SCREENING OF WINTER WHEAT DOUBLE HAPLOID POPULATION 'BUSTER' UNDER HEAT AND DROUGHT STRESS

By

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SCREENING OF WINTER WHEAT DOUBLE HAPLOID POPULATION 'BUSTER' UNDER HEAT AND DROUGHT STRESS

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Abstract: The major abiotic stresses associated with wheat production throughout the world are heat and drought. The objective of this research was to screen a double haploid (DH) 'Buster' population to identify and select DH lines with improved drought and heat resistance. Four separate studies evaluated the response of DH population to no stress, high temperature stress, drought stress, and combined high temperature and drought stress in controlled conditions at Oklahoma State University, Stillwater, OK. One hundred lines from the DH Buster population, developed from a cross of the wheat varieties 'Billings' and 'Duster', were used for the first two studies and 33 lines from the same population were used for the remaining studies. Different morpho-physiological parameters including photosynthetic pigments, tiller numbers, plant height, per unit area leaf photosynthesis, transpiration, stomatal conductance, intercellular CO_2 concentration, electron transport rate, fluorescence, instantaneous water use efficiency (IWUE), membrane thermal stability, carbohydrate remobilization, spike photosynthesis and spike and stem weights were recorded depending upon the specific objective of each study. A portable photosynthesis and fluorescence system was used to measure gas exchange parameters of leaf and spike. The defoliation treatment imposed in the drought study enabled to decipher the contribution of carbohydrate remobilization from the stem towards grain yield. Results from screening under stress free conditions showed significant differences between 100 DH lines for plant height, tiller numbers, and leaf area. Similarly, DH lines were significantly different for gas exchange and fluorescence parameters, where stomatal conductance and IWUE explained most of the variability in the population under heat stress. The IWUE was least affected in the Buster line 'DH263' under heat stress. In the drought study, the Buster lines did not differ significantly but showed similar response to different defoliation treatments. Partial defoliation increased the average spike weight demonstrating more carbohydrate remobilization from stems for grain filling under drought. The 'Buster' line 'DH236' performed well under both irrigated and drought conditions as indicated by greater carbohydrate remobilization and spike photosynthesis. In conclusion, including the identified traits (plant height, tiller number, leaf size, IWUE, and spike photosynthesis) and better performing lines (DH lines 136, 210, 236, 248, 257 and 263) into future research and breeding will accelerate development of abiotic stress tolerance in wheat.

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CHAPTER I

GENERAL INTRODUCTION

Wheat (*Triticum aestivum* L.) is the main staple food for many countries in the world including the United States of America (Bushuk, 1998; Crista et al., 2012; Shiferaw et al., 2013). It is one of the most important crops for world food security and is planted on more than 241 M ha annually across the world under different climatic conditions with a total production of 728 MT (Shiferaw et al., 2013). Wheat provides about 20% of global total dietary calories and protein (Braun et al., 2010). With world population projected to reach 9.6 billion by 2050 (United Nations, 2013), it is necessary to develop techniques to accelerate the rate of increase in crop productivity to meet the population demand. In addition to optimization of inputs and management activities, crop productivity improvement is imperative especially in response to the variable and changing climatic conditions.

Both abiotic and biotic stresses limit crop productivity and necessitate development of tolerance/resistance individually and in combination. The major abiotic stresses associated with limited wheat productivity throughout the world are increase in global temperature (Gourdji et al., 2013) and decrease in water availability (Rezaei et al., 2010; Wallace, 2000). It has been estimated that the major crops grown in the world are able to achieve only about 50% of their full potential because of different abiotic factors such as heat, freezing, drought, flooding and soil properties (Hatfield & Walthall, 2015; Wang et al., 2003). Based on a multi-model ensemble analysis, wheat production is projected to drop by 6%, which equals to approximately 42

MT, for each degree Celcius increase in temperature (Asseng et al., 2015). Wheat experiences both drought and heat during its annual growing period in several regions of the world. Drought is a period without precipitation leading to depletion of soil water. The stress resulting from drought causes injury to plants by affecting various plant processes. Likewise, heat stress in plants is a result of temperatures high enough to cause alterations in plant metabolic or physiological activities. Plants exhibit different strategies; avoidance, tolerance and escape to adjust under adverse environmental conditions such as drought and heat stress (Taiz and Zeiger, 2006). Avoidance is a mechanism in which plants make strategic changes in their life cycle to avoid the stress. Some plants show drought escape strategy by quickly completing their life cycle. Tolerance mechanism in plants is characterized by modification in different physiological processes or development of resilient structures to withstand the stress. A combination of such different mechanisms finally contribute to stress resistance in plants.

Because of the unpredictable and erratic nature of rainfall in the Southern Great Plains (SGP) of the United States (Uddin et al., 1992), there is a need for cultivars that can withstand stress with minimal loss in productivity but still be able to have optimum production under favorable situations. The SGP has been experiencing severe dry and hot weather during fall and spring seasons, which reduce tillering, leaf production and grain filling of wheat (Schonfeld et al., 1988) and more frequent and persistent droughts with an increase in global temperature is projected in the near future (Su et al., 2013). These climate projections are likely to reduce wheat production in Oklahoma. One of the major aims of the Wheat Improvement Team (WIT) at Oklahoma State University (OSU), an interdisciplinary team of scientists working on improvement of the wheat genetic resources, is to strengthen the Oklahoma wheat industry.

This study utilized the plant materials from a double haploid (DH) population developed by the OSU WIT. This population resulted from 32 F1's obtained by crossing two popular wheat varieties 'Duster' and 'Billings'. From an ancestral perspective, 'Duster' and 'Billings' probably

account for the largest segment of the elite germplasm currently flowing through OSU WIT variety development program. These two parent lines demonstrate high yield potential with impressive disease resistance and end-use quality performance. However, they reach their yield in different and complementary ways with 'Duster' having high kernel number and drought resistance, while 'Billings' has large kernel size and is susceptible to drought. In addition, 'Duster' and 'Billings' show wide pattern differences in reproductive development, yet all known genes for reproductive development were identical between them. A population developed combing these varieties would have extremely high potential value to variety development. The WIT envisions that a DH population would lead to trait discoveries, marker discoveries, knowledge of inheritance, and reduce breeding time that would have far-reaching impact in further manipulating the pipeline (B. Carver, personal communication).

To this effect, 36 F1 seeds from the single cross Duster/Billings (OK10x994) were provided to Heartland Plant Innovations (HPI, Manhattan, KS) on 10/26/10, with the expectation to produce 300 haploids (DHs). Colchicine treatment was used to develop the DHs. At HPI, the D0 and D1 plant generations were reared and D2 seed was provided to WIT at OSU in 2012. A total of 278 DHs were in sufficient supply to plant back in unreplicated single-row observation plots in 2012-2013 at Stillwater. About 271 DHs were then advanced for further evaluation in 2013-2014. The 271 lines were arbitrarily assigned to 6 sets of 42 lines each, plus one overflow set of 19 DHs, to evaluate in replicated field plots in 2014, 22015, and 2016 at Stillwater. Sets were created to reduce block size in the field, and the two parents were included in each set as a common check. Seed yield and seed of 256 DH lines including the parental varieties were available from the 2013-2014 season. For ease of referencing, the Duster/Billing DH population is called Buster population going forward (B- from 'Billings' and –uster from 'Duster') (B. Carver, personal communication).

A number of studies elucidated effects of heat and drought stresses on wheat, but most of those studies have either taken into account the whole plant life cycle or focused on the post-anthesis periods (Balla et al., 2006; Blum et al., 1994; Hassan, 2006; Zamani et al., 2014). Screening plants for the heat and drought tolerant traits during early plant growth stages can help reduce the duration for research and overall selection process. In the long process for variety release, this research can act as an intermediary for (i) identification of the drought and heat tolerant traits in different Buster lines at the plant physiological level, and (ii) selection of the Buster lines with desired characteristics for future breeding programs.

This research focuses on identifying differences between 100 Buster lines and selecting them based on measured parameters. The 100 Buster lines were selected from a yield trial conducted during the 2013-2014 growing season in Stillwater, OK. This was an extreme drought year with 270% yield difference between the low and high yielding lines (V.G. Kakani, personal communication). According to the U.S. Drought Monitor, most of Oklahoma wheat growing region was under Class 1 to Class 5 degradation due to drought as the crop season progressed (Figure 1). Similarly, a majority of crops in Oklahoma experienced severe to extreme drought during the active crop growing period during spring of 2014 (Figure 2).

Each of the 6 sub-group (described earlier) was divided into high, average and low yield based on the mean yield ± 1 standard deviation. From each yield group 5 lines were selected resulting in 15 lines for each sub group. A few additional lines with extreme yield values along with parents were selected to create the set of 100 Buster lines used in the current research (V.G. Kakani, personal communication). Similar methodology was used to develop a subset of 33 Buster lines. Selected Buster lines were evaluated under both non-stressed and stressed conditions for selection at early and late stages of plant growth. Heat and drought stress responses in wheat crop were studied individually and in combination using previously established techniques for evaluation. Four separate experiments were setup to address four specific objectives of the research project. The first experiment was conducted under greenhouse conditions without any

stress and Buster lines were assessed for differences based on photosynthetic pigments concentration, leaf morphology, tiller numbers and plant height. The second experiment was conducted in a growth chamber to study the heat stress response of the Buster lines with data on leaf gas exchange parameters and cell membrane stability. Likewise, the third experiment was conducted in the green house to study the drought response of Buster lines using data on spike photosynthesis and carbohydrate remobilization. The fourth experiment was conducted in the growth chambers to assess the response of Buster lines to heat and drought stress using leaf and spike gas exchange parameters and spikes weights.

The main objective of this research was to screen the Buster DH population for drought and heat tolerant traits and the specific objectives were:

a) To analyze differences in plant morpho-physiological traits among 100 Buster DH lines.
b) To assess variation among 100 Buster DH lines in response to heat stress.
c) To screen 33 Buster DH lines for drought responsive traits, carbohydrate remobilization and spike photosynthesis.
d) To study the variation in gas exchange parameters of leaf and spike and yield among 33 Buster DH lines under heat stress and drought conditions.

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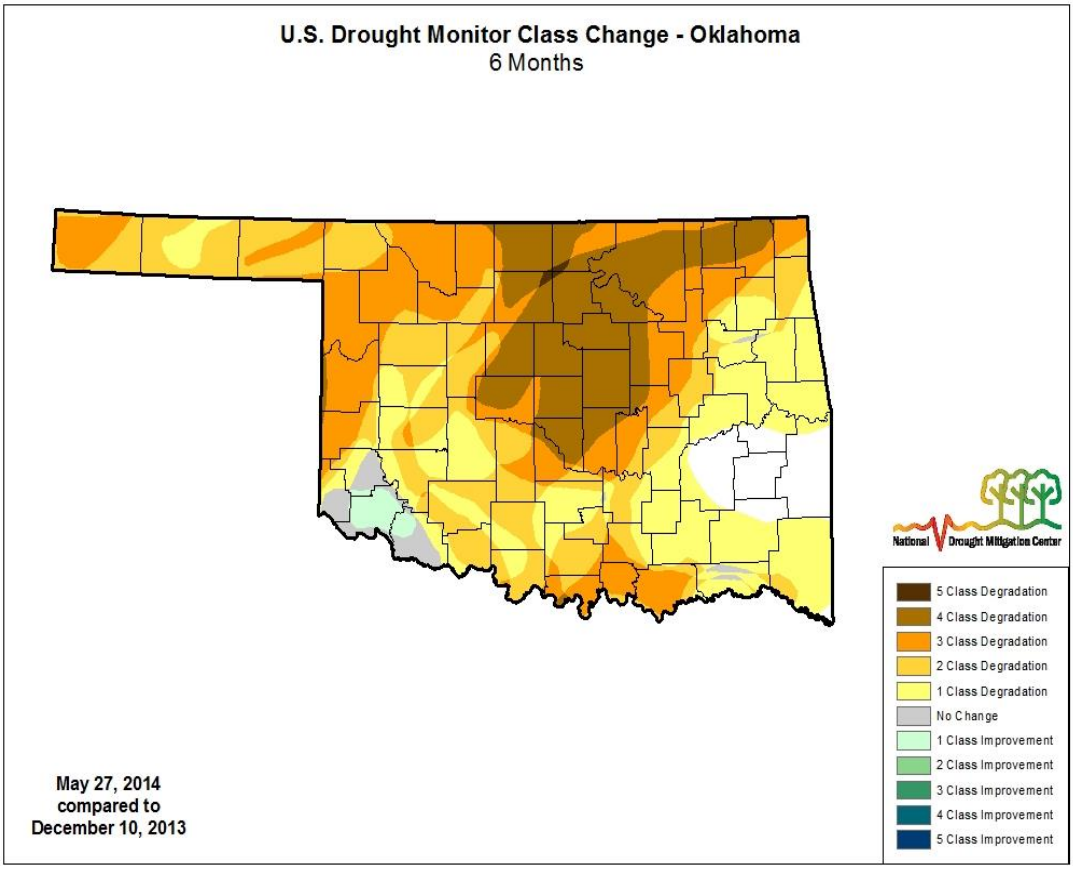

Figure 1. Change in U.S. Drought monitor class from December10, 2013 to May 27, 2014.

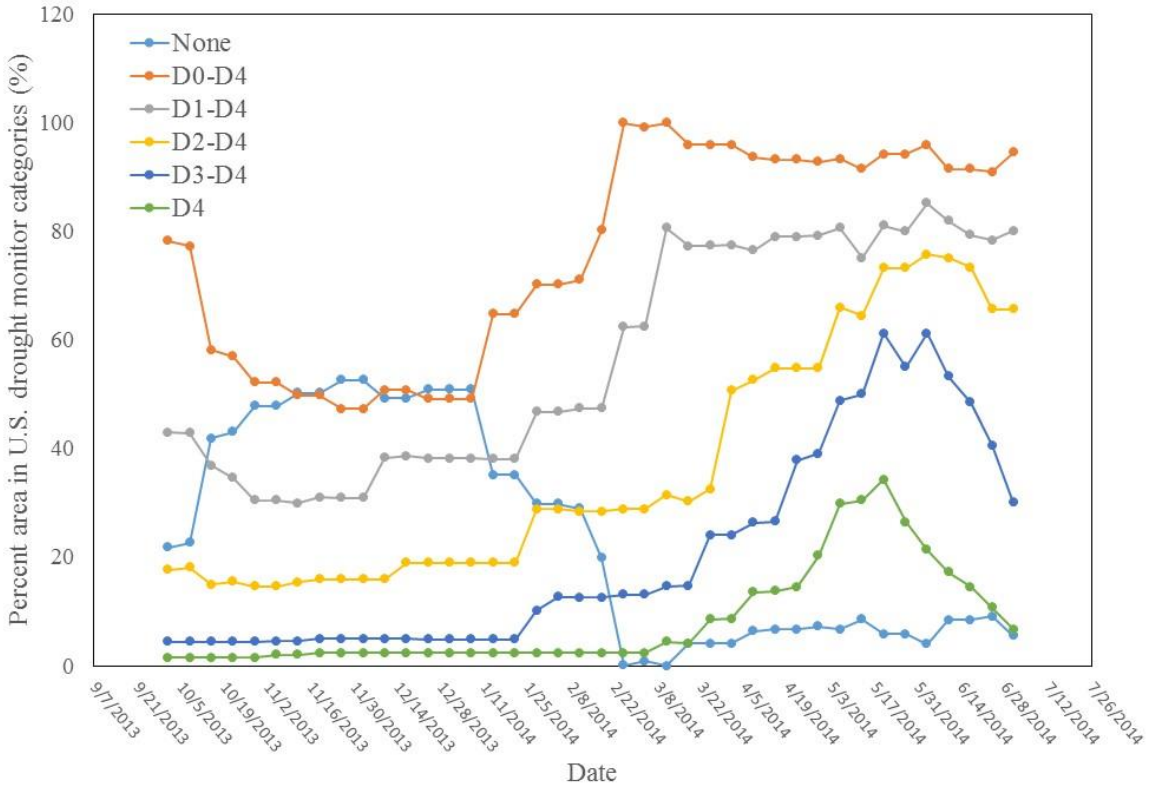

Figure 2: Progress in different drought categories in Oklahoma during the 2013-2014 wheat season. Categories described as; None- no drought; D0 – Abnormally dry, D1-Moderate drought; D2 – Severe drought; D3 – Extreme drought; and D4 – Exceptional drought. (Source: http://droughtmonitor.unl.edu)

CHAPTER II

ANALYSIS OF DIFFERENCES IN PLANT MORPHO-PHYSIOLOGICAL TRAITS AMONG 'BUSTER' LINES

Abstract

Improvement in phenotype is an important target for increased productivity of winter wheat in normal and abiotic stress conditions. This study was conducted to characterize morpho-physiological traits of 100 'Buster' lines and to identify lines for further research and variety release. Plant height, tiller number and leaf number were recorded at weekly intervals and leaf area on the main stem was recorded once. Photosynthetic pigments (chlorophyll A, chlorophyll B, carotenoids and phenolic compounds) were determined by spectrophotometry. The Buster lines showed significant differences for plant height, tiller number and leaf area but were not significantly different for the pigment concentrations and leaf number. The plant height and leaf area had a positive correlation with each other. The pigment concentrations were also positively correlated among each other. The Buster lines can potentially be used in further breeding research programs based on their available variability for morphological traits.

1. Introduction:

Wheat is grown in many parts of the world under different climatic conditions. Different varieties of wheat are developed in accordance with niche environments. Selection of genotypes is a continuous long-term process for the development of a new variety, as it takes about 8-10 years to release a variety. This study aims to identify differences on morpho-physiological traits among the Buster lines to provide information for further studies by the Wheat Improvement Team (WIT) at Oklahoma State University (OSU). The Buster lines are double haploid (DH) lines developed by OSU WIT crossing two wheat varieties, 'Duster' and 'Billings'. A detailed description on Buster lines and their development is given in Chapter I of this thesis.

Photosynthesis is the process that provides the raw materials for formation of the plant products. It is one of the most important factors influencing carbon assimilation by a plant and the overall yield (Reynolds et al., 2009; Richards, 2000). According to a review by Long et al. (2006), leaf photosynthetic rates have been known to have poor correlation with yield in the past whereas recent studies have shown positive correlation between yield increase and leaf photosynthesis. Recently, it has been reported that increased light harvesting by photosynthesis is the major cause for increase in crop yields (Zhu et al., 2010). However, due to limited information in wheat, further research is required to demonstrate the relationship between leaf photosynthesis and wheat yield.

Among different factors influencing photosynthesis, photosynthetic pigments play a significant role (Hamblin et al., 2014). Likewise, morpho-physiological improvements are one of the reasons behind increased productivity in winter wheat (Austin et al., 1980). Selection of genotypes for higher yield based on their morphological characteristics including plant height (Ilker et al., 2013), tiller number (Duggan et al., 2005) and leaf area (Reghetti et al., 2007) has been a successful approach in crop research. These morphological attributes are also taken into

account while developing a tolerant variety for abiotic stresses such as heat and drought (Ali et al., 2015; Balota et al., 2008; Ihsan et al., 2016).

1.1. Photosynthetic pigments

During photosynthesis, chlorophyll absorbs photon for CO_2 fixation (Zhao & Zou, 2002). If excess photons are absorbed by chlorophyll (more than a leaf can use for fixing CO_2), then reactive oxygen species (ROS) are formed which cause photo oxidative damage to the leaves (Asada, 1996; Richter et al., 1990). There are antioxidant compounds present in the leaves that scavenge the ROS and protect the photosynthetic apparatus (photosystem-I + photosystem-II) (Bowler et al., 1992; Salah et al., 1995). The phenolic compounds and carotenoids play an important role to protect leaves from ROS damage as they belong to the antioxidants group of compounds (Ye et al., 2000; Zhao & Zou, 2002).

1.1.1. Chlorophyll

Differences in chlorophyll content of wheat genotypes in response to drought correlates positively with yield, grain number and grain size (Izanloo et al., 2008). The chlorophyll content of wheat leaves is an effective selection criterion for screening wheat genotypes for drought tolerance. Higher chlorophyll content in leaves under drought reflects the tolerance of the varieties to drought stress (Abdipur et al., 2013). Akhkha et al. (2011) reported a significant interaction effect of drought and genotype for leaf chlorophyll content. However, reduced chlorophyll levels per unit area are desirable in plants under high temperatures because increased light absorption under high temperatures causes heat stress in plants (Hamblin et al., 2014).

1.1.2. Carotenoids

Carotenoids are one of the indispensable components of photosynthetic mechanism in plants and many studies have emphasized their importance (Cogdell, 1985; Damjanovic, Ritz, &

Schulten, 1999; Domonkos et al., 2013). Carotenoids play a major role in photosynthesis by harvesting light to extend the spectral range and protecting chlorophyll from photo-oxidative damage (Burkhardt & Bohm, 2007; Cogdell, 1985; Frank & Brudvig, 2004). Most of the carotenoids are present in thylakoid membrane of leaves, which is the site for light reactions of photosynthesis. Carotenoids improve electron transfer and light harvesting efficiency of plants to stabilize the photosynthetic apparatus and protect it from photo-destruction (Domonkos et al., 2013).

1.1.3. Phenolic compounds

The antioxidant activities of different compounds including phenolic compounds are responsible for preventing photo-oxidative damage by ROS in higher plants (Salah et al., 1995; Ye et al., 2000). Phenolic compounds have protective effect on photosynthesis since they scavenge the ROS produced during light reactions in photosynthesis under moderate and high irradiance (Zhao & Zou, 2002). The concentration of phenolic compounds correlates positively to antioxidant activities (Hatamnia et al., 2016). Hura et al. (2009) showed that phenolic compounds are reliable indicators for differences in genotypes in *Triticale* spp., especially under water deficit conditions where resistant genotypes had higher phenolic content compared to susceptible genotypes as determined by spectrofluorometer. The spectrofluorometer has specific excitation wavelengths to activate the pigments and measures emission of fluorescence by each of the pigments at specific wavelengths. The spectrophotometer used in this study measures the light absorbance by the pigments.

1.2. Leaf area

Leaf area should be taken into account while comparing genotypes for leaf parameters because comparison of genotypes having differences in per unit area leaf traits may not represent the actual differences and produce misleading results (Bhagsari & Brown, 1986; Righetti et al.,

2007). Balota et al. (2008) found that the drought tolerant wheat varieties have significantly smaller leaf area in both irrigated and drought conditions as compared to drought susceptible varieties. Negative correlation was recorded between leaf area and photosynthesis per unit leaf area as indicated by correlation analysis (Bhagsari & Brown, 1986; Oritani et al., 1979).

1.3. Growth attributes:

Growth attributes such as number of tillers and number of leaves on main stem are recorded periodically to gain insight on plant developmental phases. Number of effective tillers (fertile tillers) is one of the important yield attributes in wheat (Naruoka et al., 2011). The number of leaves on main stem affects flowering time of wheat (He et al., 2012). Delayed flowering in winter wheat can expose the crop to warmer temperatures at latter growth stages and ultimately shorten the grain-filling period of wheat. Significant differences among different wheat genotypes for leaves and tillers number were recorded (Bos & Neuteboom, 1998). Likewise, short plant height is one of the ideotypes for wheat and is one of the main reasons for increase in wheat yields in last five decades (Rybka & Nita, 2015). Ideotype is defined as a model plant with the right combination of traits that can realize the yield potential (Donald, 1968). Short wheat varieties are found to have higher yield potential under normal conditions, but tall wheat varieties can yield more than dwarf ones under severe drought conditions (Fischer & Maurer, 1978).

The current study was conducted during vegetative growth stage of plants in order to identify the potential number of tillers, number of leaves and plant height that a genotype can achieve before reproductive phase; therefore, the plants were not subjected to vernalization. This study attempted to obtain a baseline data and that is why no treatments were imposed in this experiment. In addition, screening plants at an early age can speed up the selection process. The objective of this study was to identify differences between the Buster lines based on their

morphological and physiological characteristics to provide information on morpho-physiological traits of selected Buster lines for further research and variety release.

2. Materials and Methods:

The study was conducted at Oklahoma State University in Stillwater, OK, USA. A total of hundred genotypes from 256 Buster DH lines were used for the experiment. The 100 lines were selected based on the experimental plots yield in the year 2013-14. The details on the selection process of the Buster lines for this study is provided in Chapter I of this thesis.

2.1. Experimental setup:

The greenhouse study was conducted without artificially imposing any stresses. Five seeds of each selected Buster line were sown in pots made from PVC pipes 50 cm deep and 15 cm in diameter. There were two replications with two pots per genotype. Pure sand was used as rooting medium instead of soil to obtain optimum control of water and nutrient supply to roots. Automatic drip irrigation system was used to supply 0.3 L of Hoagland's nutrient solution to the plants each time, four times a day at 8:00 AM, 12:00 PM, 4:00 PM and 8:00PM. In this study, data on leaf morphology (length, width and area), plant developmental changes (plant height, tiller number and leaf number) and pigment concentrations (chlorophyll A, chlorophyll B, carotenoids and phenolic content) were collected.

2.2. Photosynthetic pigments:

Chlorophyll A, chlorophyll B, and carotenoids were extracted using dimethyl sulfoxide (DMSO) as the extractant. Five leaf discs, 1 cm^2 each, were punched from five randomly selected leaves from each pot. The leaf discs were immersed in 5 ml of DMSO for 24 hours in the dark. The concentrations of the pigments were calculated from absorbance values obtained with a spectrophotometer (Genesys 10 Bio Spectrophotometer, Thermo Scientific) at 664 nm, 648 nm

and 470 nm for chlorophyll A, chlorophyll B and carotenoids respectively using equations by Lichtenthaler (1987):

Chlorophyll $A_c = 12.25A_{664\ nm} - 2.79A_{648\ nm}$,

Chlorophyll $B_c = 21.50A_{648\ nm} - 5.10A_{664\ nm}$,

Carotenoids$_c$ = $(1000A_{470\ nm} - 1.82$ chl $a_c - 85.02$chl $b_c) / 198$

Where,

A = absorbance at respective wavelengths,

c = pigment concentration (µg/mL of extract).

For the determination of phenolic compounds concentration, five leaves were randomly selected from a pot to get five leaf discs, 1 cm^2 each. The leaf discs were placed in the extractant solution for 24 hours at room temperature. The solution used for extraction of phenolic compounds was composed of methanol, water and hydrochloric acid in the ratio of 79: 20: 1.

Absorbance values were obtained at 330 nm for phenolic compounds and the concentration was calculated as given by Kakani et al. (2004):

C = 16.05 * A

Where,

C = concentration of phenolic compounds (µg/mL of extract),

A = absorbance at 330 nm.

2.3. Leaf area:

Three leaves per pot were randomly selected for leaf morphological data measurements after 75 days after sowing (DAS). LI-3000 (Licor Inc., NE, USA) portable leaf area meter was used for measuring leaf area, leaf length, maximum width and average width of each selected leaf. Leaves were carefully selected from same stem position in the plants to avoid differences in physiological age.

2.4. Growth attributes:

Two plants per pot were randomly selected and marked during their seedling stage. Data on tiller number, leaf number and plant height were collected from the marked plants on a weekly basis starting when leaf nodes were visible in the sampled plants for five weeks. Plant heights were recorded from base of the plant to the upper most collar on the main stem. Tiller number was counted for each of the two plants and leaf numbers were counted in the main stem of each of those two plants.

2.5. Statistical analyses:

Data collected was analyzed using SAS Version 9.4 (SAS Institute, Cary, NC). The Analysis of Variance (ANOVA) was performed using PROC GLM to see if the Buster lines were statistically significant at $P = 0.05$ probability level for the recorded parameters. Values are provided as means for chlorophyll, carotenoids, phenolic content and leaf area. Slopes were calculated for rates of increase in tiller number, leaf number and plant height from weekly observations. Correlation matrix for observed parameters was constructed using PROC CORR. Graphs were constructed using Sigma Plot.

3. Results and Discussion:

3.1. Photosynthetic pigments:

Differences in pigment concentration values were observed for different pigments concentrations among the Buster lines but were not statistically significant at 0.05 levels of significance. The obtained P-values for chlorophyll A, chlorophyll B, carotenoids and phenolic contents are 0.75, 0.95, 0.11 and 0.29, respectively. No significant differences were found by Abdipur et al. (2013) for chlorophyll content among genotypes when first spikelet of the inflorescence was visible. However, differences had been recorded for later growth stages (Abdipur et al., 2013). Studies have shown differences in chlorophyll content in different wheat cultivars and under different stress conditions (Akhkha et al., 2011; Hamblin et al., 2014). Likewise, no significant difference was found between the Buster lines for chlorophyll A/B ratio. Chlorophyll A/B ratio ranged from 2.64 to 4.9 among the Buster lines. Since the chlorophyll A and chlorophyll B concentrations were not significantly different among the lines, it is no surprise that their ratios are not significant. There is very limited information on carotenoids analysis on leaves, especially in context of wheat where studies are concentrated towards grain carotenoids content in durum wheat. Studies show differences in carotenoids content in leaves for different genotypes and/or stress combination in crops like soybeans (Dhanapal et al., 2015) and tomatoes (Barickman et al., 2014). Likewise, phenolic content in leaves and their role in scavenging ROS have been studied under various abiotic stress conditions like drought and salinity but not yet studied for non-stressed conditions. However, differential responses of phenolic compounds concentration to stresses has been observed for different growth stages where differences were mostly expressed at reproductive stages of plant growth (Ashraf et al., 2010). Therefore, the lack of significant differences in pigments concentrations among Buster lines in this study may be because of growth stage since the data were collected during the vegetative stage of plants. In addition, this might also be a result of non-stressed conditions in terms of temperature and water

availability in this study. Furthermore, the homogeneity of genes in these DH lines may be responsible for similar pigment concentrations.

The Buster lines are separately grouped for individual pigment components chlorophyll A, chlorophyll B, carotenoids and phenolic content based on mean ± 1 and 2 standard deviations. The numbers of Buster lines in each group for chlorophyll A, chlorophyll B, carotenoids and phenolic compound are shown in Figures 1, 2, 3 and 4, respectively. The mean, standard deviation, minimum and maximum values for the pigments concentrations are given in Table 1. The data for average concentration of each of the four pigments and their groups are presented in Table 2.

3.2. Leaf morphological attributes:

Significant ($P < 0.01$) differences between Buster lines were observed in leaf area, leaf length and leaf width. The leaf area ranged from 20.94 in parental line 'Duster' to 38.56 cm^2 in Buster line 'DH73' with the average of 28.85 cm^2 and s.d. of 3.59 cm^2. The data on averages of leaf area, leaf length, average width and maximum width across three leaves from a pot, and the description of the Buster lines are given in Table 3. Most of the Buster lines demonstrated greater leaf area than the parental lines, Duster and Billings, which may be because of segregation of genes in the DH population. The Buster lines are grouped based on leaf area mean ± 1 and 2 s.d. and number of Buster lines on each group is shown in the Figure 5. In a study by Morgan & Lecain (1991), significant differences were observed in leaf areas and a weak negative correlation between leaf area and water use efficiency was reported.

3.3. Growth attributes:

Significant differences ($P<0.05$) were observed between Buster lines for rates of increase in plant height and tiller number but not for the rates of increase in leaf number. The lowest rate of increase in height was observed in Buster line 'DH231' followed by the parental line 'Duster' and

highest rate was observed in the other parental line ‘Billings’. The differences in plant height might have resulted from the differences in height of their parents. ‘Duster’ and ‘Billings’ are both categorized as intermediate semi-dwarfs but differ in plant heights; ‘Duster’ (71 cm) (Edwards et al., 2012) and ‘Billings’ (73 cm) (Hunger et al., 2014). Although these plant heights represent the heights from ground level to spike tip, these differences can still be reflected on vegetative plant growth stages. In a study done by Austin et al. (1980) on identifying genetic and physiological improvements in wheat over a decade, reduced plant height was identified as one of the important characteristics for improved yield. Similar results were obtained by Donmez et al. (2001). Number of tillers and rate of increase in tiller number were highest in Buster line ‘DH136’ and lowest in ‘DH224’. Higher number of tillers are found to contribute towards higher harvest index in normal conditions but reduced number of tillers are desirable under water deficit (Duggan et al., 2005). The significant differences in tiller numbers in this case can be explained by probable segregation of genes in the population for tiller number because the parental line ‘Duster’ is known to have high tiller number while ‘Billings’ lacks this attribute. Duster and Billings showed wide pattern differences in reproductive development, yet all known genes for reproductive development were identical between them (B. Carver, personal communication). The number of tillers observed can be an indicator of tillering capacity of a genotype because the plants were putting new tillers for a long time since there was no vernalization imposed for the plants to start reproductive phase. The lack of significant differences in leaf number may be a result of definite time period in which the data was collected i.e. five weeks. Unlike tiller number and plant height, which gain measurable increments in short time period, it is required for a leaf to fully open to be counted as a leaf. The data collection duration might not have been sufficient enough to reflect the differences in leaf number. In addition, the parental lines ‘Duster’ and ‘Billings’ do not have reported differences for the leaf number or rates of increase in leaf number which further supports the results of not getting significant differences in leaf number among the studied Buster lines.

3.4. Correlation between the measured parameters:

The photosynthetic pigments positively correlated to each other at 0.01 levels of significance (Table 4). Among those, chlorophyll A and B, and carotenoids were more strongly correlated than with the phenolic compounds. Leaf area was significantly positively correlated to the height parameters whereas negatively correlated to the leaf number parameters. It seems that no such correlations have been studied specifically, but many studies have been done with the plant height and leaf area as the selection criteria. Final plant height is weakly negatively correlated to the rate of increase in leaf number. The correlation matrix for the measured parameters is shown in Table 4. The results showing the plant height and leaf area positively correlated to each other provides an opportunity to select Buster lines with shorter plant height and lower leaf area at the same time, which are the desirable characteristics.

4. Conclusions:

The studied Buster lines were significantly different in the morphological traits plant height, tiller number and leaf area but did not show a significant difference in terms of leaf pigments concentration. The physiological and morphological characteristics were not correlated with each other but were positively correlated within themselves. The tiller number recorded in this case might be an indicator of the potential tillering capacity of the genotype. There is a potential to select Buster lines in breeding programs based on the morphological characteristics, and this study provides a baseline data set on morpho-physiological attributes of selected Buster lines for further studies.

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Table 1: Mean, standard deviation (s.d.), minimum and maximum values for photosynthetic pigments chlorophyll A, chlorophyll B, carotenoids and phenolics.

	Chlorophyll A	Chlorophyll B	Carotenoids	Phenolics
mean	29.34	8.60	7.30	12.92
s.d	2.58	1.30	0.78	1.73
Minimum	15.48	5.05	3.58	4.57
Maximum	32.05	11.33	16.47	4.31

Table 2: Values for leaf pigments: chlorophyll A, chlorophyll B, carotenoids and phenolic compounds concentration of 100 DH Buster lines grouped based on mean ± 1 and 2 s.d. for each pigment. Where (A): < mean – 2 s.d., (B): mean – 2 s.d. to mean – 1 s.d., (C): mean – 1 s.d. to mean, (D): mean to mean + 1 s.d., (E): mean + 1 s.d. to mean + 2 s.d. and (F): > mean + 2 s.d.

Buster line no.	Chlorophyll A		Chlorophyll B		Carotenoids		Phenolic compounds	
1	31.14	D	10.00	E	8.03	D	16.47	F
2	31.88	D	10.73	E	7.81	D	12.14	C
3	31.37	D	10.82	E	8.25	E	11.50	C
4	31.35	D	10.03	E	8.19	E	9.87	B
5	29.34	C	8.04	C	7.59	D	11.22	C
6	30.88	D	8.85	D	7.77	D	15.98	E
7	30.47	D	9.14	D	8.02	D	10.98	B
8	28.41	C	8.53	C	7.62	D	10.72	B
9	30.17	D	9.22	D	7.82	D	13.36	D
10	29.79	D	8.80	D	7.79	D	13.84	D
11	29.06	C	8.53	C	7.52	D	11.87	C
12	28.39	C	8.01	C	7.54	D	11.28	C
13	31.67	D	10.77	E	8.27	E	12.11	C
14	30.72	D	9.76	D	7.94	D	12.54	C
15	30.79	D	10.90	E	7.93	D	11.78	C
16	30.35	D	8.25	C	7.37	D	13.21	D
17	32.05	E	10.82	E	7.97	D	12.05	C
18	28.94	C	8.21	C	7.37	D	12.70	C
19	15.58	A	5.39	A	3.86	A	4.57	A
20	29.86	D	8.33	C	7.50	D	13.30	D
21	30.09	D	9.27	D	7.70	D	14.97	E
22	30.69	D	9.72	D	8.00	D	13.92	D
23	30.91	D	9.49	D	7.99	D	13.34	D
24	31.68	D	10.71	E	8.49	E	14.99	E
25	29.72	D	8.68	D	7.49	D	12.03	C
26	30.25	D	9.10	D	7.98	D	13.19	D
27	28.42	C	7.14	B	7.20	C	12.08	C
28	29.77	D	9.00	D	7.48	D	12.37	C
29	30.91	D	10.17	E	8.08	E	13.42	D
30	30.33	D	8.42	C	7.73	D	14.71	E
31	31.80	D	9.83	D	8.26	E	14.60	D
32	30.69	D	9.10	D	7.83	D	14.43	D
33	31.56	D	9.79	D	8.26	E	15.13	E
34	31.80	D	10.24	E	8.47	E	14.05	D
35	31.43	D	9.99	E	8.40	E	15.68	E

Table 2: Continued

Buster line no.	Chlorophyll A		Chlorophyll B		Carotenoids		Phenolic compounds	
36	31.53	D	9.85	D	8.07	D	14.71	E
37	30.82	D	9.06	D	7.79	D	11.03	B
38	31.14	D	9.60	D	7.86	D	13.56	D
39	29.20	C	7.42	C	7.08	C	12.77	C
40	28.80	C	8.09	C	7.11	C	12.56	C
41	28.31	C	7.39	C	7.25	C	13.10	D
42	26.99	C	6.47	B	6.64	C	11.79	C
43	31.21	D	9.88	D	8.09	E	14.78	E
44	29.62	D	8.12	C	7.57	D	14.70	E
45	28.64	C	8.15	C	6.97	C	12.95	D
46	27.71	C	7.83	C	6.86	C	13.98	D
47	31.21	D	10.12	E	8.01	D	14.77	E
48	30.65	D	9.28	D	7.76	D	12.41	C
49	31.18	D	9.61	D	7.94	D	11.96	C
50	28.95	C	8.06	C	7.30	C	14.00	D
51	28.53	C	9.01	D	7.07	C	13.92	D
52	27.76	C	8.13	C	6.75	C	13.98	D
53	28.25	C	7.34	C	6.96	C	14.89	E
54	24.82	B	5.76	A	5.99	B	12.23	C
55	15.49	A	5.05	A	3.58	A	6.83	A
56	28.96	C	8.57	C	7.06	C	13.01	D
57	27.52	C	8.01	C	6.55	C	12.49	C
58	24.85	B	6.23	B	5.84	B	10.60	B
59	27.12	C	8.34	C	6.66	C	10.67	B
60	25.86	B	7.14	B	6.11	B	14.53	D
61	26.43	B	6.96	B	6.49	B	14.53	D
62	28.92	C	7.80	C	6.88	C	13.86	D
63	29.01	C	8.10	C	7.05	C	15.17	E
64	30.37	D	9.64	D	7.39	D	12.82	C
65	30.80	D	8.80	D	7.32	D	14.08	D
66	26.59	B	6.75	B	6.15	B	13.35	D
67	28.82	C	8.26	C	6.97	C	12.55	C
68	31.37	D	10.29	E	7.66	D	15.26	E
69	30.13	D	8.13	C	7.22	C	11.28	C
70	29.39	D	8.85	D	7.09	C	13.26	D
71	30.63	D	9.90	E	7.52	D	13.02	D
72	25.80	B	6.25	B	6.03	B	10.41	B
73	27.53	C	6.79	B	6.53	C	12.56	C

Table 2: continued

Buster line no.	Chlorophyll A		Chlorophyll B		Carotenoids		Phenolic compounds	
74	28.73	C	7.71	C	6.94	C	12.77	C
75	30.23	D	9.01	D	7.27	C	13.90	D
76	28.94	C	8.84	D	6.94	C	12.50	C
77	30.13	D	9.04	D	7.18	C	13.11	D
78	31.96	E	11.33	F	7.78	D	16.21	E
79	30.56	D	9.00	D	7.62	D	13.62	D
80	30.82	D	9.17	D	7.38	D	9.28	A
81	25.96	B	7.05	B	6.23	B	12.49	C
82	30.00	D	8.45	C	7.17	C	14.00	D
83	26.42	B	6.95	B	6.31	B	12.07	C
84	27.76	C	7.16	B	6.59	C	11.88	C
85	30.95	D	8.93	D	7.56	D	12.37	C
86	29.24	C	7.82	C	7.17	C	12.53	C
87	28.67	C	7.08	B	7.12	C	12.58	C
88	30.08	D	8.07	C	7.28	C	12.76	C
89	29.58	D	7.88	C	7.45	D	14.32	D
90	29.30	C	7.32	C	7.13	C	13.44	D
91	31.45	D	9.22	D	7.51	D	12.49	C
92	27.59	C	6.88	B	6.66	C	12.77	C
93	29.63	D	8.13	C	7.18	C	11.84	C
94	29.43	D	7.74	C	6.82	C	12.60	C
95	30.16	D	7.98	C	7.14	C	14.59	D
96	31.72	D	10.44	E	7.81	D	13.60	D
97	31.66	D	11.04	E	7.87	D	13.21	D
98	30.73	D	8.67	D	7.35	D	13.15	D
99	28.69	C	7.41	C	6.92	C	11.44	C
100	30.81	D	8.56	C	7.36	D	12.16	C

Table 3: Leaf morphological properties of 100 Buster lines under greenhouse conditions grouped based on mean ± 1 and 2 s.d. Where mean = 28.85 cm^2 and s.d. = 3.59 cm^2, group (A): < mean – 2 s.d., group (B): mean – 2 s.d. to mean – 1 s.d., group (C): mean – 1 s.d. to mean, group (D): mean to mean + 1 s.d., group (E): mean + 1 s.d. to mean + 2 s.d. and group (F): > mean + 2 s.d.

Buster line no.	Area (cm^2)	Length (cm)	Average width (cm)	Maximum width (cm)	Genotype description	Group
1	20.94	32.00	0.62	0.92	Duster	A
33	22.29	30.68	0.67	1.10	OK12D-Blgs/Dst-DH102	B
51	22.34	35.13	0.62	0.93	OK12D-Blgs/Dst-DH138	B
91	22.51	33.77	0.62	1.00	Duster sp derivative	B
58	22.98	34.40	0.63	1.02	OK12D-Blgs/Dst-DH169	B
92	23.30	37.33	0.58	1.28	OK12D-Blgs/Dst-DH261	B
97	23.37	32.73	0.67	1.02	OK12D-Blgs/Dst-DH269	B
36	23.45	33.23	0.67	1.03	OK12D-Blgs/Dst-DH110	B
87	23.57	34.47	0.62	0.98	OK12D-Blgs/Dst-DH248	B
40	23.59	33.27	0.65	1.02	OK12D-Blgs/Dst-DH121	B
96	23.97	32.92	0.67	1.00	OK12D-Blgs/Dst-DH268	B
78	24.00	29.62	0.75	1.28	OK12D-Blgs/Dst-DH224	B
19	24.01	35.50	0.62	0.95	OK12D-Blgs/Dst-DH58	B
69	24.43	32.30	0.68	1.10	OK12D-Blgs/Dst-DH193	B
82	24.46	38.83	0.60	1.17	OK12D-Blgs/Dst-DH234	B
60	24.63	31.97	0.72	1.10	OK12D-Blgs/Dst-DH173	B
49	24.83	37.17	0.63	1.05	OK12D-Blgs/Dst-DH136	B
80	25.05	36.85	0.63	1.00	OK12D-Blgs/Dst-DH228	B
100	25.21	34.80	0.67	1.05	OK12D-Blgs/Dst-DH278	B
95	25.27	34.12	0.70	1.00	OK12D-Blgs/Dst-DH266	C
37	25.87	34.13	0.70	1.07	OK12D-Blgs/Dst-DH114	C
38	25.89	32.57	0.73	1.17	OK12D-Blgs/Dst-DH117	C
79	26.02	35.48	0.70	1.07	OK12D-Blgs/Dst-DH226	C
6	26.05	34.37	0.70	1.28	OK12D-Blgs/Dst-DH14	C
2	26.28	32.50	0.77	1.18	Billings	C
54	26.38	33.62	0.73	1.12	OK12D-Blgs/Dst-DH143	C
98	26.61	33.80	0.73	1.10	OK12D-Blgs/Dst-DH270	C
65	26.69	34.12	0.73	1.08	OK12D-Blgs/Dst-DH182	C
55	26.69	39.55	0.65	1.03	OK12D-Blgs/Dst-DH145	C
34	26.98	34.85	0.73	1.05	OK12D-Blgs/Dst-DH103	C
88	26.99	40.22	0.60	1.03	OK12D-Blgs/Dst-DH255	C
94	27.43	36.73	0.68	1.07	OK12D-Blgs/Dst-DH265	C
22	27.46	39.05	0.65	1.05	OK12D-Blgs/Dst-DH67	C
10	27.57	34.93	0.73	1.18	OK12D-Blgs/Dst-DH24	C
41	27.71	34.80	0.75	1.10	OK12D-Blgs/Dst-DH123	C

Table 3: continued

Buster line no.	Area (cm^2)	Length (cm)	Average width (cm)	Maximum width (cm)	Genotype description	Group
76	27.77	33.10	0.78	1.22	OK12D-Blgs/Dst-DH215	C
17	27.98	33.05	0.78	1.13	OK12D-Blgs/Dst-DH50	C
11	27.99	36.65	0.70	1.12	OK12D-Blgs/Dst-DH25	C
62	28.00	38.33	0.67	1.08	OK12D-Blgs/Dst-DH176	C
43	28.12	33.77	0.77	1.27	OK12D-Blgs/Dst-DH128	C
21	28.20	36.13	0.72	1.17	OK12D-Blgs/Dst-DH63	C
48	28.20	35.10	0.75	1.08	OK12D-Blgs/Dst-DH134	C
56	28.28	35.77	0.75	1.17	OK12D-Blgs/Dst-DH147	C
29	28.35	37.75	0.72	1.07	OK12D-Blgs/Dst-DH81	C
86	28.37	38.40	0.70	1.10	OK12D-Blgs/Dst-DH243	C
3	28.65	38.33	0.68	1.10	OK12D-Blgs/Dst-DH1	C
18	28.73	35.92	0.77	1.22	OK12D-Blgs/Dst-DH56	C
71	28.76	35.43	0.77	1.15	OK12D-Blgs/Dst-DH207	C
12	28.86	37.28	0.73	1.15	OK12D-Blgs/Dst-DH32	D
52	28.88	38.42	0.68	1.13	OK12D-Blgs/Dst-DH140	D
32	28.91	35.58	0.75	1.12	OK12D-Blgs/Dst-DH95	D
70	29.20	35.87	0.75	1.22	OK12D-Blgs/Dst-DH206	D
74	29.26	37.95	0.73	1.07	OK12D-Blgs/Dst-DH212	D
53	29.32	39.65	0.70	1.20	OK12D-Blgs/Dst-DH142	D
81	29.38	38.88	0.72	1.08	OK12D-Blgs/Dst-DH231	D
63	29.42	38.53	0.70	1.05	OK12D-Blgs/Dst-DH178	D
46	29.53	36.13	0.77	1.13	OK12D-Blgs/Dst-DH131	D
7	29.56	37.32	0.75	1.10	OK12D-Blgs/Dst-DH16	D
61	29.60	39.45	0.68	1.15	OK12D-Blgs/Dst-DH175	D
20	29.60	37.77	0.73	1.27	OK12D-Blgs/Dst-DH59	D
39	29.62	36.25	0.78	1.10	OK12D-Blgs/Dst-DH118	D
85	29.69	35.98	0.78	1.13	OK12D-Blgs/Dst-DH240	D
50	29.70	37.32	0.75	1.12	OK12D-Blgs/Dst-DH137	D
83	29.81	37.38	0.77	1.13	OK12D-Blgs/Dst-DH236	D
84	29.94	38.72	0.73	1.10	OK12D-Blgs/Dst-DH238	D
4	30.26	36.38	0.77	1.12	OK12D-Blgs/Dst-DH8	D
72	30.33	37.57	0.77	1.15	OK12D-Blgs/Dst-DH208	D
25	30.39	38.98	0.73	1.08	OK12D-Blgs/Dst-DH75	D
90	30.42	38.93	0.73	1.17	OK12D-Blgs/Dst-DH257	D
31	30.71	35.73	0.78	1.35	OK12D-Blgs/Dst-DH91	D
23	30.78	34.45	0.85	1.27	OK12D-Blgs/Dst-DH69	D
42	30.83	36.42	0.78	1.22	OK12D-Blgs/Dst-DH126	D
59	31.02	42.22	0.70	1.07	OK12D-Blgs/Dst-DH170	D

Table 3: continued

Buster line no.	Area (cm^2)	Length (cm)	Average width (cm)	Maximum width (cm)	Genotype description	Group
93	31.06	38.90	0.75	1.20	OK12D-Blgs/Dst-DH263	D
89	31.20	36.93	0.80	1.20	OK12D-Blgs/Dst-DH256	D
15	31.24	35.93	0.82	1.22	OK12D-Blgs/Dst-DH42	D
26	31.55	39.03	0.75	1.10	OK12D-Blgs/Dst-DH76	D
45	31.80	40.58	0.73	1.22	OK12D-Blgs/Dst-DH130	D
9	31.85	38.33	0.77	1.27	OK12D-Blgs/Dst-DH22	D
5	31.88	41.52	0.75	1.22	OK12D-Blgs/Dst-DH13	D
14	31.88	37.03	0.80	1.25	OK12D-Blgs/Dst-DH40	D
66	32.01	38.30	0.77	1.17	OK12D-Blgs/Dst-DH185	D
8	32.39	39.55	0.78	1.25	OK12D-Blgs/Dst-DH19	D
67	32.42	39.52	0.77	1.22	OK12D-Blgs/Dst-DH186	D
99	32.59	37.42	0.83	1.20	OK12D-Blgs/Dst-DH275	E
77	32.65	39.52	0.75	1.17	OK12D-Blgs/Dst-DH216	E
68	32.81	37.87	0.80	1.17	OK12D-Blgs/Dst-DH187	E
13	32.90	37.70	0.82	1.20	OK12D-Blgs/Dst-DH38	E
57	32.97	38.65	0.78	1.23	OK12D-Blgs/Dst-DH167	E
16	33.04	37.57	0.82	1.22	OK12D-Blgs/Dst-DH44	E
73	33.09	40.48	0.75	1.15	OK12D-Blgs/Dst-DH210	E
75	33.23	37.12	0.85	1.23	OK12D-Blgs/Dst-DH214	E
47	33.40	37.40	0.82	1.30	OK12D-Blgs/Dst-DH132	E
44	34.02	39.95	0.78	1.23	OK12D-Blgs/Dst-DH129	E
64	34.33	39.62	0.82	1.15	OK12D-Blgs/Dst-DH181	E
27	35.50	42.35	0.80	1.18	OK12D-Blgs/Dst-DH79	E
28	35.73	41.27	0.80	1.23	OK12D-Blgs/Dst-DH80	E
35	36.02	39.88	0.83	1.25	OK12D-Blgs/Dst-DH109	E
30	37.93	40.58	0.87	1.28	OK12D-Blgs/Dst-DH84	F
24	38.56	41.72	0.87	1.32	OK12D-Blgs/Dst-DH73	F

Table 4: Matrix of simple correlation coefficients for different measured parameters, where Fht = final height, Flno = final leaf number, Ftno = final tiller number, Cha = chlorophyll A, Chb = chlorophyll B, Car = Carotenoids, Phe = phenolics, Rht = rate of increase in height per day, Rlno = Rate of increase in leaf numbers per day and Rtno = Rate of increase in tiller numbers per day.

Rtno	Rlno	Rht	Phe	Car	Chb	Cha	Area	Ftno	Flno	Fht	
-0.06	-0.17	0.87	0.01	0.05	-0.05	-0.03	0.48	-0.05	-0.03	1	Fht
-0.01	0.67	-0.06	0.02	-0.02	-0.04	0.05	-0.19	-0.03	1	-0.03^{NS}	Flno
0.99	-0.12	-0.08	-0.13	-0.16	-0.21	-0.15	-0.13	1	-0.03 ^{NS}	-0.05 ^{NS}	Ftno
-0.14	-0.25	0.38	0.06	0.13	0.01	0.07	1	-0.13 ^{NS}	-0.19*	0.48***	Area
-0.18	0.01	0.03	0.60	0.94	0.81	1	0.07 ^{NS}	-0.15 ^{NS}	0.05 ^{NS}	-0.03 ^{NS}	Cha
-0.23	0.06	-0.01	0.40	0.85	1	0.81***	0.01 ^{NS}	-0.21**	-0.04 ^{NS}	-0.05 ^{NS}	Chb
-0.19	-0.02	0.08	0.54	1	0.85***	0.94***	0.13 ^{NS}	-0.16 ^{NS}	-0.02 ^{NS}	0.05 ^{NS}	Car
-0.14	-0.01	0.03	1	0.54***	0.40***	0.60***	0.06 ^{NS}	-0.13 ^{NS}	0.02 ^{NS}	0.01 ^{NS}	Phe
-0.10	-0.11	1	0.03 ^{NS}	0.08 ^{NS}	-0.01 ^{NS}	0.03 ^{NS}	0.38***	-0.08 ^{NS}	-0.06 ^{NS}	0.87***	Rht
-0.10	1.00	-0.11 ^{NS}	-0.01 ^{NS}	-0.02 ^{NS}	0.06 ^{NS}	0.01 ^{NS}	-0.25**	-0.12 ^{NS}	0.67***	0.87*** -0.17*	Rlno
1.00	-0.10 ^{NS}	-0.10 ^{NS}	-0.14 ^{NS}	-0.19*	-0.23**	-0.18*	-0.14 ^{NS}	0.99***	-0.01 ^{NS}	-0.06 ^{NS}	Rtno

*.Significant at α = 0.1, **. Significant at α = 0.05, ***. Significant at α = 0.01, ^{NS}. Not significant

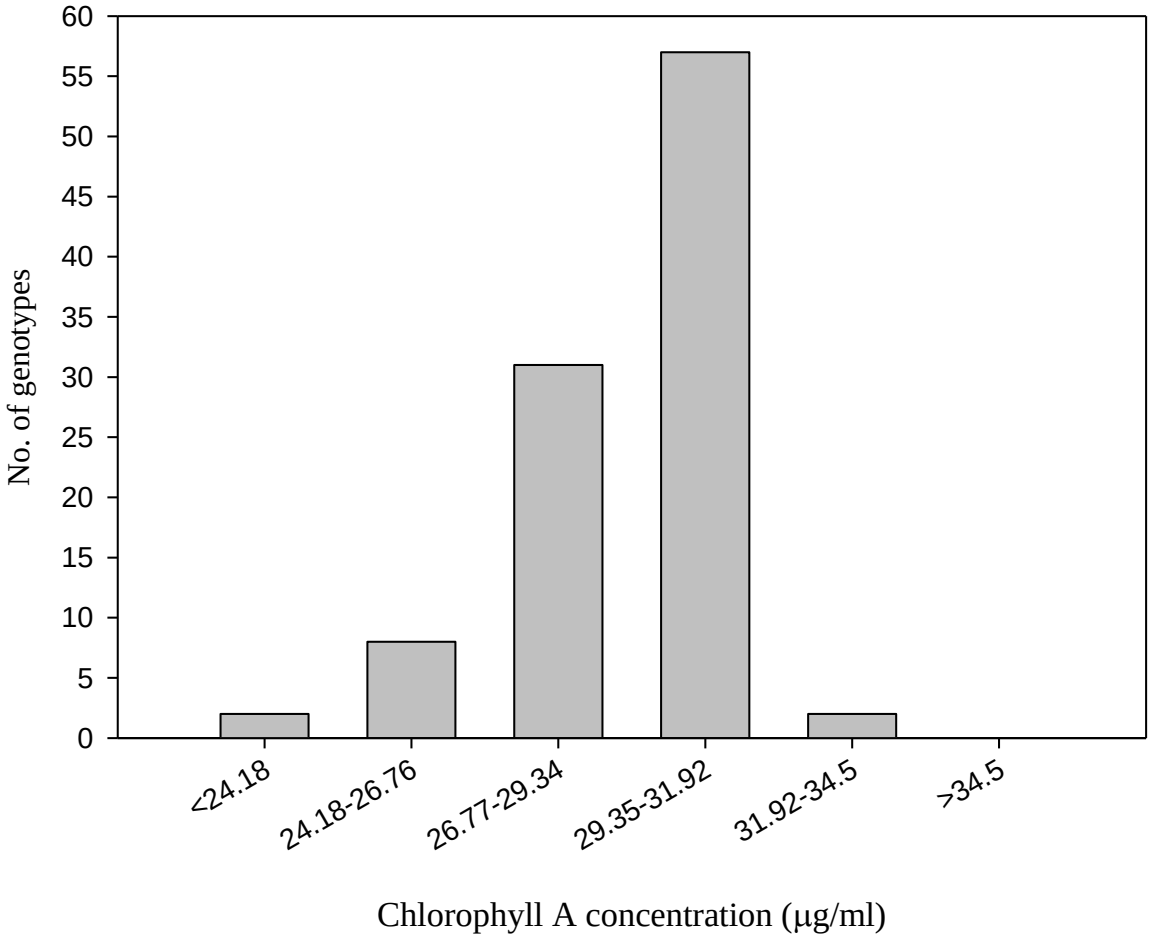

Figure 1: Number of Buster lines for each group of chlorophyll A concentrations (µg/ml) based on mean ± s.d.

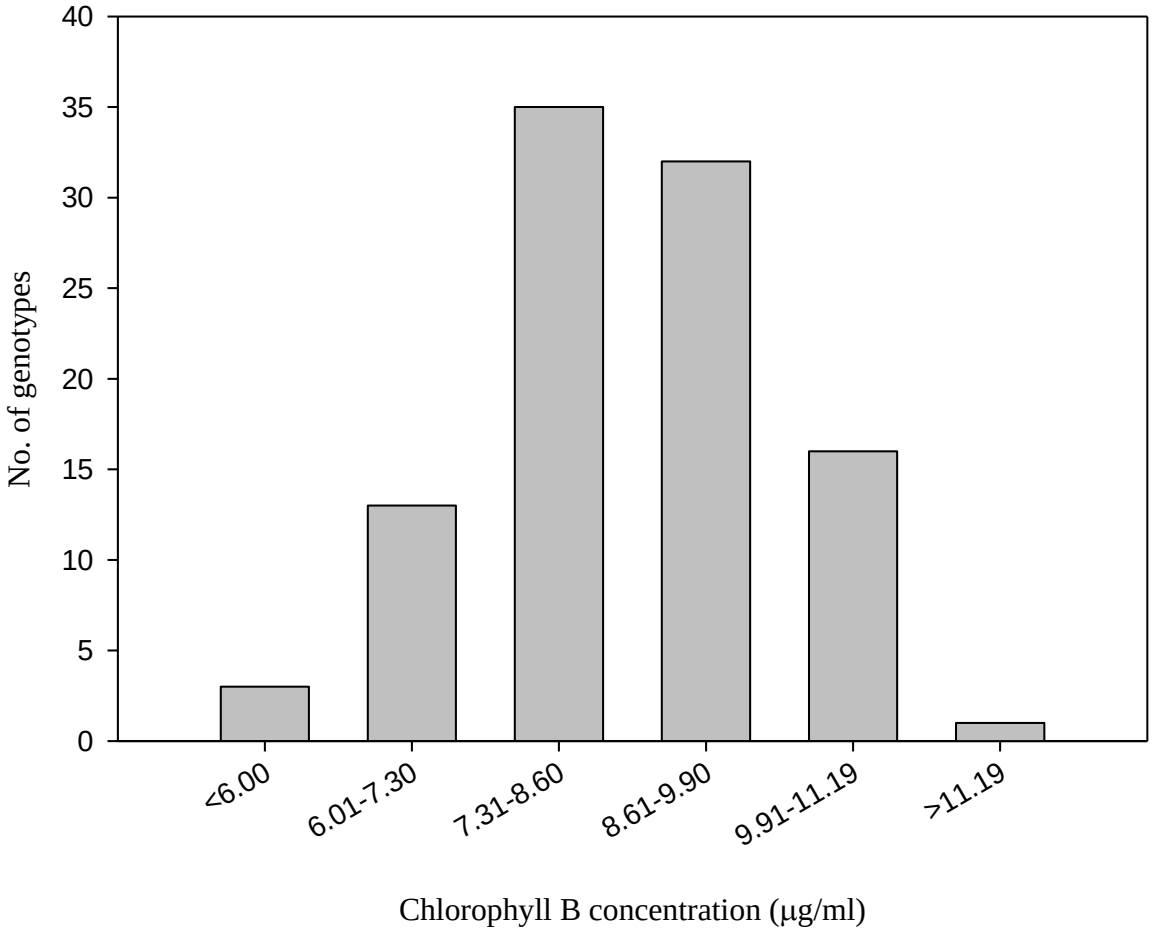

Figure 2: Number of Buster lines for each group of chlorophyll B concentrations (µg/ml) based on mean ± s.d.

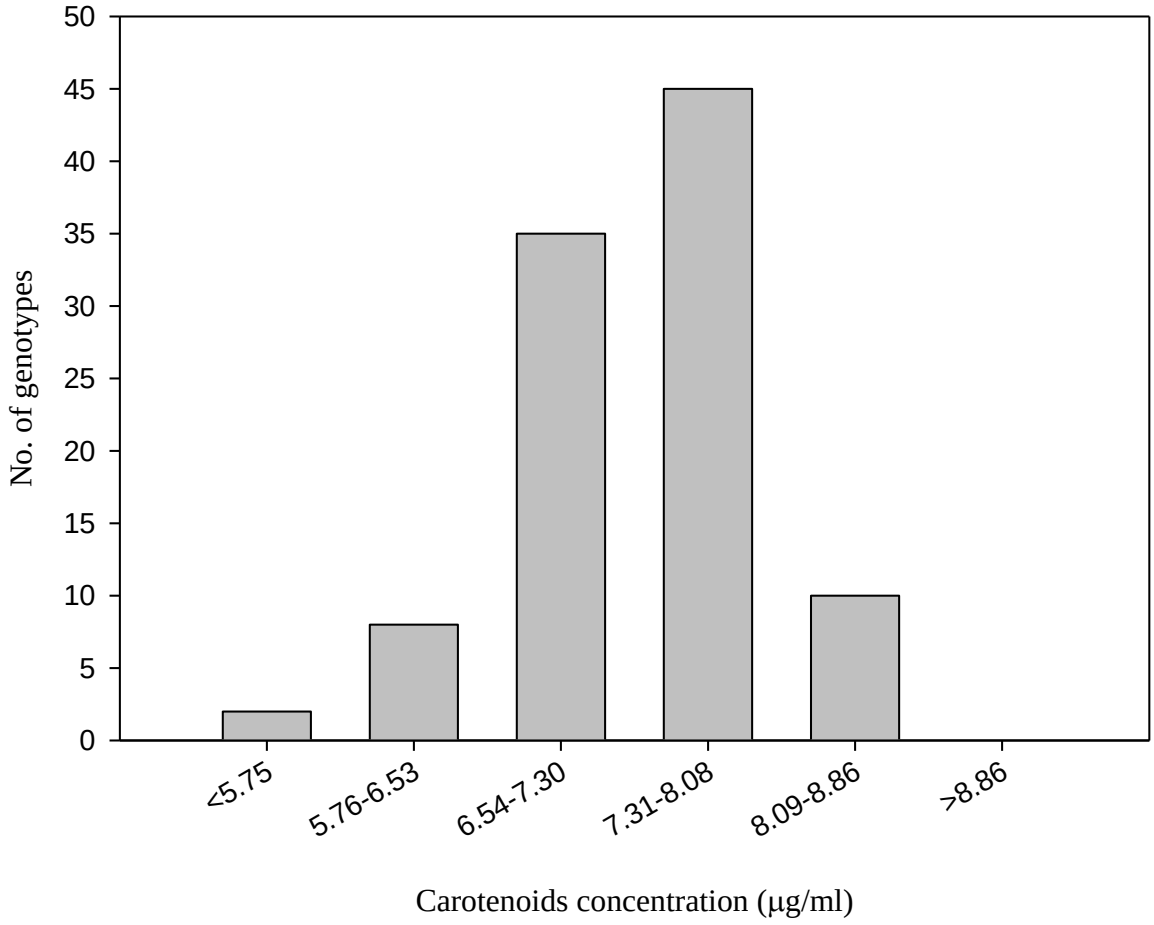

Figure 3: Number of Buster lines for each group of carotenoids concentrations (µg/ml) based on mean ± s.d.

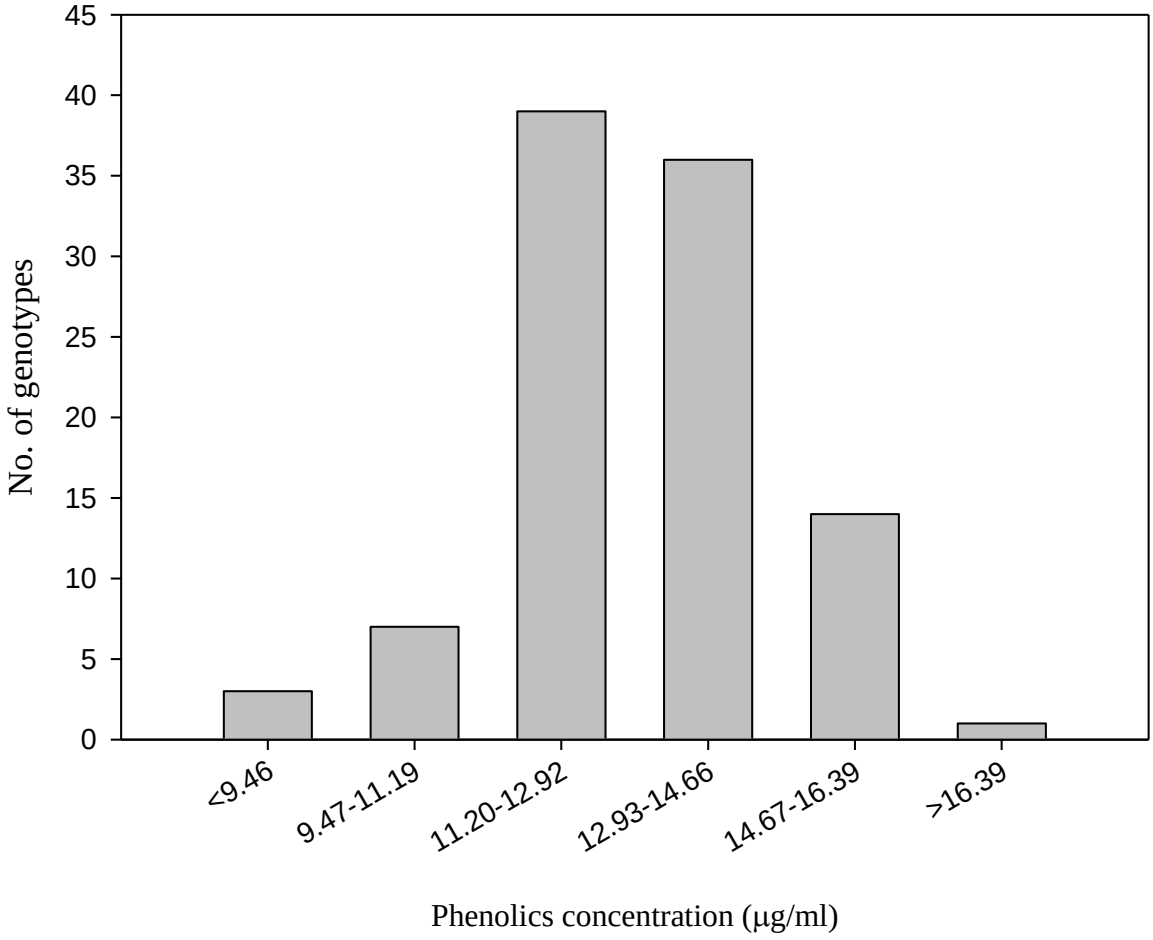

Figure 4: Number of Buster lines for each group of phenolic compounds concentrations (µg/ml) based on mean ± s.d.

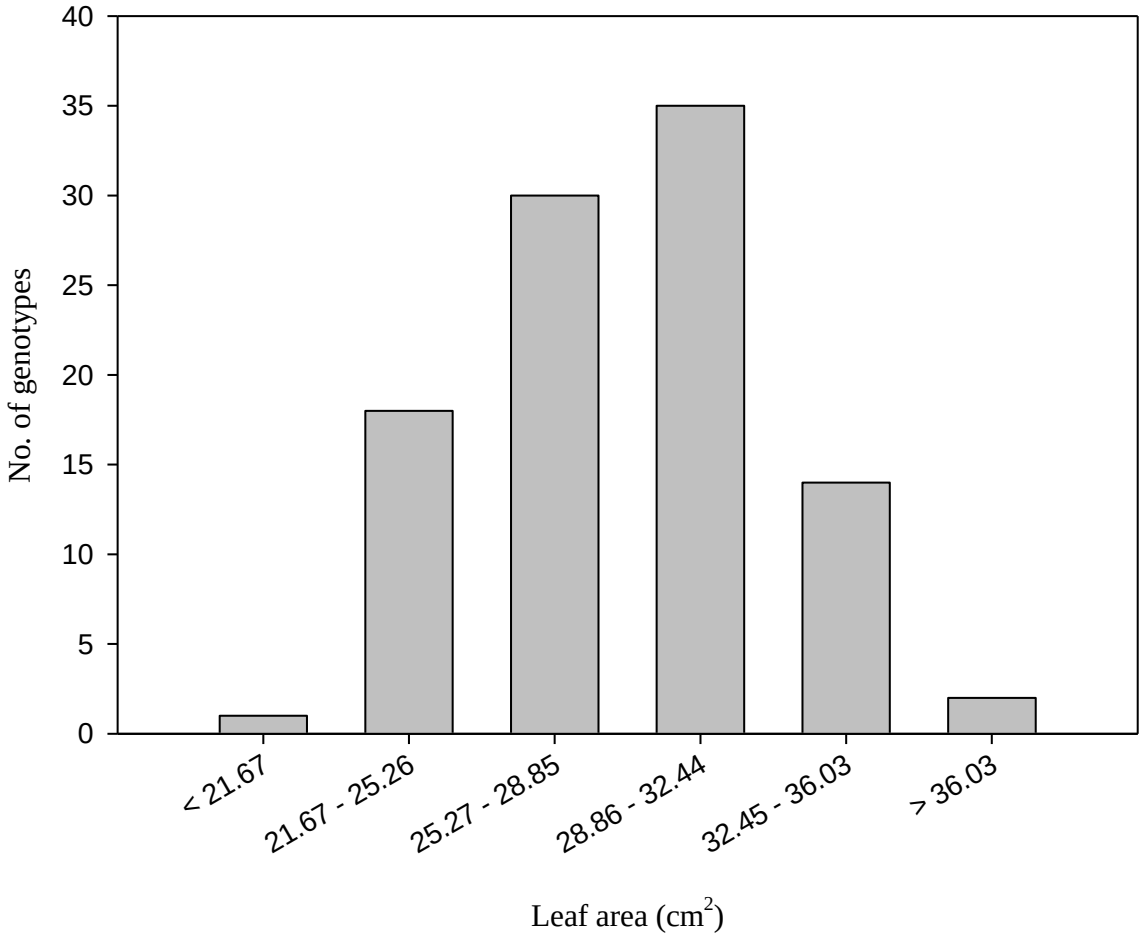

Figure 5: Number of Buster lines for each group of leaf area based on mean ± s.d.

CHAPTER III

ASSESSMENT OF VARIATION AMONG 'BUSTER' LINES IN RESPONSE TO HEAT STRESS

Abstract

Increase in wheat growing season temperature is one of the main problems associated with wheat production in a changing and variable climate. Development of suitable varieties in accordance with changing climate requires continuous site-specific research. This study was conducted to identify differences between 100 lines from a double haploid (DH) population 'Buster' at the vegetative stage of plant growth under high temperature and sufficient water conditions. Different physiological parameters (leaf photosynthesis (Pn), transpiration (E), stomatal conductance (gs), intercellular carbon dioxide concentration (Ci), electron transport rate (ETR), fluorescence (Fv'/Fm') and instantaneous water use efficiency (IWUE)) per unit leaf area were recorded using LI-6400XT, and electrical conductivities of leaves were measured with a conductivity meter. The Buster lines had significant differences in the parameters recorded and therefore can be potentially used for further breeding based on those differences. The parameters Pn, gs, E and Ci increased whereas IWUE decreased with the increase in temperature. Stomatal conductance and IWUE explained most of the variability between the temperature treatments and Buster lines.

1. Introduction:

Wheat is grown in more than 200 million hectares globally (Taylor, 2016). The wheat growing area is distributed in different regions with different geographical features, climatic conditions and weather patterns. With increase in temperature in different parts of the world at variable rates, it is important to identify and select heat tolerant wheat varieties to meet the consumer demand in near future (Mondal et al., 2016). The world wheat production is likely to decrease by 6%, which equals to 42 metric tons, for every 1 ºC rise in temperature (Asseng et al., 2015). This decrease in yield can be attributed to plant processes that are affected by increase in temperature during the crop life cycle.

Plant physiological processes like photosynthesis, nutrient and water uptake, carbon assimilation and dry matter accumulation are likely to be affected due to increase in temperature and carbon dioxide (CO_2) concentration in the environment (Gavito et al., 2001). The global temperature increase is projected to range between 1.5 ºC and 11 ºC by the year 2100 (Stainforth et al., 2005). High temperature at the beginning of spring season, coinciding with anthesis and grain-filling stages of wheat crop, substantially reduces grain number and size (Gourdji et al., 2013). Reduction in grain size and/or number ultimately decreases overall wheat productivity. Several studies have screened genotypes for heat tolerance (Rebetzke et al., 2013; Rosyara et al., 2008; Yang et al., 2002). However, most of these studies have considered either the whole plant life cycle or the post-anthesis period of crop growth. Screening plants for the heat tolerance traits at early plant growth stages can help shorten the time period of the selection process.

Photosynthesis and carbohydrate remobilization are two main sources of carbon assimilation in wheat for grain filling under heat stress (Blum et al., 1994). Heat stress reduces metabolic activities in plants, affects photosynthesis, facilitates ethylene production for higher senescence rate, cause pollen mortality, and facilitates the production of reactive oxygen species (ROS)

causing oxidative damage to chloroplasts (Nawaz et al., 2013). In addition, high temperature affects the yield parameters in wheat by reducing the duration of grain fill, grain size and single kernel weight (Blum et al., 1994; Dupont et al., 2006; Stone & Nicolas, 1995), and deteriorating the grain quality (Blumenthal et al., 1995). Studies have been conducted by exposing the plants to short duration heat stresses (heat shocks) or subjecting the plants to elevated temperatures after certain growth stages; the responses of wheat plants to heat stress are found to vary with genotypes (Blumenthal et al., 1995; Tahir & Nakata, 2005).

1.1.Photosynthesis:

Photosynthesis is one of the major factors influencing crop growth, biomass and yield (Reynolds et al., 2009; Richards, 2000; Zheng et al., 2011). Plants are able to survive the climatic extremes because of plasticity and resilience of photosynthesis (Kakani et al., 2008). Therefore, understanding the response of photosynthesis to changing environment is necessary to correctly assess the changes in plant productivity (Salvucci & Crafts-Brandner, 2004). The enzymes responsible for proper functioning of the photosynthetic apparatus (Photosystem I + Photosystem II) are degraded in temperatures above the optimum range. Rubisco activase is unstable and, electron transport chain is inhibited under high temperatures (Sharma et al., 2012). Xue et al. (2002) found positive correlation between leaf photosynthetic rates and grain yield in a few studies but no relation between them in some other studies. According to Long et al. (2006), leaf photosynthetic rates correlate poorly with yield in past, but several recent studies show increase in yield with the increase in photosynthesis. Such limited and contrasting information demands more research in this area.

1.2. Stomatal conductance:

Stomatal conductance is the rate of CO_2 moving in and water vapor moving out of the stomatal apertures in leaf. The rates of diffusion of CO_2 into leaf for photosynthesis and water

vapor out of the leaf for transpiration are controlled by the stomatal aperture openings (Sikder et al., 2015). Therefore, stomatal conductance is an important parameter that affects all gas exchange processes. Variation in stomatal conductance among genotypes can be utilized in genotypes selection for improved adaptation in wide range of growing conditions (Rebetzke et al., 2013). In agricultural areas, high temperature is often associated with dry air. This increases the evaporative demand and ultimately affects crop transpiration (Schoppach & Sadok, 2013). Increased stomatal conductance in high temperature and water unlimited conditions increases transpiration, which also allows the plant to cool their leaves. When heat stress is combined with other stresses such as drought and salinity, the transpirational cooling process does not hold well (Mittler, 2006). According to Farquhar & Sharkey (1982), photosynthesis is only slightly affected by the stomatal causes irrespective of stress conditions or C_3/C_4 mechanisms, but the high transpiration under hot conditions may lead to intrinsic water deficit in leaves, which may have an effect on photosynthesis.

1.3. Chlorophyll fluorescence:

Chlorophyll fluorescence is the process of dissipating excess light as re-emission by chlorophyll A after fulfilling the photosynthetic demands (Dobrowski et al., 2005). The three processes that light can undergo in a leaf after the chlorophyll molecules receive light are photosynthesis, dissipation as heat and chlorophyll fluorescence. These three processes always counterbalance each other's efficiency increasing one of them while the others decrease (Maxwell & Johnson, 2000). Therefore, chlorophyll fluorescence ultimately reflects the photosynthetic activities of a plant in a complex manner (Krause & Weis, 1991). Chlorophyll fluorescence measurement is one of the well-established techniques to evaluate integrity of photosynthetic apparatus for stress detection in plants (Jiang et al., 2006; Sharma et al., 2012). It has been used for detection of heat and drought stress in wheat plants in many studies (Hassan, 2006; Sharma et al., 2012; Xue et al., 2002).

1.4. Membrane thermal stability:

Cell membrane plays a vital role in ion transport and enzymatic activities in plants (Dias et al., 2010). When heat stress occurs, the plant cell membrane is structurally damaged leading to impaired transport system. Different approaches such as changes in membrane fluidity, electron transport chain, enzyme denaturation and nucleic acids damage can be used to quantify the heat stress effects in plants (Sayed, 2003). Assessment of the effects of heat stress on membrane level is a reliable approach to determine wheat sensitivity to heat stress (Dias et al., 2010). Electrical conductivity is used as an index to measure the electrolytes diffused from heat stressed wheat leaf tissues to examine the plants for heat tolerance (Blum & Ebercon, 1981). When leaf tissues are exposed to high temperatures, cell membrane is damaged and is more permeable to electrolyte leakage from the cell, which increases the electrical conductivity (Yildirim et al., 2009). The genotypes corresponding to the leaves that leak fewer electrolytes are the ones whose cell membrane is less damaged, and they are relatively more tolerant to heat stress compared to those genotypes whose leaves leak more electrolytes.

The objective of this study was to identify variation among 100 Buster lines under normal and high temperatures conditions during vegetative growth stages. The 100 Buster lines used were the same as used in the Chapter II. A detailed description of the Buster population and its development, and selection of the 100 Buster lines is provided in Chapter I. Different methods are used to identify differences between the genotypes depending upon the objective of the research. In this study, techniques that can be employed during early stages of crop growth are utilized. The 100 Buster lines were assessed using well-established techniques for studying stress response in plants - cell membrane stability of leaves, and chlorophyll fluorescence and gas exchange parameters per unit leaf area, which are well-established techniques for studying stress response in plants.

2. Materials and Methods:

This study was conducted in the controlled environment research laboratory (CERL) at Oklahoma State University (OSU) in Stillwater, OK, USA. Four growth chambers each with 50 pots (15 cm in diameter and 35 cm in depth) were used for the study. The fifty Buster lines were planted in each chamber, one Buster line per pot and four seeds of each Buster line in one pot. Therefore, a set of 100 Buster lines were split between two growth chambers. Automatic drip irrigation system was used to provide 0.3 L of Hoagland's nutrient solution to the plants each time, three times a day (8:00 AM, 1:00 PM and 6:00 PM), after germination. Sand was used as the medium for plant growth to control nutrient conditions. Plants in all chambers were grown at temperatures (22/16 ºC day/night) up to 65 days after sowing (DAS) and continued to grow in the same temperature until the end of the experiment in two chambers designated as controls. The temperature was raised to 32/26 ºC (day/night) in two of the chambers after 65 DAS to impose heat stress on one set of Buster lines. The 26 ºC was the lowest night temperature and 32 ºC was the highest day temperature. Gradual increase in temperature from night to day and vice-versa was achieved through ramping of temperature. Photoperiod was adjusted to 14 hours light and 10 hours dark period. Thus, one set of the 100 lines was under heat stress treatment, and the other set was under control conditions.

2.1. Gas exchange parameters and fluorescence:

The measurements of Pn, gs, E, Ci, ETR and Fv'/Fm' were made on attached leaves between 9 AM to 1 PM using an infrared gas analyzer (IRGA) in an open photosynthesis system, LI-6400 XT (Licor Inc., NE, USA). The two youngest fully open leaves from adjacent plants were used for the measurements in order to cover the 2 cm^2 area of the leaf cuvette. The leaves were artificially irradiated with a blue-red LED radiation source attached to the sensor head set at 1200 µmol m^{-2} s^{-1} for uniform light in all measurements. Temperature in the leaf cuvette was set in

accordance with the daytime temperature of the treatment chambers. The leaf chamber reference CO_2 was set to 400 µL L^{-1}.

The efficiency of energy harvesting by photosystem II (PSII) was calculated by built-in algorithms in LI-6400XT system using the equation:

$Fv'/Fm' = (Fm'-Fo')/Fm'$

Where,

Fo' = minimal fluorescence of a momentarily darkened leaf

Fm' = maximum fluorescence during a saturating flash light

Fv' = variable fluorescence during a saturating flash light

Instantaneous water use efficiency (IWUE) was calculated as the ratio of net photosynthesis (Pn) to transpiration (E).

The gas exchange parameters and fluorescence measurements were taken three times: first before starting the heat stress treatment, second after three days of the treatment and third after a week of the treatment. The measurements taken after introduction of heat stress are expressed as an average and this average was compared to the average before heat stress treatment. In addition, the two last measurements taken after heat stress introduction were used as replications for data analysis.

2.2. Membrane thermal stability:

After two weeks of heat stress, ten leaf samples were collected from each pot from all the chambers. Leaves were cut into 2.5 cm segments and put in two test tubes, five pieces in each test tube. The leaf segments were rinsed thoroughly with deionized water twice and 30 ml of deionized water was added to the test tubes. The test tubes were then covered with aluminum foil

and kept in the refrigerator for 16 hours to allow for diffusion of electrolytes. The test tubes were then brought back to room temperature and shaken lightly to homogenize the solution. Initial conductivity of the test tube contents was measured with an Orion 4-Star Plus pH / conductivity meter in the unit of µS/cm. The test tubes were recapped with aluminum foils and autoclaved at 120 ºC for ten minutes to kill the plant tissue and release all electrolytes. Final conductivity was measured after cooling down the tubes to room temperature. Results are expressed as percentage of total conductivity as described in Dias et al. (2010).

2.3.Statistical analyses:

Collected data was analyzed using SAS Version 9.4 (SAS Institute, Cary, NC). The Analysis of Variance (ANOVA) was performed using PROC GLM to see if the differences among the studied Buster lines are statistically significant ($P < 0.05$) for the recorded parameters. PROC CORR was used to obtain correlation coefficients between the different parameters. A principal component analysis (PCA) was conducted using PROC PRINCOMP. The PCA was performed on the differences between values of the parameters in control and treatment conditions to identify the variables that were mainly causing the differences. A biplot was constructed using PROC PRINQUAL. Biplot is a graphical representation of eigenvectors, also known as loadings, of the first two PC scores. Graphs were constructed using Sigma Plot.

3. Results and Discussion:

3.1. Gas exchange and fluorescence parameters:

There was no significant difference between the Buster lines for gas exchange and fluorescence parameters before heat stress treatment. However, after imposing heat stress treatment, significant differences ($P<0.05$) were observed between the Buster lines and in interaction with temperature for Pn, gs, E, Fv'/Fm', Ci and IWUE. The p-values for these parameters and their statistical significance are shown in Table 1. Genotypic differences were

observed for the gas exchange parameters among different wheat varieties in various studies (Ritchie et al., 1988; Wu & Bao, 2011; Xue et al., 2002).

The Pn, gs, E and Ci increased in the Buster lines in response to increased temperature. Since all of these gas exchange parameters are directly related to stomatal opening, their increase under heat stress can be attributed to increased stomatal apertures. The stomatal conductance is not limited by the high temperature unless water stress is associated with it (Baker, 2006). The higher stomatal conductance under high temperature also explains the transpirational cooling mechanism of plants in response to high temperature. Higher transpiration rates in higher temperatures allow more water vapor to exit the leaves ultimately having a cooling effect. On the other hand, increased stomatal openings allow more CO_2 to enter the leaves, which increases photosynthesis. Furthermore, increased enzymatic activity of Rubisco with increase in temperature is one of the important factors influencing photosynthesis under high temperatures and sufficient water conditions (Salvucci & Crafts-Brandner, 2004). Photorespiration is high in elevated temperatures because of increased affinity of Rubisco to oxygen, which could cause a decrease in photosynthesis (Aliyev, 2012). But at the same time, the photorespiration decreases with increase in CO_2 concentration, which serves to increase photosynthesis (Sengupta, 1988). Therefore, the effects of increased photorespiration on photosynthesis may not have been evident in our condition. A review done by Lu et al. (1998) suggested that the yields of cotton and wheat are directly correlated to the stomatal conductance under supra optimal temperatures without any influence of other stresses like drought and vapor pressure deficit. They also concluded that increase in stomatal conductance is an avoiding type of resistance in response to high temperature but the water use efficiency is decreased with the increase in temperature because of wasteful water use, which is in accordance to the results of this experiment. However, most of the studies done to assess the response of wheat cultivars to high temperatures have considered the yield and yield parameters. Photosynthetic response of wheat to high temperature was cultivar-dependent

and the gas exchange parameters did not correlate with the yield parameters (Feng et al., 2014). In addition, there is not yet any conclusive statement about the relationship between IWUE and crop water use efficiency. Therefore, this study gives an idea about the potential performance of selected Buster lines but cannot conclude on the plant responses to naturally occurring heat stress that is associated with water stress most of the time. The parameters Fv'/Fm' and ETR did not show consistent responses to the increase in temperature for the studied Buster lines. The value for fluorescence in response to high temperature decreased in 58 lines and increased in 37 lines, whereas ETR increased in 78 lines and decreased in 17 lines.

The parameters Pn, g, E and Ci were strongly positively correlated (correlation coefficients greater than 0.8) with each other and IWUE was negatively correlated with these parameters. Electron transport rate was not correlated to any of the parameters and Fv'/Fm' had a weak positive correlation with Pn and Ci. The correlation coefficients for all parameters and their statistical significance are shown in Table 2. The strongest correlation is between gs and E with a correlation coefficient of 0.97. The correlation of gs with Pn and E is obvious because the rate of CO_2 and water vapor flow to and from the leaves is controlled by the stomatal aperture. However, the negative correlation of IWUE with the gas exchange parameters suggests that water is not being efficiently used and the photosynthesis is increased at a very high cost of water. Nevertheless, a study done by Xue et al. (2002) under drought reported no correlation between the gas exchange parameters and IWUE, which is in contrast to the results of this study. The lack of correlation could possibly be because of decreased water availability in their study.

The results from PCA showed that more than 80% of the variability was explained by the first two PC scores. Therefore, a biplot was constructed plotting the eigenvectors of first two PC scores. The values of eigenvectors (loadings) and proportional and cumulative variance explained by the PC scores is shown in Table 3, and the biplot is shown in Figure 1. The parameters gs and IWUE explain most of the variability in first and second axis, respectively. The Buster lines in

upper right (first) quadrant have comparatively small increase in gs, E and Pn and the Buster lines in lower (third and fourth) quadrants are relatively less affected by heat stress as indicated by less difference in IWUE between control and treatment conditions.

The decrease in IWUE from optimum to high temperature ranged from 1.28 $\mu molCO_2/\mu molH_2O$ to 8.45 $\mu molCO_2/\mu molH_2O$. Highest decrease was observed in 'DH102' and lowest decrease was observed in 'DH263'. The decrease was 2.6 and 3.4 $\mu molCO_2/\mu molH_2O$ for the parental lines 'Duster' and 'Billings' respectively. The values for differences (control – treatment) in IWUE, Pn, E, gs, ETR, Ci and Fv'/Fm' for all Buster lines is presented in Table 4.

3.2. Membrane thermal stability:

Significant differences ($P<0.05$) were observed between the Buster lines and in interaction with heat stress based on electrical conductivity. The two-way ANOVA showing effects of genotype, heat stress treatment and the interaction of genotype*temperature on electrical conductivity of the wheat plants is shown in Table 5. The plants under controlled conditions yielded greater values for conductivity, which indicates that the electrolyte leakage was more from the plants under control conditions than the ones in heat stressed conditions. A graph for values of electrical conductivity from plant samples grown in controlled and heat stressed conditions is shown in Figure 2.

This result is in contrast to most of the previous findings in this area. In most of the studies done in this area, leaf tissues were subjected to heat stress once they were cut into segments (Blum et al., 1981; Rehman et al., 2016), whereas whole plants were heat stressed in this study. Therefore, involvement of whole plant system in this experiment may be the reason behind plants being acclimatized to the stress and leaking less electrolytes. Heat stress in plants does not occur in leaf levels under natural conditions, and so this study attempted to find the differences in electrolyte leakage when plants as a whole are heat stressed. Dias et al. (2010) conducted research

imposing heat stress at plant level and reported no significant differences in electrolyte release between the plants grown in normal and heat stressed conditions. This provides us an idea that the heat stressed plants may not necessarily show higher electrolyte leakage when whole plant system is associated. In addition, the temperature of water bath in many studies is found to be around 50 ºC (Saadalla et al., 1990; Yildirim et al., 2009; Rehman et al., 2016). This is greater than the highest temperature in this study (32 ºC) which means the variation in results may also be the outcome of difference in temperature used for the heat stress. Furthermore, the leaves were heat stressed in a water bath for a short period of time (an hour) in those studies, which is more of a heat shock. It is different from the settings in this experimental setup where the temperature was gradually increased from 26 ºC to 32 ºC and vice-versa to simulate day and night conditions. Likewise, the plants were heat stressed for two weeks as opposed to an hour in those studies. Therefore, the higher conductivity of leaves from plants under controlled conditions could be due to acclimation of plants to the heat stress in the two weeks period. The plants may have acclimated because of gradual increment in temperature. If the above-mentioned factors that possibly resulted in this outcome are studied separately and in different combinations, the actual reason behind this result can be accurately identified.

4. Conclusions:

The studied ‘Buster’ lines varied in their performances based on observed parameters. Thus, they can potentially be selected for further breeding research purposes based on these differences. The variables gs and IWUE explained most of the differences between the treatments. The IWUE decreased in response to heat stress in all Buster lines at different rates, whereas the values for gas exchange parameters increased under heat stress as compared to controlled conditions. The leaves from plants under controlled conditions turned out to have higher electrical conductivities compared to those from heat stressed set of plants possibly due to acclimation of the plants under stress.

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Table 1: P-values for photosynthesis, stomatal conductance, transpiration, ETR, fluorescence, instantaneous WUE and intercellular CO_2 showing significant differences for main factors (genotype and heat stress treatment) and their interaction.

Parameter	Genotype	Treatment	Genotype*treatment
Photosynthesis	<0.0001***	<0.0001***	0.0041**
Stomatal conductance	0.0048**	<0.0001***	0.0095**
Transpiration	<0.0001***	<0.0001***	0.0011**
ETR	0.5408^{NS}	0.8598 ^{NS}	0.5083 ^{NS}
Fluorescence	0.0275*	0.0536 ^{NS}	0.0166*
Instantaneous WUE	<0.0001***	<0.0001***	<0.0001***
Intercellular CO_2	<0.0001***	<0.0001***	0.0002***

* Significant at $\alpha = 0.05$, ** Significant at $\alpha = 0.01$, *** Significant at $\alpha = 0.001$, ^{NS} not significant

Table 2: Matrix of Pearson correlation coefficients showing correlation between photosynthesis (Pn), stomatal conductance (gs), transpiration (E), intercellular CO_2 (Ci), ETR, fluorescence (Fv'/Fm') and instantaneous water use efficiency (IWUE).

	Pn	gs	E	IWUE	ETR	Fv'/Fm'	Ci
Pn	1	0.89***	0.84***	-0.58***	0.06^{NS}	0.21***	0.71***
gs	0.89	1	0.97***	-0.78***	0.03^{NS}	0.09^{NS}	0.88***
E	0.84	0.97	1	-0.85***	0.03^{NS}	0.01^{NS}	0.85***
IWUE	-0.58	-0.78	-0.85	1	0.00^{NS}	-0.03^{NS}	-0.86***
ETR	0.06	0.03	0.03	0.00	1	0.00^{NS}	0.01^{NS}
Fv'/Fm'	0.21	0.09	0.01	-0.03	0.00	1	0.16**
Ci	0.71	0.88	0.85	-0.86	0.01	0.16	1

*Significant at $\alpha = 0.05$ **Significant at $\alpha = 0.01$ ***Significant at $\alpha = 0.001$, ^{NS}not significant

Table 3: Eigenvectors (loadings) of the principal components and proportional and cumulative variance explained by the principal components.

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7
Photosynthesis	0.42	0.36	0.06	-0.13	-0.27	0.65	-0.42
Stomatal conductance	0.47	0.06	-0.09	0.12	0.44	-0.49	-0.56
ETR	0.30	0.57	-0.10	0.04	-0.48	-0.46	0.37
Transpiration	0.46	0.00	-0.17	-0.39	0.51	0.21	0.55
IWUE	-0.22	0.59	0.37	0.45	0.46	0.16	0.13
Fluorescence	0.31	-0.27	0.88	-0.08	-0.11	-0.13	0.10
Intercellular CO2	0.38	-0.35	-0.18	0.78	-0.11	0.20	0.21
Proportional variance	0.57	0.24	0.09	0.05	0.03	0.01	0.01
Cumulative variance	0.57	0.81	0.90	0.95	0.98	0.99	1.00

Table 4: Values for differences (optimum – high temperature) in instantaneous water use efficiency (IWUE) in µmolCO_2/µmolH_2O, photosynthesis (Pn) in µmol $CO_2 m^{-2} s^{-1}$, transpiration (E) in mmol$H_2O m^{-2} s^{-1}$, stomatal conductance (gs) in mol$H_2O m^{-2} s^{-1}$, electron transport rate (ETR), intercellular CO_2 concentration (Ci) in µmolCO_2mol^{-1} and fluorescence (Fv'/Fm') for the studied 'Buster' lines where Geno no. = assigned genotype number for the Buster lines and DH no. = Double haploid number.

Geno no.	DH no.	Pn	gs	ETR	E	IWUE	Fv'/Fm'	Ci
1	Duster	0.6118	-0.1224	10.8620	-3.9332	2.6038	0.0752	-17.2890
2	Billings	-4.3992	-0.2620	-15.4180	-5.1594	3.4204	0.0337	-37.0890
3	DH1	1.6987	-0.2381	26.5310	-5.3858	3.8400	0.0334	-40.0040
4	DH8	-3.3039	-0.3088	6.5380	-6.2988	4.3992	-0.0006	-49.0110
5	DH13	-6.3118	-0.3783	1.2590	-7.7186	4.8879	0.0158	-55.9770
6	DH14	-8.5584	-0.4245	-18.6140	-8.0425	5.8721	0.0096	-79.8560
7	DH16	-7.6532	-0.3604	-1.7280	-7.3543	5.4311	0.0343	-71.9740
8	DH19	-8.4088	-0.4643	-9.0340	-8.1219	5.0789	-0.0461	-60.9270
9	DH22	-1.7546	-0.2047	9.9540	-5.0598	4.3694	-0.0494	-43.4250
10	DH24	0.9384	-0.1939	18.2810	-5.0045	4.5043	0.0353	-50.2900
11	DH25	-2.5858	-0.2777	7.6720	-6.0105	4.2715	0.0148	-44.1230
12	DH32	-5.2678	-0.3370	-10.9870	-6.6602	4.1009	0.0877	-43.5330
13	DH38	-4.8803	-0.2990	9.0130	-7.3235	5.3188	0.0676	-49.0930
14	DH40	-7.1459	-0.3449	-3.5240	-7.6710	4.7693	-0.0042	-40.0260
15	DH42	-10.4905	-0.4081	-10.0220	-8.4381	6.1104	-0.0810	-81.5900
16	DH44	-9.7420	-0.4170	-26.7420	-8.1789	5.2017	0.0293	-62.9430
17	DH50	-9.0854	-0.3150	-22.3600	-6.2409	5.0991	-0.0166	-52.5070
18	DH56	-4.6751	-0.3554	-9.6510	-6.2545	4.7521	-0.0146	-60.7250
19	DH58	-0.2894	-0.2178	28.0340	-5.2154	4.1496	-0.0119	-47.8480
20	DH59	-4.3606	-0.3305	-1.4930	-6.7350	4.3603	-0.0160	-47.2870
21	DH63	-8.5523	-0.3651	-17.2830	-7.2109	5.6340	0.0070	-69.0810
24	DH73	-10.1953	-0.4584	-28.7140	-8.5489	7.3107	0.0164	-117.3800
25	DH75	-12.5287	-0.4786	-57.6703	-7.9053	6.7567	-0.0557	-100.7140
26	DH76	-9.1810	-0.4428	-25.2160	-7.4249	5.9579	-0.0634	-92.5050
27	DH79	-4.8322	-0.3430	9.6460	-6.5550	5.8993	-0.0624	-72.4660
28	DH80	-6.3809	-0.3547	-4.7300	-7.2357	4.9419	0.0081	-49.3570
29	DH81	-4.4973	-0.3385	18.4510	-7.1888	5.1978	-0.0584	-58.8780
30	DH84	-4.6453	-0.2701	-12.1210	-6.7688	4.0758	0.0510	-30.7930
31	DH91	-12.2290	-0.3228	-40.5350	-7.1304	3.2395	-0.0226	-17.1300
32	DH95	-9.2459	-0.4254	-19.7630	-8.2012	4.5761	0.0084	-52.3320
33	DH102	-9.9560	-0.4728	-12.8750	-7.8497	8.4506	-0.0870	-113.5470
34	DH103	-9.9457	-0.4830	-21.3320	-7.9877	6.3027	0.0142	-83.2170
35	DH109	-11.7369	-0.5642	-24.6250	-8.7147	6.3894	-0.0449	-88.3500
36	DH110	-9.8610	-0.5281	-24.9780	-8.7140	7.3006	-0.0332	-109.2610
37	DH114	-11.1238	-0.4934	-14.1450	-8.7804	6.5938	-0.0487	-110.4450

Geno no.	DH no.	Pn	gs	ETR	E	IWUE	Fv'/Fm'	Ci
38	DH117	-5.4113	-0.2599	-0.3590	-6.6149	5.5757	0.0419	-61.2220
39	DH118	-7.0054	-0.3327	-6.2090	-7.1806	4.9290	0.0220	-57.8280
40	DH121	-5.1389	-0.3290	-13.7150	-7.5882	3.8864	0.1001	-30.4220
41	DH123	-5.1422	-0.3573	-3.0670	-6.6980	4.9567	0.0187	-46.4460
42	DH126	-7.2739	-0.3806	-23.1980	-6.8655	5.4416	0.0386	-63.7290
43	DH128	-2.9547	-0.2950	-0.5050	-6.3975	5.5207	0.0730	-55.5860
44	DH129	-11.6930	-0.5551	-27.1690	-8.6880	7.0964	0.0214	-100.7610
45	DH130	-6.9643	-0.3263	-12.7390	-6.6734	4.5946	0.0229	-51.4650
46	DH131	-6.4278	-0.4418	0.2810	-7.8155	5.5912	-0.0286	-80.4900
47	DH132	-10.4554	-0.4403	-34.7950	-8.1669	4.7546	-0.0011	-62.7760
48	DH134	-4.5219	-0.3795	10.7480	-7.6939	5.6761	-0.0654	-76.3910
49	DH136	-6.4664	-0.3157	-1.7620	-7.3127	5.2847	-0.0166	-48.8350
50	DH137	-7.8216	-0.3012	-11.7980	-7.2139	4.7856	-0.0468	-40.5760
51	DH138	-8.0556	-0.3487	-41.8960	-7.4242	2.6802	0.0302	-46.3490
52	DH140	-6.2344	-0.3537	-10.4820	-7.5075	3.0206	-0.0817	-52.9230
53	DH142	-11.7973	-0.3747	-35.3100	-7.7326	3.1983	0.0109	-65.0850
54	DH143	-6.0020	-0.2962	-31.6400	-5.6172	2.4153	0.0449	-47.6970
55	DH145	-9.6813	-0.4364	-41.0860	-7.1248	2.2797	0.0339	-67.2370
56	DH147	-12.3194	-0.5208	-55.3380	-8.0171	5.2155	-0.0104	-126.9930
57	DH167	-6.3170	-0.4415	-20.1200	-6.7011	2.4773	0.0132	-71.1920
58	DH169	-7.6479	-0.3533	-17.5570	-5.8943	1.8064	-0.0176	-50.6400
59	DH170	-5.2147	-0.3497	-12.6210	-7.2154	2.2847	0.0338	-32.8920
60	DH173	-6.1671	-0.2957	-28.1680	-6.5451	2.7093	0.0431	-47.6520
61	DH175	-9.7880	-0.4135	-28.7180	-8.2059	3.4606	0.0082	-84.7510
63	DH178	-9.1107	-0.3635	-56.4720	-6.9649	2.2022	0.0745	-37.9740
64	DH181	-7.3213	-0.3435	-14.2990	-6.4947	2.5513	0.0653	-65.7500
65	DH182	-9.9292	-0.4506	-33.3240	-7.3985	2.2622	0.0114	-63.2370
66	DH185	-10.7653	-0.4256	-29.8840	-6.9886	2.7311	-0.0776	-83.2540
67	DH186	-10.8352	-0.4347	-47.2010	-7.6904	2.1769	0.0544	-40.9320
68	DH187	-14.0879	-0.4512	-67.6680	-7.7336	3.1862	0.0034	-82.9450
69	DH193	-10.4154	-0.4562	-36.5840	-7.5203	3.7577	-0.0723	-97.3460
71	DH207	-11.4225	-0.5273	-36.6110	-8.9603	3.6656	-0.0468	-99.6940
72	DH208	-9.1987	-0.3600	-29.0250	-7.2796	3.8293	0.0192	-94.8050
73	DH210	0.9745	-0.1841	28.8280	-4.6282	3.1425	0.0994	-57.7970
74	DH212	-11.4693	-0.4357	-36.8710	-7.9338	3.2277	-0.0353	-77.6420
75	DH214	-10.0010	-0.3463	-34.9850	-6.7692	1.7032	0.0209	-28.5140
76	DH215	-3.2607	-0.1895	-10.6690	-5.3599	1.5432	0.0698	-15.0040
77	DH216	-9.9961	-0.4668	-33.0160	-8.1682	2.5126	-0.0324	-70.2510
78	DH224	-9.3293	-0.4531	-24.8450	-7.8414	2.3134	0.0508	-61.2220
79	DH226	-11.5228	-0.4776	-18.1950	-8.2236	2.4992	-0.0661	-76.5200

Geno no.	DH no.	Pn	gs	ETR	E	IWUE	Fv'/Fm'	Ci
80	DH228	0.1801	-0.2814	29.2150	-5.6956	2.9542	0.0397	-77.2030
81	DH231	-7.5960	-0.3404	-27.4840	-5.8809	1.3838	0.0608	-24.9150
82	DH234	-4.6303	-0.2439	-12.5220	-4.8516	1.6476	0.0426	-28.3080
84	DH238	-4.5507	-0.4223	-3.7240	-7.4847	2.9942	0.0069	-79.2660
85	DH240	0.0843	-0.0652	35.8360	-3.5006	2.2013	0.1395	-27.6580
86	DH243	-7.6506	-0.2515	-9.8430	-5.5850	1.6790	0.0276	-19.2670
87	DH248	-9.0065	-0.3424	-14.9830	-7.2642	2.2532	-0.0063	-49.5640
88	DH255	-6.6139	-0.2609	-9.1720	-5.6295	1.7733	0.0226	-29.6410
89	DH256	-8.7569	-0.3554	-23.9400	-7.3475	2.9939	-0.0326	-57.3280
90	DH257	-9.1091	-0.2783	-36.8440	-5.9787	1.4146	0.0490	-10.2860
91	Duster derivative	-8.2267	-0.3490	-29.3170	-6.8602	1.4078	0.0394	-25.1710
92	DH261	-7.8770	-0.3868	-24.5650	-6.9661	1.4659	0.0418	-28.1580
93	DH263	-12.7472	-0.3828	-49.9850	-7.2010	1.2802	-0.0110	-20.5390
94	DH265	-8.9437	-0.4345	-29.9790	-7.1320	1.3562	0.0333	-23.3530
95	DH266	-4.0691	-0.1970	-3.0120	-4.5150	1.6017	0.0921	-20.4410
96	DH268	-9.7485	-0.4079	-19.5570	-6.8984	2.0997	-0.0444	-51.3660
97	DH269	-7.5721	-0.3540	-24.7760	-5.8720	1.5919	0.0612	-38.3230
98	DH270	-8.4970	-0.3769	2.4400	-6.4760	2.8743	-0.0689	-90.6700
99	DH275	-8.5166	-0.3761	-30.2980	-7.0807	2.2248	0.0420	-49.0140
100	DH278	-9.1302	-0.4098	-37.7200	-7.6690	2.8812	0.0620	-64.4500

Table 5: ANOVA showing significant differences in electrolyte leakage indicated by electrical conductivity as affected by genotype, heat stress treatment and their interaction effects.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
genotype	99	780.577295	7.884619	2.88	<.0001
treatment	1	1041.02241	1041.022407	379.94	<.0001
genotype*treatment	95	718.811805	7.56644	2.76	<.0001
Error	196	537.033005	2.739964		

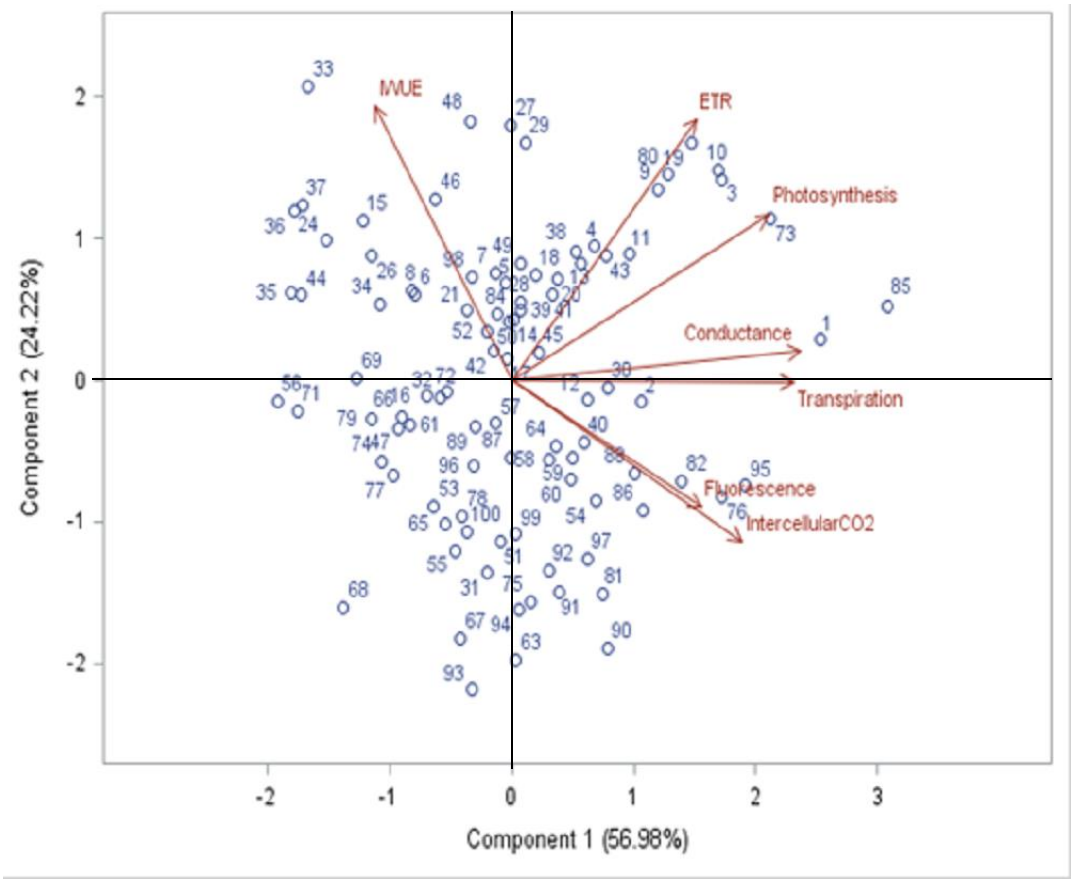

Figure 1: Biplot of the eigenvectors of first two principal component scores. The genotype numbers correspond to genotype numbers in Table 4.

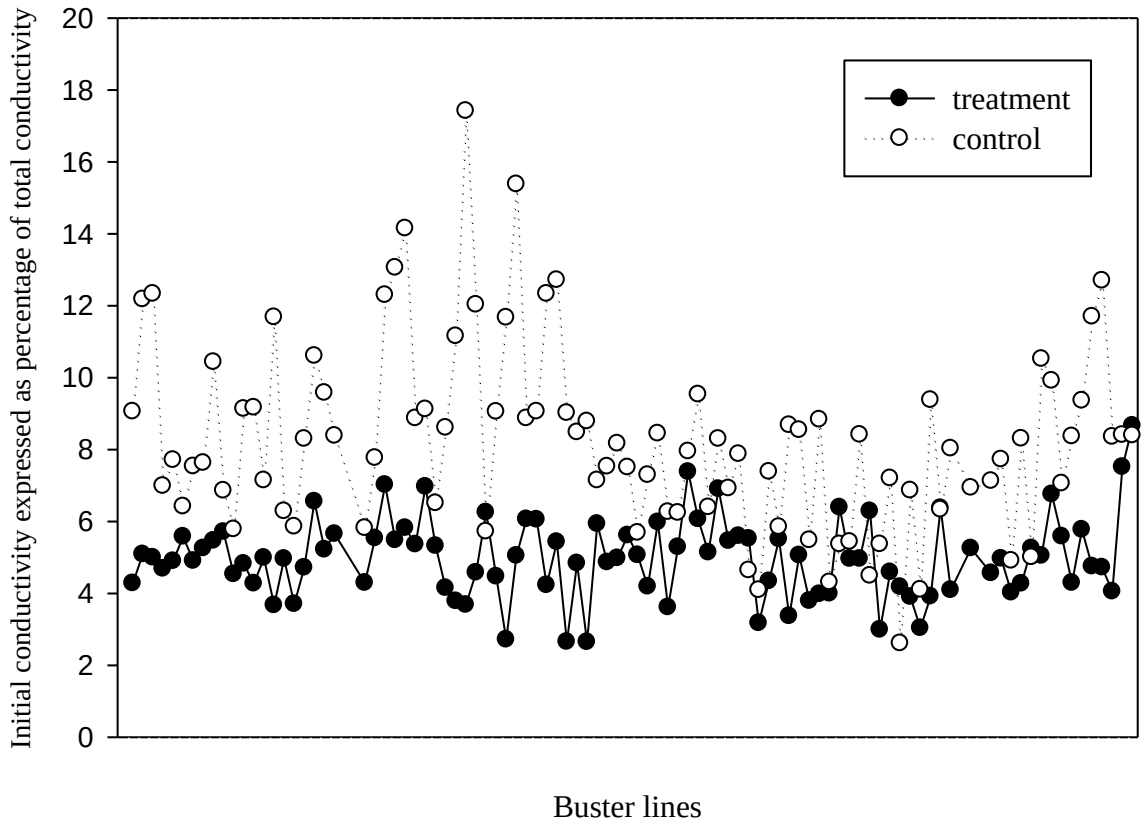

Figure 2: Initial conductivity expressed as percentage of total conductivity for the plants grown in controlled optimum environmental conditions (control) and heat stressed conditions (treatment).

CHAPTER IV

SCREENING OF THE WINTER WHEAT 'BUSTER' POPULATION FOR DROUGHT RESPONSIVE TRAITS

Abstract

Drought is one of the important limiting factors for wheat production in Southern Great Plains (SGP) of the United States. In this study, 33 selected genotypes from a double haploid (DH) winter wheat population 'Buster' were screened for drought responsive traits. Carbohydrate remobilization and spike photosynthesis, well-known parameters that aid in grain filling of wheat under stress, are used to distinguish the Buster lines' responses to drought stress. Six defoliation treatments (spike covered with no leaves, spike covered with all leaves, spike uncovered with no leaves, spike uncovered with no flag leaf, spike uncovered with only flag leaf and spike uncovered with all leaves) were employed to all the lines under two irrigation levels, drought and irrigated. Spikes' and stems' dry weights and spike photosynthesis were measured. Based on spike weights, the Buster lines were found to be significantly different for all the main factors genotype, treatment and defoliation and for genotype*irrigation and defoliation*irrigation interactions but not significantly different for genotype*defoliation and the three-way interaction. The Buster line 'DH236' performed better than other genotypes under irrigated and drought conditions in terms of both carbohydrate remobilization and spike photosynthesis.

1. Introduction:

Wheat is one of the most important crops for global food security fulfilling a large proportion of the total calories and proteins (Braun et al., 2010). Wheat crops are produced throughout the world under different climatic conditions (Shiferaw et al., 2013). Different types of wheat varieties are developed in accordance with the environment they are grown in. As a result of climate change and global warming, the tropics and sub-tropics will have to suffer more heat and drought whereas the northern high latitudes will be warmer and moister in coming days (Dixon et al., 2009). This has placed a challenge on crop scientists to keep up with the crops production in order to meet the demands of the increasing population. Various effects of the climate change that affect wheat production include changes in air and soil temperature, drought, flooding, increase in CO_2 concentration, soil salinity and so on. Drought is an important limiting factor for wheat production in the SGP because most of the wheat grown in this region is rainfed. In addition, these areas have been experiencing an erratic and unpredictable pattern of precipitation and there is a very low confidence in prediction of drought dynamics in this region because of the inconsistent trends (Hoerling et al., 2012). This indicates the need for developing wheat cultivars that can withstand water stress with a minimal loss but can still produce optimally under favorable conditions. SGP account for almost 50% of the total wheat production in the United States (Raz-Yaseef et al., 2015). Therefore, sustenance of wheat production in adverse environmental conditions and increase in productivity under normal conditions are equally crucial for the US wheat industry to fulfil wheat demands.

Drought affects morpho-physiological processes in plants including photosynthesis, respiration, transpiration, nutrient mobilization and translocation, growth and development of above and below ground plant parts and timing of phenological phases. Photosynthesis and carbohydrate accumulation and remobilization are two main processes responsible for grain formation in wheat (Blum et al., 1994; B. Ehdaie et al., 2008). In case of stresses like heat and

drought, photosynthesis can be significantly reduced which makes grain filling more dependent on carbohydrate remobilization (Ehdaie et al., 2008; Yang et al., 2002).

1.1. Carbohydrate accumulation and remobilization:

Largest amount of carbohydrates that contribute to grain filling of wheat are those that are accumulated in the stems (Zhang et al., 2013). Under normal conditions, these stored reserves are mobilized to the grains during grain filling and the remobilization process is accompanied by flag leaf and spike photosynthesis. Nonetheless, grain filling is mostly dependent on the stored reserves when the plants are in heat or drought stress. Water deficit accelerates senescence and promotes carbohydrate remobilization from stem to grains (Xue et al., 2006; Yang et al., 2001). However, the rate of water-soluble carbohydrates (WSC) remobilization varies from genotype to genotype (Blum, 1998). Significant variation was found among wheat genotypes for WSC concentration and remobilization (Ehdaie et al., 2008; Yang et al., 2002; Zamani et al., 2014). An increase in the rates of WSC remobilization in wheat was found under water stress (Ehdaie et al., 2008; Zhang et al., 2013). Increased carbohydrate remobilization as senescence proceeds is one of the desired characteristics for maintaining wheat production in hot and dry conditions (Yang et al., 2001;Asseng & Herwaarden, 2003). Likewise, WSC concentration in the stems is an important trait because there is a strong association between WSC concentration and its remobilization (Zamani et al., 2014). Greater accumulation of WSC in stem and its efficient mobilization to grains during grain filling are desired traits for drought resistance. Changes in stem weight after anthesis is an appropriate measure to study carbohydrate remobilization (Ehdaie et al., 2008).

In addition, several studies were conducted by employing different defoliation treatments to study the contribution of stored reserves to grain filling in wheat (Dodig et al., 2016; de Souza et al., 2013). The defoliation treatment employed ten days after anthesis was found to increase the

stem reserve mobilization attributes and the effective partitioning between stem and grain (Dodig et al., 2016). In a study by Ahmadi et al. (2009), no effect of defoliation was found on grain yield and they reported that other sources like spike photosynthesis and carbohydrate remobilization can meet the demands of grain formation. In addition, the defoliation of leaves in wheat is reported not to affect grain yield under drought but cause a decrease in biomass and yield under well-irrigated conditions (Hu et al., 2015).

1.2. Spike photosynthesis:

Inflorescence or spike photosynthesis in wheat is one of the important components contributing to grain yield. Although, the importance of spike photosynthesis in wheat had been recognized a long time ago, this parameter is only gaining attention in recent decades (Sanchez-Bragado et al., 2016; Tambussi et al., 2007). The contribution of spike photosynthesis to grain yield of wheat was found to range from 10% to 44% by (Kriedemann, 1966). The photosynthetic contribution by awned varieties of wheat was found to be considerably greater than contribution by the upper two leaves (Carr & Wardlaw, 1965). Photosynthetic activity by spikes can be more important under water stressed conditions in comparison to well-watered conditions (Araus et al., 1993; Johnson et al., 1974; Tambussi et al., 2007). This is because the spikes exhibit higher tolerance to water stressed conditions as compared to flag leaves (Tambussi et al., 2007). According a review by Jia et al. (2015), non-leaf organs including spikes are more tolerant to water deficit therefore are important for photosynthetic carbon assimilation under stress. A study by Maydup et al. (2010) reported spikes photosynthesis to contribute from 13% to 33% under non-stressed conditions and from 22% to 45% under resource limited conditions to final yield. Likewise, the spikes contribution towards assimilates from photosynthesis was reported to be greater under drought as compared to irrigated conditions (Evans et al., 1972). Moreover, the spike photosynthesis is also reported to have a positive correlation with final grain yield in wheat (Olszewski et al., 2014). Therefore, photosynthetic capacity of wheat spikes needs to be

considered while developing wheat varieties to grow in places that experience unpredictable weather conditions. In addition, it may also be useful in terms of increasing atmospheric CO_2 because the spikes show greater stimulation in response to increase in CO_2 than the flag leaf (Maydup et al., 2010).

2. Materials and Methods:

Two sets of experiments were conducted, the first under normal field conditions and the second in a greenhouse with drought treatment imposed after anthesis. Selected genotypes from a DH population of winter wheat 'Buster' were used for the experiments. A detailed description on Buster population is provided on Chapter I of this thesis. A total of 100 Buster lines, the ones used in Chapter II and III were utilized for the field experiment. The number was reduced to 33 lines selected based on yields from year 2013-14 for the green house experiment. The number of lines was reduced because the space constraints in controlled conditions did not allow to have replicated study when 100 Buster lines were used. The yields from the crop of that particular year were taken as reference because of natural drought stress that occurred during that period. A detail on the selection process of the Buster lines is provided in Chapter III of this thesis.

2.1. Experimental setup:

In the field, plants were sown at Oklahoma State University (36.1270° N, 97.0737° W) on October 10th, 2014. Each line was planted with John Deere Seed Drill in plots measuring 3 m^2. Plots were 3 m long and 1 m wide. Each plot had four rows planted 25 cm apart. The soil at the location was Easpur loam with 0 to 1 percent slope. The experimental design was a randomized complete block with four replications. Plants were grown under rainfed conditions and no supplemental irrigation was provided.

The greenhouse study was conducted at Oklahoma State University, Stillwater, OK, USA. Four seeds of each Buster line were sown in small one gallon pots. Initially, six sets of pots, each

set with 33 pots were prepared. Sand was used as a medium instead of soil to control the nutrient conditions. The seedlings were hand-watered. The seedlings were subjected to vernalization at 4-6 ^{o}C at 4-6 leaves stage in a cold room for six weeks. The plants were later transplanted in pots of PVC pipes with 50 cm depth and 15 cm diameter in the green house in a split-split plot design to employ irrigation as the main factor, genotypes as sub factor and defoliation as sub-sub factor. Automatic drip irrigation system was used to supply 0.3 L of Hoagland's nutrient solution to the plants each time, four times a day at 8:00AM, 12:00 PM, 4:00PM and 8:00 PM. Drought treatment was imposed on three sets of plants after 50% anthesis was observed in the plants and the other three sets were left as such, as control. The amount of water the plants received was decreased to half to impose drought. In total, there were three replications for each of the genotype in control and drought treatments. Six different defoliation treatments were imposed in six different spikes from all the pots in the same day the drought treatment was started. Spikes with same stage of anthesis were chosen for defoliation to avoid differences in spike physiological age. The defoliation treatments were:

i) Spike covered with all leaves removed from the tiller.
   Grain filling was solely relying on the remobilization of stored reserves.

ii) Spike covered with no leaf removed from the tiller.
   Grain filling relied on leaves photosynthesis and stored reserves mobilization

iii) Spike uncovered with all leaves removed from the tiller.
   Grain filling relied on spike photosynthesis and stored reserves mobilization.

iv) Spike uncovered with flag leaf removed from the tiller.
   Grain filling relied on lower leaves and spike photosynthesis and stored reserves mobilization.

v) Spike uncovered with all leaves removed except flag leaf from the tiller.

Grain filling relied on flag leaf photosynthesis, stored reserves mobilization and spike photosynthesis.

vi) Control tiller – All leaves retained, spike uncovered.

Grain filling relied on leaves and spike photosynthesis and stored reserves mobilization.

2.2. Spike and stem weights:

For the field study, 15 cm row length of each Buster line was sampled twice; immediately after heading and at harvest. Bulk stem and spike weights as well as five individual spikes and stems weights were recorded for each line.

For the green house study, no sampling was done before harvest. The plants were harvested at harvest maturity and dried at around 60 °C for a week and spikes and stems dry weights were recorded separately for individual tillers with defoliation treatments. Bulk dry weights were recorded for rest of the tillers from a pot.

2.3. Spike photosynthesis:

No photosynthesis measurements were taken for the field experiment. Spike photosynthesis was measured after a week of treatment in the green house using an infrared gas analyzer (IRGA) in an open photosynthesis system LI-6400 XT (Licor Inc., NE, USA) using a special conifer chamber that is designed to contain the whole organ. The spikes were artificially irradiated with a light source attached to the sensor head set at 1500 $\mu molm^{-2}s^{-1}$. Temperature in the leaf cuvette was set to 28 °C and chamber reference CO_2 was set to $400\mu LL^{-1}$.

2.4. Statistical analyses:

SAS Version 9.4 (SAS Institute, Cary, NC) was used for the statistical analyses of the data. The Analysis of Variance (ANOVA) was performed using PROC GLM to see if the differences

among the Buster lines are statistically significant at $\alpha = 0.05$ for the recorded parameters. Correlation analysis was done using PROC CORR. For the green house study, irrigation was the main factor, genotypes were sub factor and defoliation treatments were used as sub-sub factors. Graphs were constructed using Sigma Plot.

3. Results and Discussion:

3.1. Spike and stem weights:

3.1.1. Field study:

There were significant differences between the spike weight and stem weight of the Buster lines as inferred by the ANOVA. Changes in average spike weights and stem weights from anthesis to maturity and their relation for the 100 Buster lines is presented in Figure 1. The values for initial and final spikes and stem weights and their differences under field conditions of the 33 Buster lines selected for green house study from the 100 lines is shown in Table 1. Significant positive correlation with a R^2 of 0.18 was observed between the change in stem weights and spike weights from anthesis to maturity. Therefore, 18% of the increase in spike weight can be attributed to the contribution from stems on an average under normal field conditions. The contribution of stem reserves towards final grain weight were found to be 10-29% and 21% in the studies done by Gebbing et al. (1999) and Borrell et al. (1989) respectively, which are in accordance with the results of this study. However, the year 2014/15 experienced a wet winter in Stillwater, OK and so there was no discernable water stress in the field. The carbohydrate concentration and remobilization are most of the times used to study the plants under drought conditions (Hu et al., 2015; Kaur et al., 2012; Yang et al., 2000). This is because the grain filling is dependent on stored reserves under stressed conditions as compared to the normal conditions (Ehdaie et al., 2006).

3.1.2. Green house study:

Results showed different responses of the Buster lines to irrigation and defoliation treatments as indicated by the spike weights. A three-way ANOVA showing differences in spike weights as affected by genotype, irrigation treatment and defoliation treatment and their interaction is shown in Table 2. Among the two-way interactions, the differences were significant for the genotypes*irrigation and defoliation*irrigation interactions but insignificant for genotypes*defoliation interaction. A three-way interaction (genotype*defoliation*irrigation) was not observed in this study. Since there was no genotypes*defoliation interaction, all the Buster lines responded to defoliation treatments in a similar way but the water availability influenced their response. Therefore, the spike weights of the 33 Buster lines were averaged across the defoliation treatments in two irrigation regimes. The graph showing average unit spike weight for each defoliation treatment across the two irrigation regimes is shown in Figure 2. Under irrigated and spike uncovered conditions, the spike weights were similar for the tillers with no flag leaf, only flag leaf and all leaves. Ahmadi et al. (2009) found that grain weight is not significantly affected by post anthesis defoliation, which is in accordance with our results. However, under drought, the average spike weight was significantly more for the tiller with only flag leaf and uncovered spike compared to other defoliation treatments. This may be a result of higher water use efficiency of the plants as the evapotranspiration was reduced by removal of the leaves. The reduction in evapotranspiration by defoliation can help to increase water use efficiency under dry conditions (Shao et al., 2010).

In the first defoliation treatment (spike covered with all leaves removed), both leaves and spikes photosynthesis were excluded from the plant system so it was assumed that the grain filling was dependent on carbohydrates remobilization. Therefore, this defoliation treatment provides information on potential contribution of stem reserves to the spike weight. Nonetheless, the remobilization of stem reserves under other defoliation treatments may not be same as in this

treatment because of the leaf and spike photosynthesis processes going on. In this defoliation treatment, Buster lines 'DH16' and 'DH236' had smallest differences in spike weight between irrigated and drought conditions. Differences in spike weights for each defoliation treatment and Buster line are shown in Figure 3 and the values for differences in same defoliation treatment across two irrigation regimes are shown in Table 3. This indicates the suitability of these lines to be grown on both water limited and unlimited conditions. The spike weights from this defoliation treatment were significantly positively correlated to the average spike weight calculated from bulk measurement with correlation coefficients of 0.42 and 0.50 for drought and irrigation treatments respectively. This indicates that the genotypes with better carbohydrate remobilization under resource limited conditions i.e. defoliation have chances of performing better under better resource conditions.

For the stem weights, the differences were statistically significant for genotype, defoliation and genotype*irrigation ($P < 0.05$) but not for irrigation, genotype*defoliation, irrigation*defoliation and genotype*irrigation*defoliation. No further analyses were done with stem weights as a response variable since this variable did not have significant difference for the main factor irrigation.

In reference to the bulk measurements of the stems and spike weights and number of effective tillers of the remaining tillers after defoliation treatment, there was no significant difference in total spikes weight and average spike weight among genotypes and in interaction with irrigation treatment but the parameters were significantly different ($P < 0.05$) across irrigation treatments. The average spike weight in this case was calculated by dividing the total weight of spikes by number of effective tillers i.e. fertile tillers. Nonetheless, the total stem weight was significantly different among genotypes and irrigation treatments but depicted no interaction effects. Differences in average spike weight and average number spikes between irrigated and drought conditions is shown in Figure 4. There was no relation observed between number of effective

tillers and average spike weight. No further analyses were done because of no significant differences. This similarity in response of the Buster lines can be attributed to the level of homogeneity in these DH lines.

3.1.3. Spike photosynthesis:

Because of unfavorable conditions to, spike photosynthesis could not be recorded on all plants. Among the recorded plants, the spike photosynthesis did not seem to differ much between the irrigation treatments. The undiminished spike photosynthesis under drought can be an indication of spike tolerance to water deficit stress. However, the values were not always greater for either of the irrigation treatments rather were greater for irrigated treatment for nine Buster lines whereas they were greater for the drought treatment in fourteen Buster lines. The values for spike photosynthesis for the Buster lines are provided in Table 4. In rest of the plants under drought, the spikes were not green enough to record photosynthesis. The measurement was taken in all plants under irrigated conditions but there was no significant difference among the genotypes (p-value = 0.24). Also, no correlation was observed between the spike photosynthesis and spike weight under irrigated conditions. The contribution of spike photosynthesis to grain yield is not yet well understood (Sanchez-Bragado et al., 2016). The lack of correlation in this case may also be because the irrigated treatment was only taken into consideration. According to literatures, spike photosynthesis has a greater role in grain formation under drought stress as compared to irrigated conditions (Inoue et al., 2004; Tambussi et al., 2007). Buster line ‘DH269’ and ‘DH236’ performed well in terms of spike photosynthesis regarding the values and differences between irrigated and drought conditions.

4. Conclusions:

The studied genotypes from the Buster population showed similar response to different defoliation treatments. The greater spike weight under partial defoliation as compared to other

treatments under both irrigation regimes may be because of increased water use efficiency and reduced of loss of assimilates through respiration. The extent of carbohydrate mobilization from stems to spikes was significantly influenced by the defoliation treatments. Spike photosynthesis was not reduced to a great extent with decrease in water availability and was not correlated to the spike weight. The Buster line 'DH236' performed well among the studied genotypes in terms of carbohydrate remobilization and spike photosynthesis.

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Table 1: Values for initial and final spikes' and stems' weights and their differences under field conditions of the 33 Buster lines later used for greenhouse condition

Buster line no.	Genotype description	Initial average spike weight (gm.)	Initial average stem weight (gm.)	Final average spike weight (gm.)	Final average stem weight (gm.)	Difference in spike weight (final - initial)	Difference in stem weight (final - initial)
1	Duster	0.272	0.7404	1.0714	0.6402	0.7994	-0.1002
2	Billings	0.4606	1.2928	1.5988	0.6948	1.1382	-0.598
6	OK12D-Blgs/Dst-DH14	0.3892	1.3144	1.2834	0.8976	0.8942	-0.4168
7	OK12D-Blgs/Dst-DH16	0.4072	1.1388	1.082	0.638	0.6748	-0.5008
16	OK12D-Blgs/Dst-DH44	0.3156	1.0764	1.5924	0.89	1.2768	-0.1864
19	OK12D-Blgs/Dst-DH58	0.2996	0.9644	0.9302	0.667	0.6306	-0.2974
20	OK12D-Blgs/Dst-DH59	0.334	0.829	0.8584	0.7968	0.5244	-0.0322
22	OK12D-Blgs/Dst-DH67	0.3422	0.9678	0.8948	0.577	0.5526	-0.3908
30	OK12D-Blgs/Dst-DH84	0.3504	1.0042	1.0276	0.5916	0.6772	-0.4126
32	OK12D-Blgs/Dst-DH95	0.3738	1.061	1.0584	0.5328	0.6846	-0.5282
36	OK12D-Blgs/Dst-DH110	0.3414	0.9808	1.02	0.5454	0.6786	-0.4354
38	OK12D-Blgs/Dst-DH117	0.2442	0.7868	1.4446	0.7838	1.2004	-0.003
39	OK12D-Blgs/Dst-DH118	0.4018	1.1844	1.4202	0.8262	1.0184	-0.3582
44	OK12D-Blgs/Dst-DH129	0.404	1.2274	1.6138	0.9672	1.2098	-0.2602
48	OK12D-Blgs/Dst-DH134	0.4482	1.2586	1.2222	0.705	0.774	-0.5536
54	OK12D-Blgs/Dst-DH143	0.36	0.958	1.1256	0.6224	0.7656	-0.3356
55	OK12D-Blgs/Dst-DH145	0.336	0.8828	1.469	0.8552	1.133	-0.0276
58	OK12D-Blgs/Dst-DH169	0.2052	0.7724	0.6594	0.6268	0.4542	-0.1456
60	OK12D-Blgs/Dst-DH173	0.3046	0.9176	1.2256	0.6678	0.921	-0.2498
61	OK12D-Blgs/Dst-DH175	0.3872	1.0338	1.5206	0.7784	1.1334	-0.2554
65	OK12D-Blgs/Dst-DH182	0.3694	1.061	1.136	0.525	0.7666	-0.536
67	OK12D-Blgs/Dst-DH186	0.2678	0.7656	1.1044	0.603	0.8366	-0.1626
68	OK12D-Blgs/Dst-DH187	0.3266	0.7708	0.917	0.5114	0.5904	-0.2594
73	OK12D-Blgs/Dst-DH210	0.2848	0.6964	0.8888	0.5556	0.604	-0.1408

Table 1: continued

Buster line no.	Genotype description	Initial average spike weight (gm.)	Initial average stem weight (gm.)	Final average spike weight (gm.)	Final average stem weight (gm.)	Difference in spike weight (final - initial)	Difference in stem weight (final - initial)
80	OK12D-Blgs/Dst-DH228	0.314	1.0186	1.071	0.5506	0.757	-0.468
83	OK12D-Blgs/Dst-DH236	0.3372	0.8766	0.931	0.4934	0.5938	-0.3832
86	OK12D-Blgs/Dst-DH243	0.2646	0.6878	0.7392	0.3902	0.4746	-0.2976
87	OK12D-Blgs/Dst-DH248	0.2766	0.7022	0.6656	0.3528	0.389	-0.3494
90	OK12D-Blgs/Dst-DH257	0.2436	0.5604	0.6974	0.2794	0.4538	-0.281
92	OK12D-Blgs/Dst-DH261	0.2216	0.685	0.9274	0.4618	0.7058	-0.2232
95	OK12D-Blgs/Dst-DH266	0.2722	0.8642	1.0904	0.62075	0.8182	-0.24345
97	OK12D-Blgs/Dst-DH269	0.2766	0.8058	0.7102	0.321	0.4336	-0.4848

Table 2: A three-way ANOVA showing effect of genotype, irrigation and defoliation treatments and their interaction on spike weight.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Genotype	32	11.57099	0.361593	5.03	<.0001
Irrigation	1	59.629	59.629	829.39	<.0001
Defoliation	5	5.236924	1.047385	14.57	<.0001
Genotype*Irrigation	32	9.499352	0.296855	4.13	<.0001
Genotype*Defoliation	160	4.899867	0.030624	0.43	1
Irrigation*Defoliation	5	1.128432	0.225687	3.14	0.0083
Genotype*Irrigation*Defoliation	160	5.135659	0.032098	0.45	1

Table 3: Differences in average spike weights (irrigated – drought) of the 33 Buster lines for each of the defoliation treatments 1, 2, 3, 4, 5 and 6. Where (1) spike covered with no leaves, (2) spike covered with all leaves, (3) spike uncovered with no leaves, (4) spike uncovered without flag leaf, (5) spike uncovered with only flag leaf and (6) spike uncovered with all leaves.

Buster line no.	Genotype description	1	2	3	4	5	6
1	Duster	0.54	0.38	0.40	0.58	0.69	0.66
2	Billings	0.42	0.56	0.68	0.55	0.68	0.93
6	OK12D-Blgs/Dst-DH14	0.58	0.69	0.55	0.60	0.54	0.60
7	OK12D-Blgs/Dst-DH16	0.21	0.49	0.30	0.39	0.48	0.91
16	OK12D-Blgs/Dst-DH44	0.46	0.42	0.70	0.60	0.45	0.68
19	OK12D-Blgs/Dst-DH58	0.40	0.49	0.40	0.57	0.36	0.70
20	OK12D-Blgs/Dst-DH59	0.37	0.60	0.56	1.04	0.72	0.70
21	OK12D-Blgs/Dst-DH63	0.37	0.34	0.21	0.75	0.69	0.64
22	OK12D-Blgs/Dst-DH67	0.89	0.95	0.68	1.01	0.91	0.85
30	OK12D-Blgs/Dst-DH84	0.39	0.12	0.44	0.64	0.26	0.11
32	OK12D-Blgs/Dst-DH95	0.22	0.08	-0.14	0.58	0.07	0.29
36	OK12D-Blgs/Dst-DH110	0.37	0.21	0.15	0.52	0.20	0.45
38	OK12D-Blgs/Dst-DH117	0.49	0.64	0.46	0.82	0.51	0.82
39	OK12D-Blgs/Dst-DH118	0.62	0.55	0.54	0.76	0.65	1.11
44	OK12D-Blgs/Dst-DH129	0.73	0.38	0.54	0.75	0.45	0.90
48	OK12D-Blgs/Dst-DH134	0.42	0.79	0.53	0.46	0.96	0.65
54	OK12D-Blgs/Dst-DH143	0.04	0.17	0.36	0.53	0.26	0.06
55	OK12D-Blgs/Dst-DH145	0.46	0.42	0.60	0.52	0.55	0.75
58	OK12D-Blgs/Dst-DH169	0.47	0.68	0.30	0.77	0.72	0.62
60	OK12D-Blgs/Dst-DH173	0.46	0.52	0.36	0.66	0.25	0.45
61	OK12D-Blgs/Dst-DH175	0.20	0.04	0.19	0.24	0.54	0.15
65	OK12D-Blgs/Dst-DH182	0.85	0.92	0.74	0.84	0.51	0.70
67	OK12D-Blgs/Dst-DH186	0.75	0.82	0.37	0.55	0.54	0.80
68	OK12D-Blgs/Dst-DH187	0.58	0.76	0.42	0.93	0.99	0.59
73	OK12D-Blgs/Dst-DH210	0.43	0.46	0.90	0.60	1.05	0.25
80	OK12D-Blgs/Dst-DH228	0.53	0.31	0.31	0.63	0.92	0.46
83	OK12D-Blgs/Dst-DH236	0.27	0.21	-0.12	0.22	0.28	0.04
86	OK12D-Blgs/Dst-DH243	0.43	0.43	0.36	0.68	0.40	0.68
87	OK12D-Blgs/Dst-DH248	0.43	0.53	0.48	0.58	0.61	0.73
90	OK12D-Blgs/Dst-DH257	0.19	0.31	0.21	0.11	0.13	-0.01
92	OK12D-Blgs/Dst-DH261	0.07	0.34	0.14	0.16	0.16	0.15
95	OK12D-Blgs/Dst-DH266	0.40	0.58	0.29	0.37	0.72	0.55
97	OK12D-Blgs/Dst-DH269	0.17	0.29	0.33	0.28	0.30	0.45

Table 4: Values of spike photosynthesis (μmol$CO_2$$m^{-2}$$s^{-1}$) for 33 Buster lines under irrigated and drought conditions.

Buster line no.	Genotype description	Drought	Irrigated
2	Billings	15.111	13.129
7	OK12D-Blgs/Dst-DH16	11.707	13.210
16	OK12D-Blgs/Dst-DH44	12.686	16.805
19	OK12D-Blgs/Dst-DH58	15.453	9.604
20	OK12D-Blgs/Dst-DH59	9.432	12.194
21	OK12D-Blgs/Dst-DH63	14.702	13.036
30	OK12D-Blgs/Dst-DH84	14.103	12.485
32	OK12D-Blgs/Dst-DH95	13.128	12.482
36	OK12D-Blgs/Dst-DH110	10.559	12.000
38	OK12D-Blgs/Dst-DH117	11.224	10.171
44	OK12D-Blgs/Dst-DH129	6.641	9.554
54	OK12D-Blgs/Dst-DH143	13.737	10.726
60	OK12D-Blgs/Dst-DH173	10.730	9.927
61	OK12D-Blgs/Dst-DH175	13.978	7.271
65	OK12D-Blgs/Dst-DH182	10.464	15.308
67	OK12D-Blgs/Dst-DH186	11.226	13.852
73	OK12D-Blgs/Dst-DH210	11.314	6.185
80	OK12D-Blgs/Dst-DH228	12.294	10.239
83	OK12D-Blgs/Dst-DH236	15.887	13.585
87	OK12D-Blgs/Dst-DH248	5.318	10.217
92	OK12D-Blgs/Dst-DH261	14.282	12.680
95	OK12D-Blgs/Dst-DH266	7.272	13.054
97	OK12D-Blgs/Dst-DH269	17.882	17.055

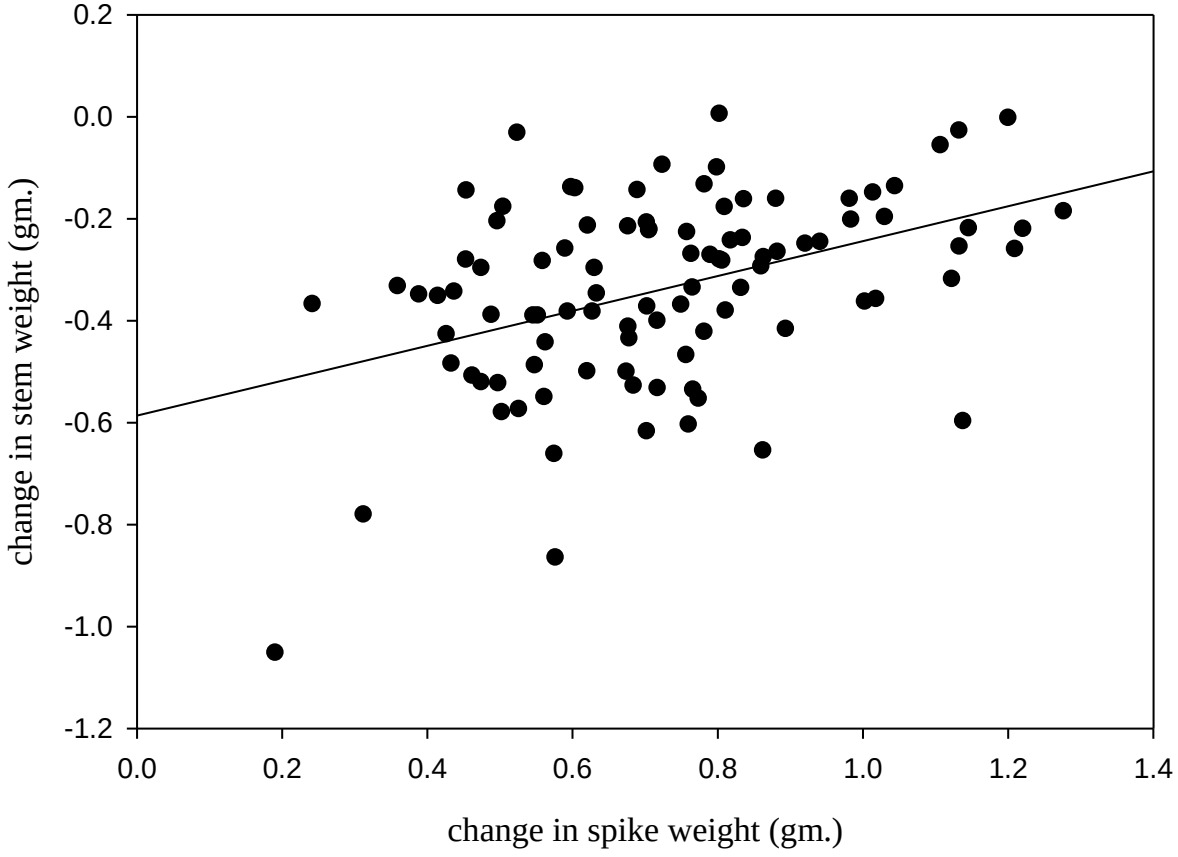

Figure 1: Scatterplot showing relationship between change in spike and stem weights (final weights at harvest – initial weights at anthesis) among 100 Buster lines, $R^2 = 0.18$, significant correlation.

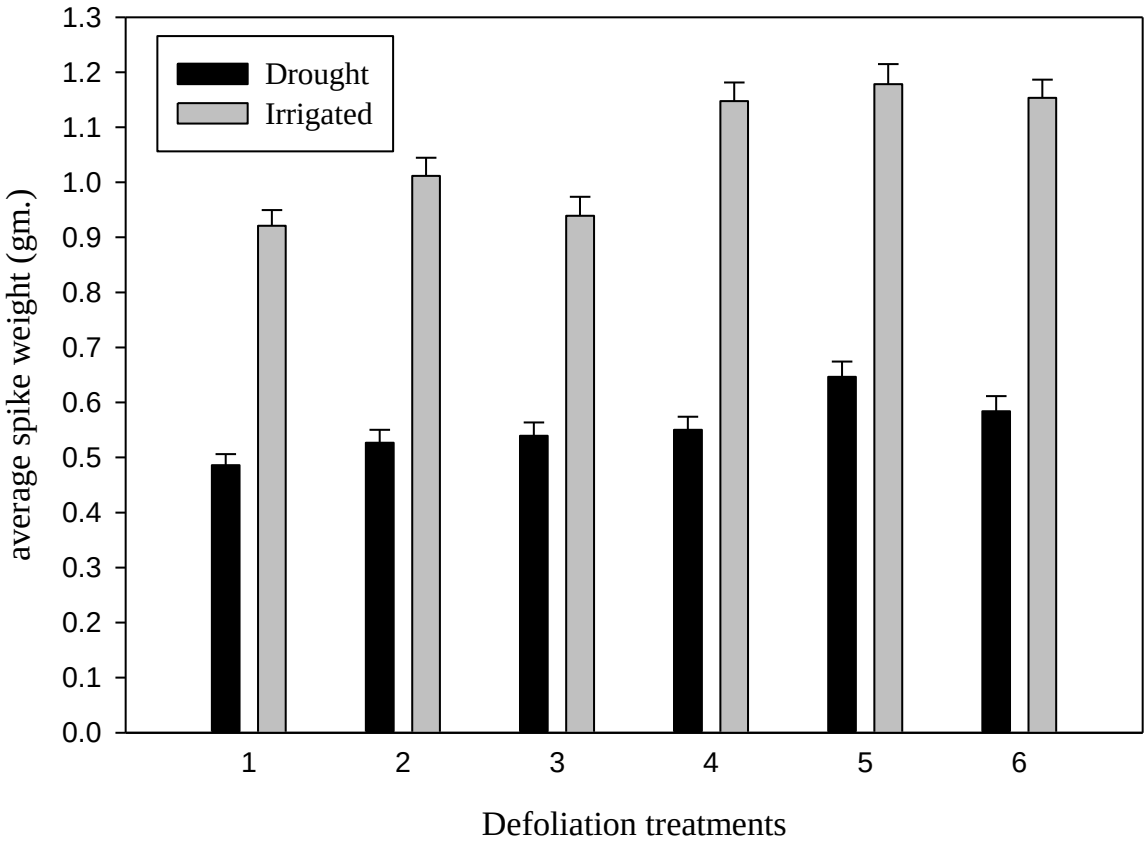

Figure 2: Differences in average spike weights across irrigation regimes for the defoliation treatments 1, 2, 3, 4, 5 and 6. Where, (1) is spike covered with no leaves, (2) is spike covered with all leaves, (3) is spike uncovered with no leaves, (4) is spike uncovered without flag leaf, (5) is spike uncovered with only flag leaf and (6) is spike uncovered with all leaves.

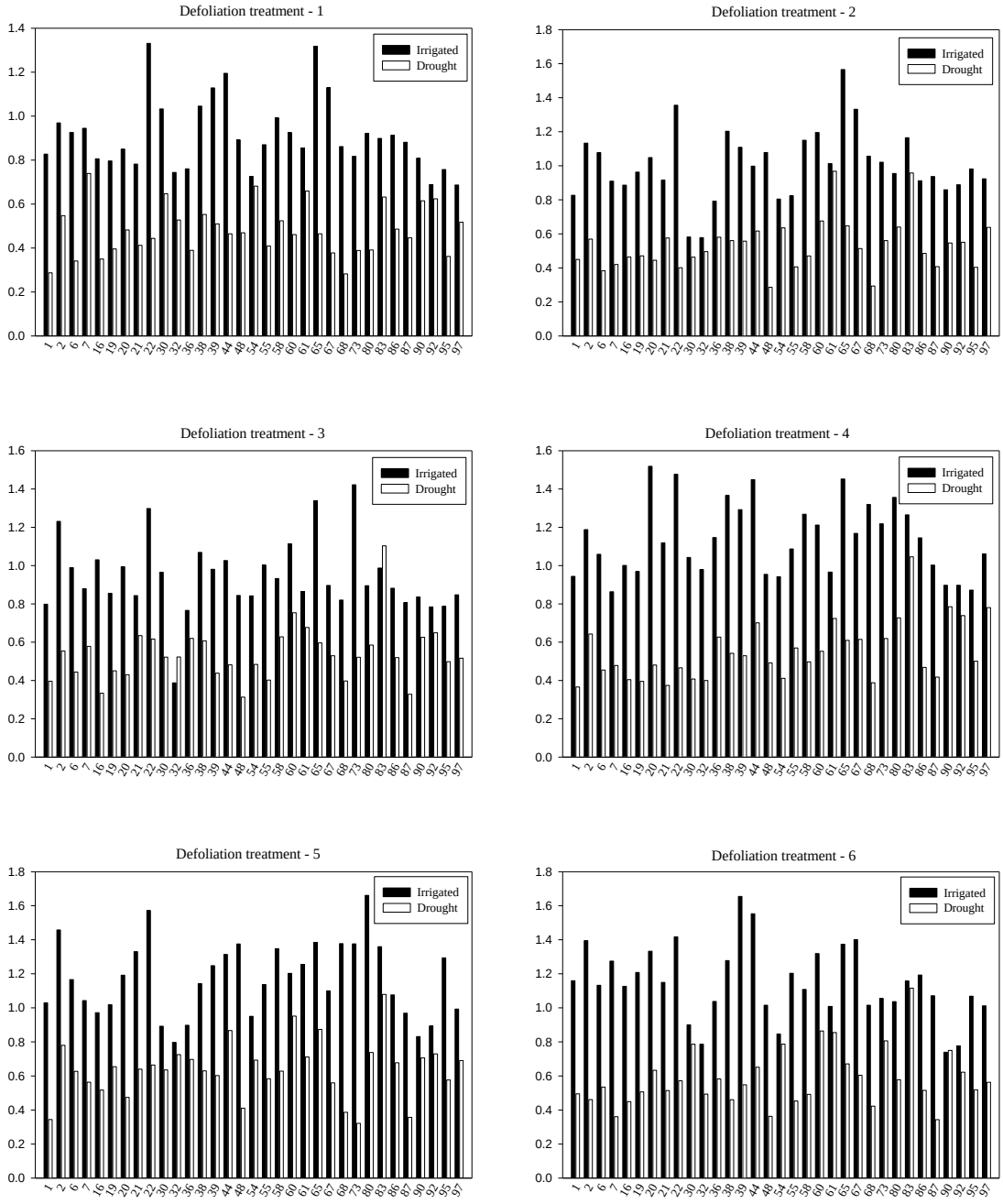

Figure 3: Differences in average spike weights across irrigation regimes for each Buster lines and the defoliation treatments. Where, (1) spike covered with no leaves, (2) spike covered with all leaves, (3) spike uncovered with no leaves, (4) spike uncovered without flag leaf, (5) is spike uncovered only with flag leaf and (6) spike uncovered with all leaves and y-axis = average spike weight in grams, x-axis = numbers assigned for the 33 Buster lines.

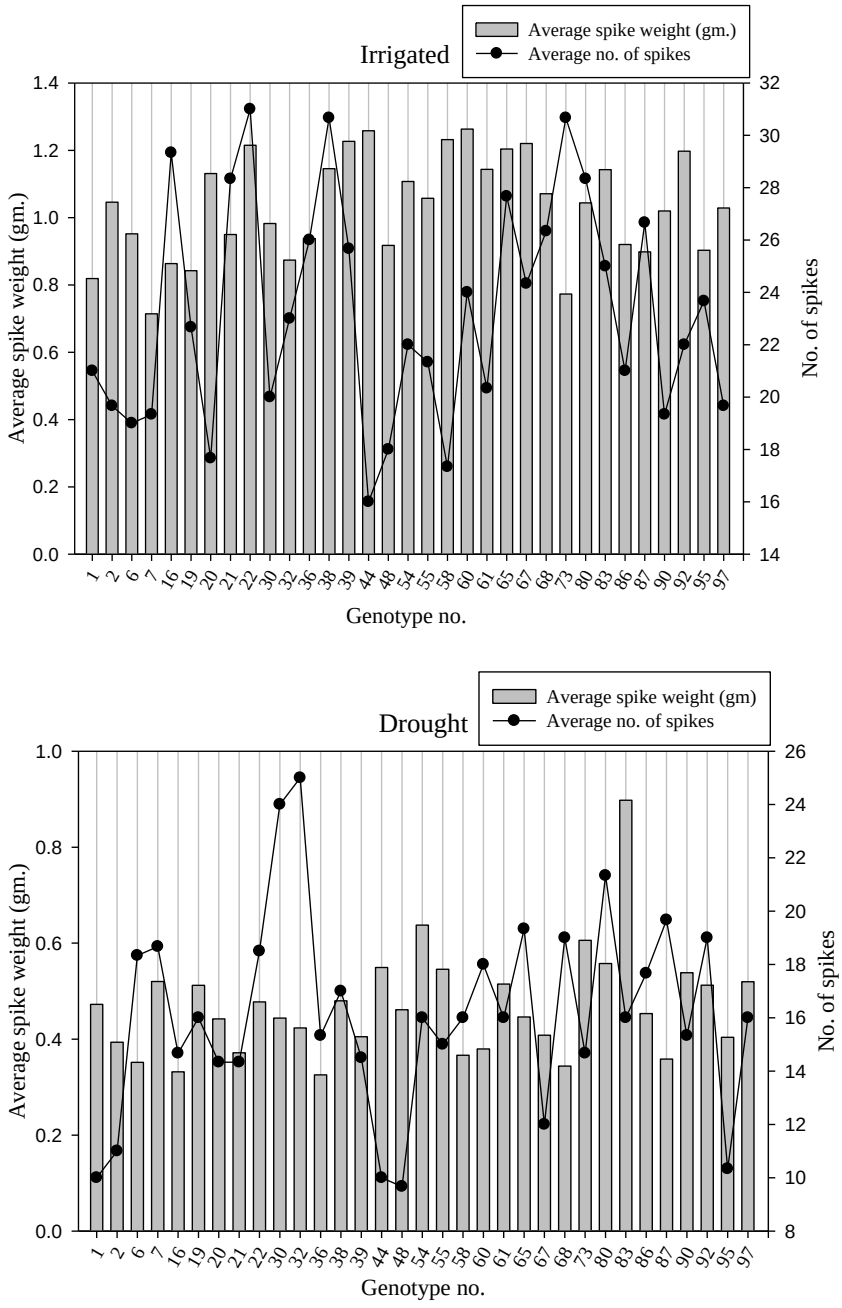

Figure 4: Average unit spike weights (bar graphs) and number of spikes (line graph) of selected 33 Buster lines across the two irrigation regimes.

CHAPTER V

DIFFERENTIAL RESPONSES OF WHEAT 'BUSTER' LINES TO HEAT AND DROUGHT STRESS

Abstract

The major abiotic stresses associated with wheat production throughout the world are high temperature and low water availability. The objective of this study is to screen 33 double haploid (DH) 'Buster' lines against heat and drought stress. This study assesses changes in different physiological plant parameters and yield parameters in response to controlled conditions, high temperature irrigated, and high temperature drought conditions. Leaf and spike gas exchange parameters were measured using LI-6400 (Licor Inc., NE, USA), and spike and stem dry weights were recorded. Interaction between Buster lines and treatments was not significant. The main effect of treatment was significant for all observed parameters and the main effect of genotype was significant for spike gas exchange parameters and leaf fluorescence. The spikes that underwent anthesis before stress treatments had greater weight than those, which underwent anthesis under stress. The spike photosynthesis was positively correlated to average spike weight under stressed conditions.

1. Introduction:

Wheat is grown more than any other crops in the world in terms of area (Curtis, 2002). It fulfils the greatest proportion of calories for the world population. With the prediction of world population to reach 9.6 billion by 2050 (Searchinger et al., 2014), it has been important to address and solve the problems associated with wheat production to fulfil the consumer demands in the near future. The major abiotic problems associated with wheat production worldwide are drought (Rezaei et al., 2010) and high temperature (Gourdji et al., 2013). More frequent and persistent droughts are predicted in the world with increase in global temperature in the near future (Su et al., 2013). Because of unpredictable and erratic nature of rainfall and continuously increasing temperature every year, there's a need of wheat cultivars that can withstand stress with minimal loss but continue to produce to their full potential under favorable conditions. High temperature and water stress affect different physiological processes in plants such as tillering, leaf production and grain filling, and metabolic processes such as photosynthesis, transpiration and respiration. Studies have been conducted evaluating wheat performance under water stressed or increased temperature conditions. However, these two stresses often occur together in the field and therefore need to be dealt in combination. In addition, the interaction between these two factors contributes to a complex response of plants and the results of combined heat and drought stress can be more severe than the results of individual stresses. This study attempts to identify the differences in wheat response between optimum environment, high temperature with adequate water and high temperature combined with water stress. From the Buster DH population, 33 lines were evaluated based on different physiological (leaf and spikes gas exchange) parameters and yield attributes in order to identify heat and drought resistant traits in them. The Buster DH population is developed by Wheat Improvement Team (WIT) at Oklahoma State University (OSU) by crossing two varieties 'Duster' and 'Billings'. A detailed description on Buster

population development is provided in Chapter I of this thesis. The 33 lines used in this experiment are same as the ones used in Chapter IV.

The primary physiological parameters evaluated in this study are photosynthesis and carbohydrate remobilization, because these are the main processes responsible for grain formation in wheat (Blum et al., 1994; Ehdaie et al., 2008). Furthermore, the drought and heat stress conditions have a negative effect on photosynthetic processes, which makes grain filling more dependent upon the carbohydrate reserves.

The gas exchange parameters (photosynthesis (Pn), stomatal conductance (gs), internal CO_2 concentration (Ci), transpiration (E) and instantaneous water use efficiency (IWUE)) measured on both leaves and spikes, and electron transport rate (ETR) and fluorescence (Fv′/Fm′) measured only on leaves are the physiological parameters considered in this study. In addition, number of spikes and average weight of the spikes are used as yield parameters.

1.1. Leaf gas exchange parameters:

The most responsive leaf gas exchange parameters under stress are stomatal conductance, photosynthesis and transpiration. This is mostly because the movement of carbon dioxide and water to and from the leaves takes place via stomatal openings (Sikder et al., 2015). High stomatal conductance correlates to higher rate of photosynthesis and at the same time to higher rate of transpiration. When there is an increase in temperature, the plants respond by opening their stomata, which also allows them to cool the leaves. However, under drought, the stomata are closed and leaf temperature increases leading to metabolic alterations. Therefore, in case of combined heat and drought stress the evaporative cooling does not hold well (Mittler, 2006). A study done by Johnson et al. (1974) found a decrease in both photosynthesis and transpiration under low leaf water potential. A detailed discussion on leaf gas exchange is done in Chapter III of this thesis.

1.2. Spike photosynthesis:

Spike photosynthesis is believed to contribute more towards grain filling under drought conditions as compared to full resource conditions (Araus et al., 1993; Johnson et al., 1974). It is more efficient under limited moisture conditions because of relative water content of spikes higher than that of the leaves (Tambussi et al., 2007). Increase in spike photosynthesis can be one of the main approaches to improve overall photosynthetic efficiency of wheat plants (Parry et al., 2011). The contribution of spike photosynthesis to grain yield is only significant when plants are grown in resource-limited conditions (Evans et al., 1972). However, spike photosynthesis have been found to contribute towards final yield from 13% to 33% under optimal conditions and from 22% to 45% under stressed conditions as found by a study done by (Maydup et al., 2010). This parameter is also discussed in Chapter IV of this thesis.

1.3. Carbohydrate remobilization:

Carbohydrate remobilization is the second most important factor contributing to grain formation in wheat after photosynthesis. From different related studies, carbohydrates accumulated in stems are found to contribute towards final grain yield from 10% to 62% under normal conditions and from 40% to 100% under stress depending upon the severity of the stress (Ehdaie et al., 2008). Grain filling in wheat is highly influenced by the carbohydrate remobilization efficiency of the cultivar, especially under heat and drought stressed conditions because of decreased leaf photosynthetic efficiency (Zhang et al., 2014). In addition, water deficit induces water-soluble carbohydrates mobilization from stems to spikes with higher efficiency as the senescence is accelerated (Xue et al., 2006; Yang et al., 2001). An increase in remobilization of water-soluble carbohydrates was observed under heat stress by Zamani et al. (2014) and under drought stress by Zhang et al. (2013). Therefore, carbohydrate remobilization is an important trait

to be considered when screening genotypes for drought and heat resistance. The carbohydrate remobilization was also considered in our third study i.e. Chapter IV of this thesis.

2. Materials and methods:

2.1. Experimental setup:

This was a growth chamber study conducted in the controlled environment research laboratory (CERL) at OSU, Stillwater, OK. Six growth chambers were used for the study and each chamber consisted of 33 pots of 50 cm depth and 15 cm diameter filled with fine sand. Four seeds of each of the 33 lines were sowed per pot in each chamber. The seedlings were vernalized at 4-6 leaves stage in a cold room (4-6 ºC) for six weeks. The plants were later transplanted in pots of PVC pipes with 35 cm depth and 15 cm diameter in the growth chamber. Hoagland's solution was provided to the plants three times a day at 8:00 AM, 1:00 PM and 6:00 PM, 0.3 L each time, through an automatic drip irrigation system. Photoperiod was adjusted as 14 hours of day length and 10 hours dark period. The six chambers were divided into three groups (two chambers in each group) after 50% of plants in all the chambers had undergone anthesis in order to subject them to different treatments. The tillers that had already undergone anthesis were tagged in the chambers at the time of treatment introduction. This allowed separation of spikes, which flowered before treatment introduction from the spikes that flowered under stress. Plants were grown under temperatures of (22/16 ºC day/night) before introducing the treatments. The two chambers designated for control group had unchanged conditions throughout the plant's life cycle. Whereas, the temperature was increased to (32/26 ºC day/night) in rest of the four chambers after 50% anthesis. Among those four chambers, two chambers received the water-nutrient solution similar to control treatment thus the plants received high temperature irrigated treatment. The irrigation was cut into half (0.15 L each time) along with the high temperature for the remaining two chambers. In short, the three groups of treatments can be described as:

Treatment 1: Control (Optimum temperature and adequate water)

Treatment 2: Heat stressed (High temperature with adequate water)

Treatment 3: Combined heat and drought stressed (High temperature with reduced water supply)

2.2. Leaf gas exchange parameters and spike photosynthesis:

The gas exchange parameters on both leaves and spikes were measured after a week of the stress treatment with the use of an infrared gas analyzer (IRGA) in an open photosynthesis system LI-6400 (Licor Inc., NE, USA).

For the leaf gas exchange parameters and fluorescence measurements, two youngest fully open leaves from adjacent plants were used to cover the 2 cm^2 of the leaf cuvette. The leaves were artificially irradiated with a blue-red LED radiation source attached to the sensor head set at 1200 $\mu molm^{-2}s^{-1}$ for uniform light in all measurements. Leaf chamber reference CO_2 was set to 400 μLL^{-1} and temperature in the cuvette was set according to the day time temperature of the respective growth chambers.

A conifer chamber designed to contain the whole organ was used for spike photosynthesis measurements. A light source was attached to the sensor head set at 1200 $\mu molm^{-2}s^{-1}$ for artificial irradiation of the spikes. Temperature was set in accordance to the day time temperature of the growth chamber and reference CO_2 was set to 400 μLL^{-1}.

2.3. Spike and stem weights:

At the time of the treatment, the spikes that had undergone anthesis were tagged to differentiate them from the spikes, which had not already flowered because they were likely to be affected by the stress treatments in different ways. Plants were harvested at harvest maturity and oven dried at around 60 ºC for about a week. Spikes and stem dry weights were measured. The

two groups of spikes, those flowered before stress and the ones that flowered after stress, were weighed separately. Number of spikes was recorded for each pot.

2.4. Statistical analyses:

SAS Version 9.4 (SAS Institute, Cary, NC) was used for the statistical analysis of the data. The analysis of variance and correlation analyses were conducted using PROC GLM and PROC CORR respectively. PROC DISCRIM was used for canonical analysis to see if the three treatments are different from one another. Then a contrast analysis was done using PROC GLM to see if the differences in individual parameters are significant across the treatments. Sigma plot was used to construct graphs.

3. Results and Discussion:

The ANOVA showed that genotypes and treatments interaction was not significant. The main effect of temperature and drought treatments was significant for all parameters and the main effect of genotype was significant only for the spike gas exchange parameters (Pn, gs, E, Ci and IWUE) at 0.05 levels of significance. The two non-zero canonical correlation coefficients from canonical correlation analysis proved that the three treatments are significantly different from one another. A contrast analysis was done to identify one to one differences between the treatments. The p-values for main and interaction effects and contrast between three treatments are presented (Table 1).

Spikes that flowered before stress were not much affected by the stresses compared to those, which flowered after stress. Differences in average spike weight for both of the spikes that flowered before and after stress between (i) control and high temperature irrigated treatment and (ii) control and high temperature drought treatment are shown in Figures 1(a) and 1(b) respectively.

Since the Buster lines were found to significantly differ only for the spike gas exchange parameters, further consideration was given to these criteria. The differences across three treatments for spike Pn and IWUE are presented in Table 2. The Buster lines 'DH248' had highest photosynthesis of 7.11 $\mu molCO_2m^{-2}s^{-1}$ under control condition whereas the lines 'DH210' (15.38 $\mu molCO_2m^{-2}s^{-1}$) and 'DH257' (14.29 $\mu molCO_2m^{-2}s^{-1}$) had highest spike photosynthesis in high temperature irrigated condition and high temperature drought respectively.

A set of values of Pearson correlation coefficients between plant physiological parameters and yield parameters from the results of the correlation analysis are presented in Table 3. The spike photosynthesis was significantly positively correlated to total spikes weight and average spike weight for the spikes that underwent anthesis before treatment introduction, with Pearson correlation coefficients of 0.31 and 0.40 respectively. A study done by Olszewski et al. (2014) exhibited positive correlation between spike photosynthesis and grain yield. The spike photosynthesis of all the Buster lines was higher in stress as compared to control conditions. The optimum temperature for carbon exchange per unit area for spikes was determined to be 32 °C or more (Blum, 1986) which is in accordance with our results where spike photosynthesis increased from a temperature of 22 °C to 32 °C. Three graphs constructed to show the relation between spike photosynthesis and average spike weight under different treatment conditions are shown in Figures 2(a), 2(b) and 2(c). It reveals that the spike photosynthesis is not correlated to average spike weight under normal conditions (p-value for correlation coefficient is large), but is significantly positively correlated at 0.05 levels of significance under high temperature irrigated and high temperature drought conditions with the Pearson's correlation coefficient of 0.4 in both cases. This is reasonable because spike photosynthesis has an important contribution to grain formation under stress as compared to normal conditions (Tambussi et al., 2007; Inoue et al., 2004).

4. Conclusion:

In conclusion, the Buster lines did not show significant differences in interaction with temperature treatment based on studied parameters but the treatments alone had significant effects on all studied plant parameters. Significant differences were observed with genotype as main effect for five spike parameters (Pn, gs, E, Ci, and IWUE). The high temperature alone and combined with drought significantly reduced the spike weight which underwent anthesis after stress compared to those which had already flowered. Spike photosynthesis was significantly positively correlated to average spike weight under stress conditions.

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Table 1: P-values for the variables showing differences for main factors (genotype and treatment) and their interaction and for the contrasts between each of the two treatments among the total of three treatments. In the table, Geno = genotype, Trt = treatment, HI = high temperature irrigated and HD = High temperature drought. The suffixes L- and S- signify leaf and spike measurements and B- and A- signify before and after where before and after means the spikes which underwent anthesis before and after treatment introduction respectively.

	P-values for factors			P-values for contrast analysis		
	Geno	Trt	Geno*Trt	Control-HI	HI-HD	Control-HD
L-Photosynthesis	0.79	0.01	0.68	0.008	0.005	0.84
L-Conductance	0.82	<0.0001	0.87	<0.0001	0.09	<0.0001
L-Ci	0.53	<0.0001	0.86	<0.0001	0.24	<0.0001
L-Fv'/Fm'	0.03	<0.0001	0.8	<0.0001	0.0001	<0.0001
L-ETR	0.4	<0.0001	0.2	<0.0001	<0.0001	<0.0001
L-Transpiration	0.84	<0.0001	0.97	<0.0001	0.53	<0.0001
L-IWUE	0.86	<0.0001	0.9	<0.0001	0.0093	<0.0001
S-Photosynthesis	0.04	<0.0001	0.14	<0.0001	0.56	<0.0001
S-Conductance	0.03	0.0015	0.49	0.04	0.0005	0.13
S-Ci	0.02	<0.0001	0.61	<0.0001	<0.0001	<0.0001
S-Transpiration	0.02	<0.0001	0.44	<0.0001	<0.0001	<0.0001
S-IWUE	0.04	<0.0001	0.22	<0.0001	<0.0001	0.1376
Total biomass	0.01	<0.0001	0.64	<0.0001	0.06	<0.0001
No. of spikes	0.48	<0.0001	0.48	<0.0001	0.03	<0.0001
B-Total spike wt.	0.63	<0.0001	0.78	<0.0001	0.74	<0.0001
B-Total stem wt.	0.25	<0.0001	0.27	<0.0001	0.93	<0.0001
B-Avg. unit spike wt.	0.41	<0.0001	0.97	<0.0001	<0.0001	0.0005
A-Total spike wt.	0.46	<0.0001	0.88	<0.0001	0.54	<0.0001
A-Total stem wt.	0.002	<0.0001	0.53	<0.0001	0.04	<0.0001
A-Avg. unit spike wt.	0.79	<0.0001	0.3	<0.0001	<0.0001	<0.0001

Table 2: Differences in spike photosynthesis (sPhoto) and IWUE (sIWUE) leaf across three treatments; control, high temperature irrigated and high temperature drought.

Buster line number	Genotype description	Control - High temperature irrigated		Control - High temperature drought		High temperature (irrigated - drought)	
		sPhoto	sIWUE	sPhoto	sIWUE	sPhoto	sIWUE
1	Duster	-6.10	-0.06	-2.07	0.01	4.03	0.07
2	Billings	-5.74	0.35	-7.10	0.33	-1.36	-0.02
6	OK12D-Blgs/Dst-DH14	-4.38	0.09	-8.93	-0.26	-4.55	-0.35
7	OK12D-Blgs/Dst-DH16	-1.65	0.27	-5.66	-0.17	-4.01	-0.43
16	OK12D-Blgs/Dst-DH44	-4.09	0.35	-4.99	0.05	-0.91	-0.30
19	OK12D-Blgs/Dst-DH58	-2.28	0.52	-5.29	0.28	-3.01	-0.24
20	OK12D-Blgs/Dst-DH59	-3.96	0.43	-5.56	0.23	-1.59	-0.20
21	OK12D-Blgs/Dst-DH63	-6.23	0.13	-7.54	-0.04	-1.31	-0.17
22	OK12D-Blgs/Dst-DH67	-7.47	0.07	-6.42	0.07	1.05	0.00
30	OK12D-Blgs/Dst-DH84	-5.91	0.18	-0.91	0.50	5.00	0.32
32	OK12D-Blgs/Dst-DH95	-4.25	0.00	-7.90	-0.43	-3.65	-0.43
36	OK12D-Blgs/Dst-DH110	-4.94	0.52	-1.87	0.47	3.07	-0.05
38	OK12D-Blgs/Dst-DH117	-3.84	0.19	-3.13	0.14	0.71	-0.05
39	OK12D-Blgs/Dst-DH118	-1.71	0.49	-5.10	-0.16	-3.40	-0.64
44	OK12D-Blgs/Dst-DH129	-6.14	0.16	-3.60	-0.11	2.54	-0.27
48	OK12D-Blgs/Dst-DH134	-5.87	0.12	-2.08	0.05	3.79	-0.08
54	OK12D-Blgs/Dst-DH143	-4.32	0.09	-8.35	-0.43	-4.03	-0.53
55	OK12D-Blgs/Dst-DH145	-5.01	0.18	-8.27	-0.11	-3.26	-0.29
58	OK12D-Blgs/Dst-DH169	-5.48	0.23	-6.48	-0.05	-0.99	-0.28
60	OK12D-Blgs/Dst-DH173	-2.42	0.32	-3.10	-0.02	-0.68	-0.34
61	OK12D-Blgs/Dst-DH175	-5.86	0.31	-4.49	0.34	1.38	0.03
65	OK12D-Blgs/Dst-DH182	-7.74	-0.12	-5.16	-0.16	2.58	-0.04
67	OK12D-Blgs/Dst-DH186	-4.78	0.29	-4.25	0.31	0.53	0.02
68	OK12D-Blgs/Dst-DH187	-6.87	0.07	-6.07	-0.01	0.80	-0.08
73	OK12D-Blgs/Dst-DH210	-9.88	0.14	-7.88	0.00	2.01	-0.14
80	OK12D-Blgs/Dst-DH228	-6.62	0.05	-3.40	0.15	3.22	0.10
83	OK12D-Blgs/Dst-DH236	-5.25	0.24	-7.50	-0.03	-2.25	-0.27
86	OK12D-Blgs/Dst-DH243	-5.60	0.06	-8.74	-0.20	-3.14	-0.26
87	OK12D-Blgs/Dst-DH248	-3.57	0.57	-3.18	0.33	0.38	-0.24
90	OK12D-Blgs/Dst-DH257	-5.10	0.13	-10.25	-0.35	-5.15	-0.48
92	OK12D-Blgs/Dst-DH261	-2.47	0.64	-0.92	0.63	1.55	-0.01
95	OK12D-Blgs/Dst-DH266	-5.75	0.36	-3.63	0.23	2.12	-0.12
97	OK12D-Blgs/Dst-DH269	-4.81	0.17	-3.88	0.20	0.93	0.03

Table 3: Pearson's correlation coefficients between different physiological parameters and yield parameters where, the prefixes L- and S- stand for leaf and spike respectively and the prefixes A- and B- stand for spikes that underwent anthesis after and before stress introduction respectively.

	Total BM	No. of spikes	B-Total spike wt.	B- Total stem wt.	B- Avg unit spike wt	A-Total spike wt.	A-Total stem wt.	A- Avg unit spike wt
L-Pn	-0.22	-0.22	-0.16	-0.20	0.16	0.15	-0.19	0.18
L-gs	-0.26*	-0.18	-0.16	-0.17	0.05	0.16	-0.29*	0.22
L-Ci	-0.11	-0.05	-0.08	-0.02	-0.12	0.07	-0.14	0.13
L-Fv'/Fm'	0.12	0.27*	0.23	0.07	-0.03	0.06	-0.05	0.01
L-ETR	-0.07	-0.28*	-0.18	-0.17	0.22	0.28*	-0.01	0.24
L-Trmmol	-0.21	-0.13	-0.10	-0.15	0.07	0.15	-0.27*	0.19
S-Pn	0.00	0.14	0.31*	-0.16	0.40***	0.11	-0.29*	0.19
S-gs	0.02	0.14	0.35**	-0.11	0.47***	0.17	-0.32*	0.16
S-Ci	0.00	0.01	0.14	0.13	0.32**	0.13	-0.15	-0.05
S-Trmmol	0.01	0.12	0.34**	-0.17	0.53***	0.17	-0.33**	0.19
L-IWUE	0.06	-0.03	-0.01	0.02	0.08	-0.06	0.14	-0.08
S-IWUE	-0.02	0.08	0.08	-0.17	-0.01	-0.07	-0.07	0.12

*Significant at $\alpha = 0.05$, **Significant at $\alpha = 0.01$, ***Significant at $\alpha = 0.001$.

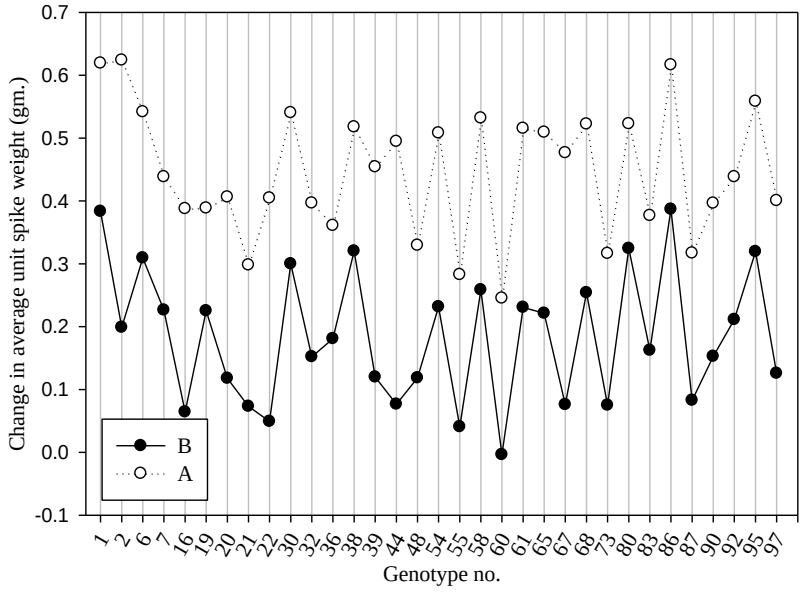

Fig.1 (a)

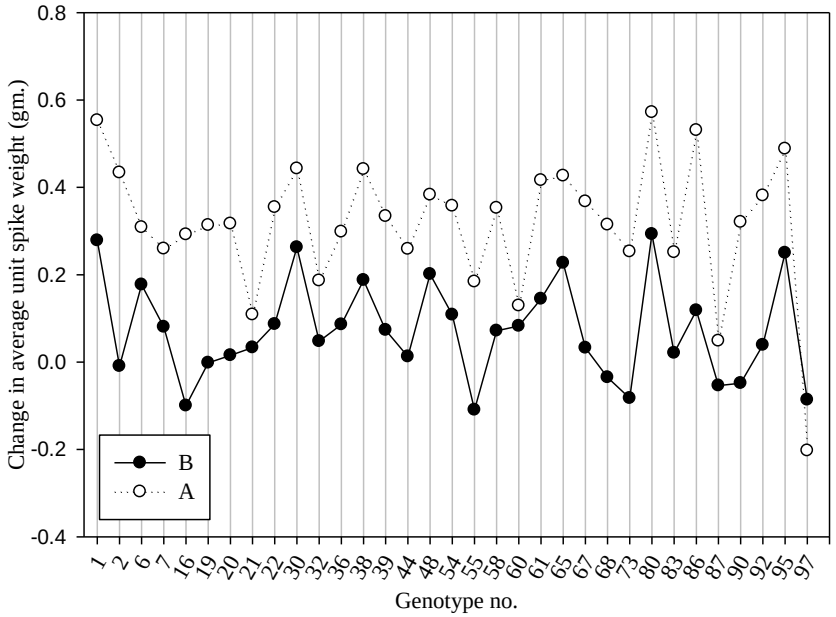

Fig. 1(b)

Figure 1: (a) Change in average unit spike weight between control and high temperature irrigated treatment and (b) Change in average unit spike weight between control and high temperature drought treatment, where B = difference between the spikes that underwent anthesis before treatment introduction and A = difference between the spikes that underwent anthesis after treatment introduction.

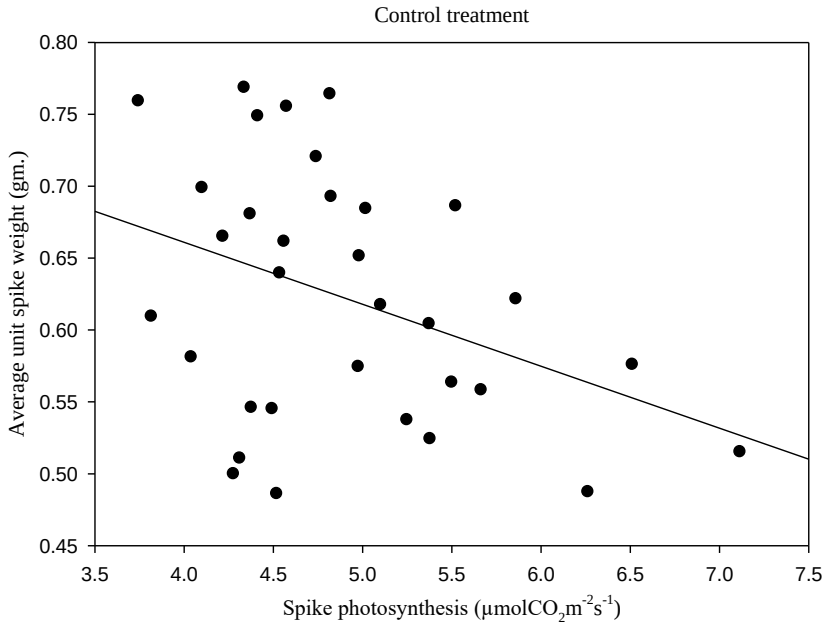
Control treatment
Average unit spike weight (gm.)
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
Spike photosynthesis (µmolCO2m-2s-1)

Fig. 2(a)

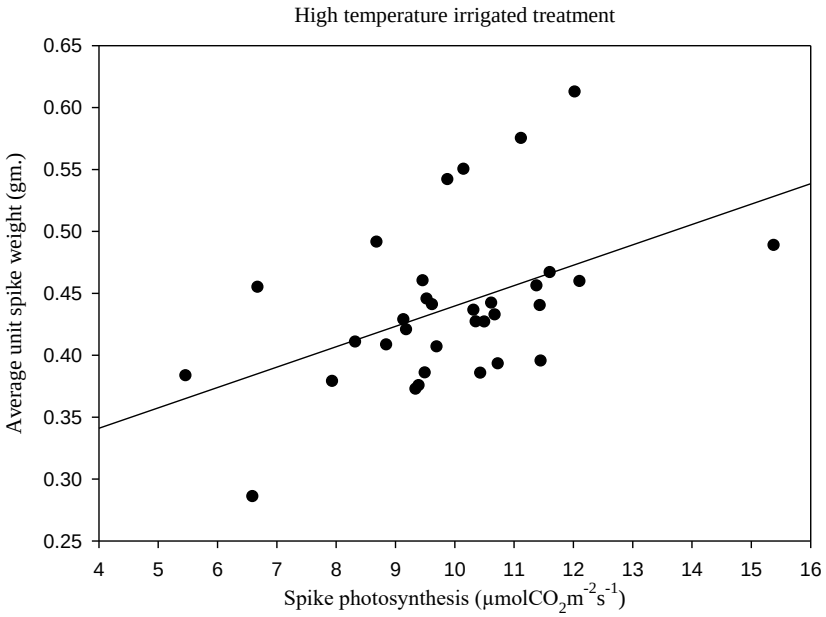
High temperature irrigated treatment
Average unit spike weight (gm.)
0.65
0.60
0.55
0.50
0.45
0.40
0.35
0.30
0.25
4
5
6
7
8
9
10
11
12
13
14
15
16
Spike photosynthesis (µmolCO2m-2s-1)

Fig. 2 (b)

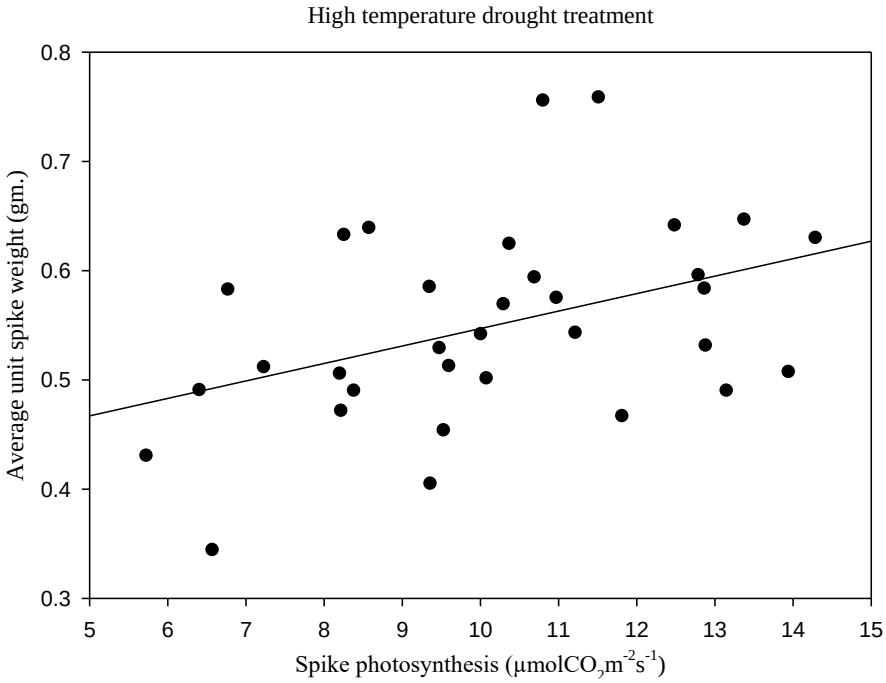

Fig. 2 (c)

Figure 2: Relationship between average unit spike weight and spike photosynthesis under (a) control condition, (b) high temperature irrigated condition and (c) high temperature drought

CHAPTER VI

GENERAL CONCLUSIONS

The Buster DH lines developed by Wheat Improvement Team (WIT) at Oklahoma State University (OSU) are a unique resource for wheat variety development for Oklahoma. The DH lines, although being developed from the same parents and fairly homogenous, were considerably different from each other and showed different responses under different stress conditions.

Under normal greenhouse conditions and vegetative growth stage, the 100 Buster lines were different for the morphological attributes such as plant height and tiller number but were not significantly different for photosynthetic pigments concentrations. The shortest Buster line was 'DH231'; the parental line 'Duster' was shorter than most of the DH progenies while 'Billings' had the maximum height amongst all. The smallest leaf area was observed in 'Duster' and maximum leaf area in 'DH73'. The number of tillers was highest in 'DH136' and was lowest in 'DH224'. When grown in the growth chambers, these 100 Buster lines showed significant difference among themselves and in interaction with the heat stress. The gas exchange processes were accelerated under heat because sufficient water was supplied, but the IWUE of plants decreased due to increase in transpiration. The genotype 'DH263' was least affected by the heat stress in terms of IWUE. However, the morphological traits measured under greenhouse conditions (Chapter II) did not seem to correlate with the gas exchange parameters under growth chamber conditions (Chapter III).

In the greenhouse under simulated drought, the 33 Buster lines did not show significant differences for the average spike weight. Nonetheless, there were differences in carbohydrate mobilization and spike photosynthesis inferred by the defoliation treatments. The Buster line 'DH236' was superior based on both carbohydrate remobilization and spike photosynthesis. In addition, the partial defoliation treatment with only flag leaf yielded significantly higher spike weight under drought. Likewise, in the experiment in the growth chambers, the lines showed significant differences in gas exchange parameters of spike but did not show any significant differences in terms of spikes weight or leaf gas exchange parameters. The Buster lines 'DH248', 'DH210' and 'DH257' had the highest rate of spike photosynthesis under control, high temperature irrigated and high temperature drought conditions respectively. The spike photosynthesis correlated positively with average spike weight under stress conditions. Yet, the gas exchange parameters recorded under stress in vegetative crop growth stages (Chapter III) did not correlate with the gas exchange parameters under similar stress conditions in their reproductive stage (Chapter IV). A schematic diagram showing the four studies, traits evaluated, main results and Buster lines identified from each study is presented in Figure 1.

The Buster lines are significantly different among themselves for a number of characteristics under different conditions and accelerate breeding program for abiotic stress tolerance. The results of this experiment provide the information on performance of the selected Buster lines under various stress conditions such as heat, drought and heat plus drought, and under different growing conditions such as the greenhouse and the growth chambers. In conclusion, including the identified traits (plant height, tiller number, leaf size, IWUE, and spike photosynthesis) and better performing lines (DH lines 136, 210, 236, 248, 257 and 263) into future research and breeding will accelerate development of abiotic stress tolerance in wheat.

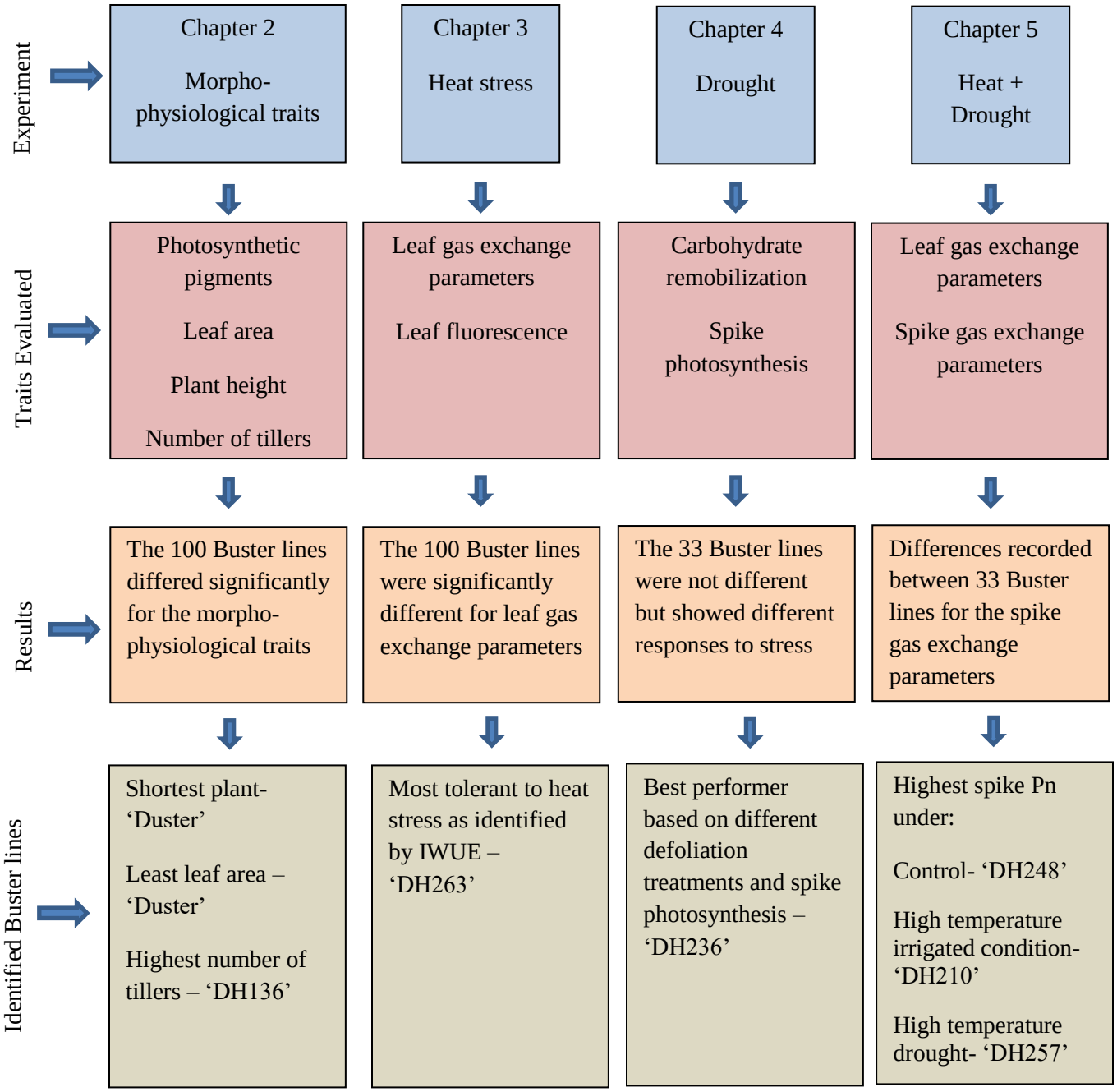

Figure 1: Schematic diagram summarizing the four studies, traits evaluated, results, and identified Buster lines from each study.

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