.89	0.3459	1.59	0.2089	0.01	0.9283	0.04	0.8335
Temperature	10.43	0.0014	3.73	0.0544	2.79	0.0959	0.01	0.9203
Spike section	465.47	<.0001	320.29	<.0001	0.68	0.5066	210.08	<.0001
Cultivar × 4-Cl-IAA	0.49	0.612	0.96	0.3829	2.37	0.095	0.27	0.7598
Cultivar × temperature	2.07	0.1278	0.21	0.8068	0.29	0.7451	0.65	0.5211
4-Cl-IAA × temperature	6.75	0.0099	9.4	0.0024	5.1	0.0247	3.52	0.0618
Cultivar × spike section	6.04	0.0001	5.41	0.0003	1.86	0.1176	8.52	<.0001
4-Cl-IAA × spike section	0.04	0.9566	0.13	0.8765	0.55	0.5793	0.32	0.7247
Temperature × spike section	10.68	<.0001	6.91	0.0012	4.1	0.0176	1.1	0.3339
Cultivar × 4-Cl-IAA × temperature	3.85	0.0224	8.61	0.0002	2.43	0.0895	1.57	0.2104
Cultivar × 4-Cl-IAA × spike section	0.72	0.5766	0.76	0.5506	1.02	0.3954	0.48	0.748
Cultivar × temperature × spike section	3.9	0.0043	2.24	0.0649	0.51	0.7297	0.41	0.8012
4-Cl-IAA × temperature × spike section	1.25	0.2872	0.48	0.6209	2.26	0.1068	0.04	0.9608
Cultivar × 4-Cl-IAA × temperature × spike section	0.31	0.874	0.14	0.9659	1.07	0.3731	0.36	0.8399
Spike 2								
Cultivar	100.17	<.0001	139.09	<.0001	387.21	<.0001	37.1	<.0001
4-Cl-IAA	0.53	0.4665	0.86	0.3536	0.67	0.4147	0.01	0.9265
Temperature	13.23	0.0003	9.2	0.0027	1.89	0.1705	0.38	0.5363
Spike section	473.16	<.0001	348	<.0001	3.59	0.0289	174.81	<.0001
Cultivar × 4-Cl-IAA	0.7	0.4974	1.12	0.3278	1.69	0.1863	1.15	0.3179

Cultivar × temperature	6.96	0.0011	2.88	0.0576	1.89	0.1529	1.63	0.1987
4-Cl-IAA × temperature	10.95	0.0011	14.62	0.0002	0.02	0.8975	5.26	0.0226
Cultivar × spike section	12.73	<.0001	13.76	<.0001	2.19	0.0701	24.48	<.0001
4-Cl-IAA × spike section	1.48	0.23	0.22	0.8003	0.06	0.9446	0.71	0.4945
Temperature × spike section	9.77	<.0001	9.37	0.0001	0.27	0.764	3.82	0.0232
Cultivar × 4-Cl-IAA × temperature	1.73	0.1791	6.19	0.0023	0.82	0.4407	2.08	0.1267
Cultivar × 4-Cl-IAA × spike section	0.19	0.9413	0.21	0.9306	0.63	0.6402	0.46	0.7662
Cultivar × temperature × spike section	1.21	0.3064	1.12	0.3484	0.66	0.6208	0.21	0.933
4-Cl-IAA × temperature × spike section	0.01	0.992	0.07	0.9279	0	0.9955	0.82	0.4422
Cultivar × 4-Cl-IAA × temperature × spike section	0.49	0.7437	1.07	0.3706	0.06	0.9926	0.59	0.6706
Spike 3								
Cultivar	45.57	<.0001	57	<.0001	330.67	<.0001	19.83	<.0001
4-Cl-IAA	0.24	0.6233	0.09	0.7695	1.37	0.2434	0.62	0.433
Temperature	13.79	0.0002	6.54	0.0111	0.57	0.4515	0.41	0.5238
Spike section	380.91	<.0001	276.01	<.0001	1.39	0.252	189.81	<.0001
Cultivar × 4-Cl-IAA	0.76	0.4691	1.61	0.202	0.04	0.9628	1.48	0.2301
Cultivar × temperature	4.1	0.0176	1.38	0.2526	1.57	0.2092	1.21	0.2994
4-Cl-IAA × temperature	8.18	0.0046	10.06	0.0017	1.43	0.2335	2.62	0.1067
Cultivar × spike section	10.37	<.0001	10.07	<.0001	0.54	0.7086	20.79	<.0001
4-Cl-IAA × spike section	0.95	0.3861	0.47	0.6272	0.16	0.8546	0.06	0.9375
Temperature × spike section	7.88	0.0005	6.13	0.0025	0.49	0.6111	1.32	0.2687
Cultivar × 4-Cl-IAA × temperature	4.01	0.0193	4.46	0.0124	0.92	0.3997	1.51	0.2231
Cultivar × 4-Cl-IAA × spike section	0.14	0.9659	0.15	0.964	0.9	0.4638	0.35	0.8454
Cultivar × temperature × spike section	2.47	0.0449	2.04	0.0884	1.82	0.1244	0.57	0.6858
4-Cl-IAA × temperature × spike section	0.5	0.6042	0.47	0.6269	0.03	0.9688	0.06	0.9417
Cultivar × 4-Cl-IAA × temperature × spike section	0.09	0.9864	0.02	0.9991	0.79	0.5325	0.44	0.7764

* spklts = spikelets

Table D.6 ANOVA F-values and probabilities for the main effect means of cultivar, 4-Cl-IAA, temperature, and spike section and their interactions, on reproductive parameters at the basal, central, and the distal sections (average of first three spikes) of wheat cultivars Attila, CDC Go, and CDC Teal presented in Table 2.6.

Treatment	Grain weight (g)		Grain number		Total spklts per spike		Fertile spklts per spike	
	F value	Pr > F	F value	Pr > F	F value	Pr > F	F value	Pr > F
Cultivar	114.32	<.0001	157.02	<.0001	569.89	<.0001	44.09	<.0001
4-Cl-IAA	0.01	0.9317	1.28	0.2582	0.4	0.5257	0.12	0.7269
Temperature	20.83	<.0001	11.14	0.001	0.02	0.8988	0.29	0.5881
Spike section	709.01	<.0001	529.18	<.0001	2.93	0.0549	328.12	<.0001
Cultivar × 4-Cl-IAA	0.54	0.5862	1.64	0.1967	1.53	0.2176	0.8	0.4492
Cultivar × temperature	6.67	0.0015	2.08	0.1263	2.27	0.1049	1.87	0.1567
4-Cl-IAA × temperature	14.24	0.0002	19.67	<.0001	4.52	0.0344	7.06	0.0084
Cultivar × spike section	14.47	<.0001	15.18	<.0001	0.85	0.4952	29.69	<.0001
4-Cl-IAA × spike section	0.81	0.4481	0.17	0.8459	0.16	0.8553	0.07	0.9336
Temperature × spike section	15.57	<.0001	12.79	<.0001	2.56	0.0788	3.36	0.0362
Cultivar × 4-Cl-IAA × temperature	5.11	0.0067	10.89	<.0001	3.17	0.0435	2.88	0.0577
Cultivar × 4-Cl-IAA × spike section	0.25	0.9116	0.24	0.9184	0.14	0.9676	0.49	0.7401
Cultivar × temperature × spike section	3.7	0.0059	2.92	0.0217	0.65	0.6292	0.54	0.7091
4-Cl-IAA × temperature × spike section	0.69	0.5032	0.53	0.59	0.62	0.5362	0.3	0.7429
Cultivar × 4-Cl-IAA × temperature × spike section	0.13	0.9729	0.21	0.9311	0.15	0.9643	0.53	0.7142

* spklts = spikelets

Table D.7 ANOVA F-values and probabilities for the main effect means of cultivar, 4-Cl-IAA and temperature, and their interactions, for the reproductive parameters of spike 1 of nine wheat cultivars presented in Table 3.1.

Treatment	Grain weight (g)		Grain number		Days to anthesis		Plant height (cm)		Flag leaf area (cm^2)		Total spklts per spike		Fertile spklts per spike		Grains per spklt		Grains per fertile spklt	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Cultivar	15	<.0001	27.4	<.0001	40	<.0001	8.3	<.0001	5.8	<.0001	35.64	<.0001	13.36	<.0001	16.49	<.0001	24.5	<.0001
4-Cl-IAA	6	0.0186	5.5	0.0209	0.2	0.6679	1.8	0.183	3.5	0.066	2.32	0.1313	4.44	0.0376	4.06	0.0468	3.71	0.0572
Temperature	7	0.0123	13.8	0.0003	5.5	0.0213	34	<.0001	6.7	0.011	1.85	0.1767	7.4	0.0077	10.88	0.0014	8.97	0.0035
Cultivar × 4-Cl-IAA	0.8	0.6251	0.8	0.6264	0.5	0.8659	0.3	0.951	1.1	0.355	0.39	0.9225	0.67	0.7127	0.94	0.4911	1.01	0.4332
Cultivar × temperature	0.6	0.7951	1.5	0.1864	0.7	0.7259	3.1	0.004	1.2	0.335	0.9	0.5211	2.11	0.0423	1.32	0.2403	0.72	0.6774
4-Cl-IAA × temperature	1.3	0.2521	4	0.0492	0.5	0.4721	0.02	0.894	2.1	0.1469	1.11	0.2946	1.01	0.317	0.9	0.3458	0.55	0.4598
Cultivar × 4-Cl-IAA × temperature	0.5	0.8706	0.6	0.7711	2.7	0.011	0.7	0.677	0.7	0.6581	0.64	0.746	0.79	0.6139	0.38	0.9311	0.68	0.7052

* spklts = spikelets

Table D.8 ANOVA F-values and probabilities for the main effect means of cultivar, 4-Cl-IAA and temperature, and their interactions, for the reproductive parameters of spike 1 of 'Attila' and 'CDC Go' grown under the controlled environmental conditions presented in Table 3.2.

Treatment	Grain weight (g)		Grain number		Days to anthesis		Plant height (cm)		Flag leaf area (cm2)		Total spklts per spike		Fertile spklts per spike		Grains per spklt		Grains per fertile spklt	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
Cultivar	187	<.001	309	<.0001	454	<.0001	134	<.0001	60	<.0001	518	<.0001	323	<.0001	190	<.001	179	<.001
4-Cl-IAA	3.5	0.061	1.5	0.2206	0.1	0.7849	12.2	6E-04	4	0.0476	0.4	0.512	1.6	0.2063	1.9	0.173	0.6	0.437
Temperature	13	0.003	21	<.0001	16.6	<.0001	66.2	<.0001	0.1	0.7706	1.2	0.2775	13	0.0003	35	<.001	37	<.001
Cultivar × 4-Cl-IAA	0.0	0.861	0.0	0.9011	0.3	0.5586	0.1	0.794	3.3	0.072	0.6	0.4464	0.0	0.9184	0.1	0.753	0.3	0.561
Cultivar × temperature	8.5	0.004	2.5	0.1185	0.4	0.5564	0	0.956	3.7	0.0549	1.7	0.1986	14.5	0.0002	5.3	0.022	1.6	0.206
4-Cl-IAA × temperature	2.6	0.109	0.5	0.4758	1.8	0.1784	0.2	0.656	3.4	0.0671	1.8	0.1809	0.1	0.7742	2.0	0.164	1.7	0.199
Cultivar × 4-Cl-IAA × temperature	1.1	0.290	1.9	0.1744	0.2	0.6445	2.7	0.102	1.9	0.1646	0	0.97	1.1	0.3053	1.9	0.165	2.3	0.131

* spklts = spikelets

Table D.9 ANOVA F-values and probabilities for the main effect means of cultivar, spike section, and temperature and their interactions, on grain weight and grain number at the basal, central, and the distal sections of wheat cultivars Attila and CDC Go grown under the controlled environmental conditions presented in Table 3.3.

Treatment	Grain weight (g)		Grain number	
	F	Pr > F	F	Pr > F
Cultivar	181.6	<.0001	326.7	<.0001
Spike section	71.82	<.0001	45.33	<.0001
Temperature	26.67	<.0001	30.2	<.0001
Cultivar × temperature	14.97	0.0001	9.17	0.0026
Cultivar × spike section	10.1	<.0001	3.18	0.0428
Temperature × spike section	2.52	0.0819	2.3	0.1015
Cultivar × temperature × spike section	0.92	0.3978	0.57	0.5658

* spklts = spikelets

Table D.10 ANOVA F-values and probabilities for the main effect means of RILs, 4-Cl-IAA and temperature, and their interactions, for the reproductive parameters of spike 1 of the 'Attila' × 'CDC Go' RIL population grown under the controlled environmental conditions presented in Table 3.4.

Treatment	Grain weight (g)		Grain number		Days to anthesis		Plant height (cm)		Flag leaf area (cm2)		Total spklts per spike		Fertile spklts per spike		Grains per spklt		Grains per fertile spklt	
	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F	F	Pr > F
RIL	9.29	<.0001	12.7	<.0001	22.8	<.0001	25.2	<.0001	11.2	<.0001	9.96	<.0001	6.87	<.0001	8.68	<.0001	8.53	<.0001
4-Cl-IAA	5.85	0.0157	0.75	0.387	0.9	0.3434	8.51	0.004	15.7	<.0001	0.12	0.725	6.96	0.0084	3.36	0.067	0.01	0.9069
Temperature	361	<.0001	482	<.0001	42.4	<.0001	320	<.0001	28.6	<.0001	0	0.9842	239	<.0001	621	<.0001	371	<.0001
RIL × 4-Cl-IAA	0.91	0.7935	0.83	0.9477	0.8	0.9691	0.79	0.974	1.01	0.4641	0.81	0.964	0.9	0.8118	0.84	0.934	0.95	0.655
RIL × temperature	1.72	<.0001	1.7	<.0001	1.04	0.3531	0.9	0.808	1.03	0.3763	1.05	0.3233	1.63	<.0001	2.22	<.0001	1.67	<.0001
4-Cl-IAA × temperature	0.08	0.7752	1.1	0.2934	1.05	0.3066	0.64	0.423	2.91	0.0881	0.63	0.4271	2.61	0.1064	0.62	0.4313	0.06	0.8076
RIL × 4-Cl-IAA × temperature	0.77	0.9864	0.82	0.9578	0.8	0.9704	0.84	0.924	1.07	0.2588	1.01	0.4373	0.84	0.9311	0.87	0.8725	1	0.4918

* spklts = spikelets

Table D.11 ANOVA F-values and probabilities for the main effect means of RILs, spike section, and temperature, and their interactions, for the grain number and weight of spike 1 of the 'Attila' × 'CDC Go' RIL population grown under the controlled environmental conditions presented in Table 3.5.

Treatment	Grain weight (g)		Grain number	
	F	Pr > F	F	Pr > F
RIL	9.23	<.0001	12.17	<.0001
Spike section	1086	<.0001	799	<.0001
Temperature	327	<.0001	422.5	<.0001
RIL × temperature	2.4	<.0001	2.42	<.0001
RIL × spike section	1.87	<.0001	2.05	<.0001
Temperature × spike section	27.75	<.0001	20.74	<.0001
RIL × temperature × spike section	0.65	1	0.6	1

* spklts = spikelets