Advanced breeding methodologies for wheat improvement in Bangladesh

by

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B.Sc.Ag. (Hons.), Bangladesh Agricultural University, 2002
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AN ABSTRACT OF A DISSERTATION

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Abstract

Crop improvement is a central objective to address global food security of the increasing population. Breeders and geneticists around the world are trying to find out the best ways and means that can select the superior lines of any crops. A 2% genetic gain is needed to keep up with the increasing global population and increasing food demand. To accelerate rapid genetic gain conventional breeding methods of crop selection should be complemented with the advanced molecular selection methods that encompasses with the genotyping technology. Rapid advances in technology like next generation sequencing that resulted in many sequenced genomes and the ability to quickly genotype thousands of individuals are providing the datasets to match genotyping to phenotyping. Here, we will describe the advanced breeding methodologies that can be used to improve any crop with specific focus on wheat improvement for the heat stressed environments of Bangladesh. Advanced breeding methodologies includes – predicting yield with the secondary traits, genome wide association studies to identify the significant genomic region for a specific trait and using whole-genome prediction models to calculate the genomic estimated breeding values to make genomic selection.

There are two ways of predicting important traits of any crops – phenotypic prediction and genotypic prediction. Yield prediction is the final target of any breeding program but selection for yield is limited by the extent of field trials, fluctuating environments, and the time needed to obtain multi-year assessments. Proximal sensing data collection is increasingly implemented with high-throughput platforms that provide powerful and affordable information, while efficiently using this data is challenging. The objective of this study was to monitor wheat growth and predict grain yield in wheat breeding trials using high-density proximal sensing measurements under extreme terminal heat stress that is common in Bangladesh. We used several models and different secondary traits for this purpose. Our results showed that optimized phenotypic prediction models can leverage secondary traits to deliver accurate predictions of wheat grain yield, allowing breeding programs to make more robust and rapid selections.

A genome wide association study (GWAS) was conducted for grain yield, yield components and other secondary traits in elite spring wheat germplasm grown in natural heat stressed environment in Bangladesh to identify genomic regions that control component traits and contribute to yield potential. A total of 2682 unique advanced wheat lines from the CIMMYT bread wheat program were planted in cohorts of ~540 lines in each of the five wheat growing seasons with measurement of important traits including grain yield and yield component traits and proximal sensing data including normalized difference vegetation index (NDVI) and canopy temperature (CT). To understand the genetic architecture of these traits, genome-wide association study (GWAS) was conducted using 39,912 SNPs from genotyping-by-sequencing. GWAS result were insignificant and variable for CT and NDVI supporting a hypothesis of highly polygenic genetic architecture. In contrast, large effect loci associated with days to heading and days to maturity were found on chromosomes 5A, and 5B at the Vrn-A1 and Vrn-B1 loci and the frequency and impact of these alleles was observed to vary over successive cohorts. We were able to find significant association in chromosome 3B and 4A for grain yield that colocalized with loci identified for thousand grain weight. Overall, this study highlights the utility of secondary traits including sensor based NDVI and CT to identify chromosome regions that contribute to yield and stress tolerance in South Asian spring bread wheat and better understand the genetic architecture, particularly for heading date and maturity which are critical targets of selection to avoid extreme terminal heat stress.

By matching the dense genotyping data with the phenotyping data, we can successfully predict and select the best performed cultivar. Predicting crop performance and selecting them using genetic information is a major challenge for 21st century plant breeders. This is because a complex trait is controlled by thousands of genes and their interactions with the environment where the crops are grown. We have developed a genomic selection model for the heat stressed environment in South Asia. With the advanced wheat lines collected from CIMMYT, Mexico, a training population was created, and genomic selection was done for the breeding population. We found low to high prediction accuracy across the years and how to moderate prediction accuracy across trials. Days to heading and maturity showed the highest and consistent prediction accuracy while thousand grain weight and grains per spike had good predictability among the yield components. This genomic selection approach can be used in any unbalanced dataset that are common to any breeding program. It will ultimately accelerate the rate of crop improvement that is important to secure the global food security.

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Dedication

This work is dedicated to my parents – Mokshed and Khadiza, my sons – Zawad and Tahmid, and my wife – Rafeza for their unconditional support and enormous mental support during my stay at KSU in USA.

Preface

Wheat is the second staple grain crop and its demand and consumers are increasing day by day in Bangladesh. Wheat research in Bangladesh follows only conventional methodologies and the genetic gain is not up to the mark that is required to improve wheat for addressing the country's increasing population and growing demand. Conventional breeding should be complemented by the advanced molecular breeding that can reduce land, labor, and time and increase genetic gain. To address this issue, we have developed some advanced breeding methodologies including yield prediction, genome wide association study (GWAS), and genomic selection models those can be used by the researchers to improve wheat in Bangladesh. Breeders can use secondary trait measurements, obtained during the growing season, to increase selection accuracies prior to harvesting the plots and ensure that high yielding plots are harvested. This is of particular interest if these secondary traits can be measured on smaller plots at earlier generations in the breeding cycle enabling more intense selection prior to lines entering into replicated yield testing. We already have implemented these models along with high throughput phenotyping (HTP) to select the superior wheat lines from the advanced lines collected from CIMMYT, Mexico. Over the previous five years we selected several wheat lines and due to their superior performance than the national check variety they can be released as new variety. I think these advanced breeding methodologies can help to improve the wheat breeding research in Bangladesh.

Chapter 1 - Scope of Wheat Improvement in Bangladesh

Abstract

Wheat is the second most important cereal crop after rice in Bangladesh and its consumption has increased by more than 10% in recent years. The crop is subjected to various abiotic and biotic stresses with terminal heat stress having the greatest constraint. Recently the discovery of wheat blast, caused by *Magnaporthe oryzae pathotype Triticum*, poses significant additional challenges for wheat breeding and production. The Bangladesh Wheat and Maize Research Institute (BWMRI) is the organization in Bangladesh is tasked with wheat and maize improvement. Using traditional breeding methods, it usually takes 8 to 10 years to develop and an additional 3 to 5 years to release a new wheat variety. Classical breeding assisted with molecular breeding will accelerate the improvement. With the conjunction of low cost new high-throughput genotyping technologies and more routine statistical methods the genomic selection can greatly accelerate the breeding cycle and thus genetic gain beyond what is possible with phenotypic selection. Our objectives are - to harness genetic resources for wheat improvement through systematic characterization and use of genetic diversity to accelerate breeding gains to address climate change issues, - develop high-throughput phenotyping system and genomic selection models to identify useful alleles/traits for stress tolerance and rapidly make predictions and selections of the most promising candidate wheat varieties. In this context, breeding heat and drought tolerant cultivars, and developing new technologies comparable with those in more advanced countries in collaboration with various national and international organizations like CIMMYT, ICARDA, ICRISAT, CIDA, AusAID, USAID are most important.

Introduction

During liberation war the production of number one cereal crop, rice in Bangladesh decreased due to a series of disasters. Then people came to realize that the rice couldn't alone mitigate the food requirement of the country (Bangladpedia, 2006). The country received a lot of wheat grain as food relief from international communities. In course of time, Bangladesh became highly dependent on wheat imports while dietary preferences were changing such that wheat was becoming a highly desirable food supplement to rice. Now wheat is the second most important cereal crop in Bangladesh and its consumption increased by 13% annually due to changes in dietary habit, socioeconomic upliftment, and increasing per capita income (Hossain, Islam, and Islam 2020). The country's wheat imports constitute 70% of total wheat consumption. Moreover, demand for wheat across the developing world is projected to increase 60 % by 2050. Recently, the production cost of wheat became lower compared to irrigation dependent boro rice and the market price of wheat is higher than rice. There had been recorded a depletion of ground water table for irrigation in boro rice cultivation in the barind region in the country. Moreover, government took initiative to purchase wheat with some added incentives. All these might rejuvenate the farmers' waning interests to grow wheat again in their field.

Scope and challenges of increasing wheat production in Bangladesh

There are two ways of wheat expansion in Bangladesh. A vertical expansion – increasing wheat productivity by minimizing yield gap through adoption of new wheat varieties and other technologies and a horizontal Expansion – Southern belt (1 million ha) can be brought under wheat cultivation. Charlands in different districts are also very much suitable for growing wheat (0.8 m ha.). Encouraging wheat instead of growing Boro rice in high land and sandy soils in north-western districts and Barind areas can be brought under wheat by 1-2 light irrigations (75,000 ha). Greater Sylhet can be brought under wheat by improving irrigation facility.

The abiotic constraints of wheat production here are heat especially terminal heat stress (global warming), moisture stress (drought), salinity, sterility (B deficiency), and soil acidity (low pH). The most important stress is the heat stress. CIMMYT and ICARDA (2011) estimated that 20–30 % wheat yield losses would occur by 2050 in developing countries as a result of a predicted temperature increase of 2–3 °C. The Geophysical Fluid Dynamics Laboratory transient model (Manabe et al. 1991) projected that, in Bangladesh, temperatures would rise 1.3 °C by 2030 and 2.6 °C by 2070, compared with mid-20th-century levels. The annual mean temperature of Bangladesh is 25.75 °C and is expected to rise by 0.21 °C by 2050 (Karmakar 2000). By 2050, rice yield could drop by 8 % and wheat yields by 32 % (Action 2010). With a change in average temperature of 2 – 4 ^{0}C over 34/16^{0}C day/night temperature, the prospect of growing wheat and potato would be severely impaired and production loss may exceed 60 % of the achievable yields (Karim 1993). The major stress faced by wheat in South Asia is high temperature, mainly terminal heat stress (Joshi et al. 2007), which was defined by (Fischer and Byerlee 1991) due to mean daily temperature above 17.5 °C in the coolest month. In Bangladesh, mean temperature in winter (rabi) has risen by 0.66 °C since 1990 and a further warming of 2.13°C by 2050 is predicted (Rawson 2011).

The biotic constraints are Bipolaris leaf blight (BpLB) caused by Bipolaris sorokiniana (Sacc.in Sorok.) Shoemaker, leaf rust caused by Puccinia triticina Eriks. (= Puccinia recondite f. sp. Tritici), black point caused by B. sorokiniana, Alternaria alternata, etc., head blight caused by B. sorokiniana. Among them Bipolaris leaf blight is the most important. A rise in temperature can be expected to increase the severity of Bipolaris leaf blight and other wheat diseases in the future because a warm and humid climate favors the development and spread of the pathogen. Unfortunately, wheat blast, a devastating wheat disease caused by the Triticum pathotype of *Magnaporthe oryzae* B.C. couch (Synonym *Pyricularia oryzae* Cavara) emerged for the first time in 2016 in several south-western and southern districts of Bangladesh. Since then, it is being a focus and concern in the international scientist community.

History of wheat research in Bangladesh

The research on wheat crop was initiated by the first testing of two Mexican varieties ('Sonora 64' and 'Penjamo 62') in the northern part of Bangladesh in 1965 (BARI, 2010). Their spectacular performance encouraged scientists to introduce wheat more generally to this part of the country. Since then, there were so many ups and downs in the acreage and production of wheat crop in Bangladesh. It was recorded that wheat production reached more than 1 million ton per year in the first half of eighties (BARI 2010). This was possible due to the release of 'Sonalika' in 1972 that created a true breakthrough in wheat production. It was an early maturing high yielding widely adapted wheat variety. It became a mega variety in the early eighties (WRC 2009). Another breakthrough was recorded when Kanchan was released along with other three varieties viz. Ananda, Barkat, and Akbar in 1983 by the scientists of WRC, BARI. These four varieties were higher yielding (yield 2–3 tons/ha) than Sonalika. Gradually wheat variety Sonalika was replaced by the new variety Kanchan, which became the predominant variety in Bangladesh by the early 90s. The highest production of wheat was recorded at 19.08 lakh tons in fiscal year 1998-99. In the meantime, Kanchan became susceptible to Bipolaris leaf blight and for this disease yield of Kanchan was dramatically decreased. Later the acreage and production of wheat decreased due to the competition with other winter crops like maize, potato, and winter vegetables that promised higher profits. In 2006 the lowest wheat production was recorded. The lower acreage, which is due to crop competition, lack of stress tolerant wheat varieties, etc. is one of the most important causes of its low production. The acreage of wheat was the lowest in 2011-12 fiscal year. Then total area increased by 1.83% from 436814 ha in 2014-15 to 44805 in 2015-16. But the yield decreased by 1.78% due to blast disease. Currently, Bangladesh only produces one fifth of its annual wheat demand.

Current wheat breeding strategy in Bangladesh

Bangladesh Wheat and Maize Research Institute (BWMRI) is the only organization solely assigned to research on the development and improvement of wheat and maize crops. BWMRI has develops all the wheat varieties that are being cultivated by the farmers throughout the country. Scientists of BWMRI are continuing their research to develop biotic and abiotic stress tolerant wheat varieties. The institute has released so far 36 wheat varieties.

The main objective of variety improvement is to develop high yielding wheat varieties with a wide range of adaptability with a view to enhance wheat productivity in Bangladesh. Development of heat tolerant varieties has been given the highest research priority under the context of global climate change. Due emphasis has also been given to develop varieties against other abiotic stresses like drought, salinity, Boron-deficiency etc. Genetic improvement through incorporating stress adaptive traits into good agronomic background is being duly emphasized in the variety development program. In addition, research thrust has been put forwarded towards developing varieties for improved bread making quality. Efficient deployment of resistance genes into the genotypes with good agronomic background for the major diseases like bipolaris leaf blight, leaf rust, stem rust, etc. is also considered as a priority area. The performance of newly developed wheat lines from national and international sources specially from CIMMYT is being evaluated under different growing environments across the country and promising lines superior to the standard check varieties are selected.

Shuttle breeding with Kenya & Ethiopia

Every year some selected lines were sent to Kenya for screening against stem rust (Ug99 race). The BWMRI maintains a unique crossing block having germplasm from diverse sources and those are utilized for hybridization. Strategic crosses are made based on the pyramiding yield potential, disease resistance, physiological traits conferring tolerance to biotic and abiotic stresses, etc. in the agronomical superior adapted varieties. The crossing block materials are consisted of high yielding commercial varieties, early maturing varieties/lines, BpLB tolerant varieties/lines, rust resistant including Ug99 tolerant varieties/lines, short height varieties/lines, high biomass and high harvest index varieties/lines, genotypes with more grains/spike, genotypes with excellent grain filling under late planting condition, genotypes with good bread making quality, sterility tolerant genotypes, etc. Single cross, three-way cross or top cross, and limited-backcross strategies are followed for developing breeding populations. Crosses are then evaluated in respect of their female parent and superior crosses are selected to proceed through segregating generations. Segregating generations are advanced following selected bulk method. In the segregating generations selections are made based on good vigor, earliness, medium height, disease and sterility tolerance and resistance, etc. The F_6 nursery is planted as plant to plot. Yield of the F_6 plots are not usually recorded. Genotypes are selected on the basis of visual agronomic characteristics like homogeneity in plant height, good arrangement of spikelets on the spike, short plant height, early maturing, disease resistant/tolerant, visual seed quality viz. white seed color, bold grain, smoothly filled grain, etc. Every year new lines are being added in the nurseries/trial for performance evaluation.

The selected genotypes go directly to the national wheat-screening nursery called Bangladesh wheat screening nursery (BWSN). These nurseries are evaluated under irrigated time sown (ITS) and irrigated late sown (ILS) conditions. Selected genotypes from this nursery are evaluated in preliminary yield trial (PYT) and then in advance yield trial (AYT) both in ITS and ILS conditions. The most promising lines are then evaluated in farmers field as participatory variety selection (PVS) and then the selected lines from PVS are advanced to adaptive trial, which is tested on farmers' field and on research station. Personnel from national seed board then come to evaluate the lines in adaptive trial and finally the national technical committee approves the variety. Wheat genotypes those are not recommended as varieties are kept in germplasm maintenance nursery and there is a scope to initiate a program for core collection that can help future breeders to develop better variety in future. Normally it takes 12 to 15 years to develop a new wheat variety with this breeding method.

Future strategy of wheat breeding in Bangladesh

Breeding efforts to develop high-yielding varieties still continued with the classical methods but the breeders should be evaluating new breeding methodologies to initiate programs for addressing the future global warming. Most of the wheat varieties grown Bangladesh are sensitive to high temperature, and yield safety is in jeopardy because of the forecast climatic changes. Drought and high temperature are key stress factors with high potential for impacting negatively on crop yield. Yield safety can only be improved if future breeding is based on new knowledge concerning plant development and its responses to stress, for example by enabling the development of crop plants with improved thermo-tolerance using various genetic approaches (Wahid et al., 2007). A thorough understanding of physiological responses of plants to high temperature, mechanisms of heat tolerance and possible strategies for improving crop thermo-tolerance will be needed for this. In this context, breeding heat and drought tolerant cultivars, and developing new technologies comparable with those in more advanced countries in collaboration with various national and international organizations (i.e. CIMMYT, ICARDA, ICRISAT, CIDA, AusAID, USAID) are most important. So, the conventional breeding should be complemented with the advanced breeding methodologies like yield prediction, high throughput phenotyping, GWAS, and genomic selection. By implementing these advanced breeding methodologies, desired increase in genetic gain will be possible through which food and nutritional security in Bangladesh will be ensured.

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Chapter 2 - Improving Wheat Yield Prediction Using Secondary Traits and High-Density Phenotyping under Heat Stressed Environments

Abbreviations

BLUE, Best Linear Unbiased Estimator; NDVI, Normalized Difference Vegetation Index; CT, Canopy Temperature; DTHD, Days to heading; DAYSMT, Days to maturity; GRNSPK, Grains per spike; GRYLD, Grain yield; HELSPSEV, *Helminthosporium* severity; PH, Plant height; SN, Number of spikes per square meter; SPKLNG, Spike length; SPLN, Number of spikelets per spike. Z

Abstract

A primary selection target for wheat (*Triticum aestivum*) improvement is grain yield. However, selection for yield is limited by the extent of field trials, fluctuating environments, and the time needed to obtain multi-year assessments. Secondary traits such as spectral reflectance and canopy temperature (CT), which can be rapidly measured many times throughout the growing season, are frequently correlated to grain yield and could be used for indirect selection in large populations particularly in earlier generations in the breeding cycle prior to replicated yield testing. While proximal sensing data collection is increasingly implemented with high-throughput platforms that provide powerful and affordable information, efficiently using this data is challenging. The objective of this study was to monitor wheat growth and predict grain yield in wheat breeding trials using high-density proximal sensing measurements under extreme terminal heat stress that is common in Bangladesh. Over five growing seasons, we analyzed normalized difference vegetation index and CT measurements collected in elite breeding lines

from the International Maize and Wheat Improvement Center at the Regional Agricultural Research Station, Jamalpur, Bangladesh. We explored several variable reduction and regularization techniques followed by using the combined secondary traits to predict grain yield. Prediction accuracy was calculated via a cross-fold validation approach as the correlation between observed and predicted grain yield using univariate and multivariate models. We found that multivariate models resulted in higher prediction accuracies for grain yield than the univariate models. Stepwise regression performed equal to, or better than, other models in predicting grain yield. When incorporating all secondary traits into the models, we obtained high prediction accuracies of 0.58 to 0.68 across the five growing seasons. Our results show that optimized phenotypic prediction models can leverage secondary traits to deliver accurate predictions of wheat grain yield, allowing breeding programs to make more robust and rapid selections.

Background

Wheat is one of the most important cereal crops in the world and a staple for human consumption. It accounts for 26% of world cereal production and 44% of total cereal consumption (McGuire, 2015). Rapid economic and income growth, urbanization, and globalization are leading to dramatic dietary shifts, especially in Asia as consumers are increasing their consumption of wheat products (Pingali, 2007). Wheat production needs to increase to meet the combined growing population and expanding demand by the middle of this century (Tilman et al., 2011). Currently, wheat yield gains are estimated to be 0.9% per year, much less than the 1.5% per year that is required to meet the projected 60% increase in global production needed by 2050 (Reserach Program on Wheat, 2016). At the current rate, global production of wheat may only increase by 38%, which is far short of the projected demand.

Additionally, the effect of climate change, including less favorable growing conditions, may even further reduce wheat production (Gammans et al., 2017). Up to 6% yield declines are projected in wheat for each degree temperature increase if adaptive measures such as improved germplasm are not realized (Zhao et al., 2017). Given these challenges, wheat yield is increasing far less than what is needed to meet future demand.

While wheat is globally distributed and faces a variety of biotic and abiotic challenges, in South Asia, heat is the most important stress and critical yield limitation. Terminal heat stress is also a common problem in temperate regions where 40% of world wheat is produced. In these areas temperature ranges from 32 - 38°C can cause up to a 50% grain yield reduction (Asseng et al., 2011). Heat stress is a regulated physiological process that can affect a range of plant phenotypes including canopy temperature (Ayeneh et al., 2002). Fundamental research has shown that this response is highly complex and differs at the tissue (Thomason et al., 2018), species (Kotak et al., 2007), and developmental stage (Tricker et al., 2018) suggesting that heat tolerance is a physiologically and genetically complex trait.

In wheat, temperatures above the optimum level are deleterious and cause irreversible damage, with the duration and magnitude of temperature exposure determining the severity of yield loss. In controlled studies with supra-optimal temperatures, a 3-5% yield loss for every 1°C increase of mean temperature above 15°C has been observed (Gibson and Paulsen, 1999). In addition to reducing grain yield, high temperatures can reduce individual grain mass by up to 23% (Stone and Nicolas, 1994) further impairing grain yield and quality (Teixeira et al., 2013). Many of the global wheat production areas already have supraoptimal temperature conditions, and global temperatures are predicted to further increase between 1.7°C to 4.8°C by the end of the century (Pachauri et al., 2014). Thus, increasing grain yield under heat stress is a major global objective, and more efficient breeding methods and technology are needed to increase the rate of genetic gain in heat stressed environments.

The complexity of heat stress means that breeding programs cannot use a single strategy to improve heat tolerance. Some plant adaption mechanisms to avoid and minimize heat stress include early flowering (Aiqing et al., 2018; Ishimaru et al., 2010) and stomatal closure (Liu et al., 2018). The difference of expression of these traits provides an opportunity to improve wheat if this beneficial genetic variation can be accurately measured. Traditionally, before the implementation of molecular markers, plant breeders selected promising lines only on the basis of phenotype. By generating large numbers of crosses and evaluating successive generations in a wide range of environments superior individuals could be identified. While great improvements have been made in this fashion, as the number of lines to evaluate increase, breeders are faced with the challenge of precisely phenotyping large populations within a short time to identify the most promising candidates.

With the advent of low cost, high-throughput genotyping technologies, breeders have access to high-density genomic data (Morrell et al., 2012). While molecular markers have aided in breeding objectives (Bernardo, 2008) breeding programs continue to face a combined challenge of characterizing breeding lines precisely and rapidly (McMullen et al., 2009; Araus and Cairns, 2014). Unraveling complex traits, such as heat stress, requires precise and accurate phenotypic data to connect the phenotype to genotypic data (Cobb et al., 2013). Phenotyping is now considered the bottleneck of crop improvement, yet it is crucial to fully realize the benefits of plant breeding (Araus and Cairns, 2014).

Increasing grain yield, especially under extreme terminal heat stress is a primary goal of the national breeding program in Bangladesh. While grain yield is the primary trait of interest, it can be estimated using remote or proximal sensing data (Lillesand et al., 2014). Any trait which is correlated with the primary trait can be considered as secondary trait in selection and can potentially be used to reduce evaluation time and cost (Rutkoski et al., 2016). If the secondary traits can be accurately phenotyped within the breeding program, these secondary traits can be used to predict the primary trait and improve genetic gain particularly earlier in the breeding cycle before advancement to replicated yield trials. Two potential secondary traits that are amendable to high-throughput measurements include spectral reflectance and canopy temperature (CT) (Pask et al. 2012).

Remote sensing of spectral reflectance is based on the ability to measure the electromagnetic reflectance of plants. Plants' cells and tissues have wavelength specific absorbance and reflectance properties which make spectral reflectance a trait that can be rapidly and quantitatively measured (Montesinos-López et al., 2017). Remote sensing has been widely used in agriculture with different vegetation indices providing a non-destructive, real-time measure of crop growth. Normalized Difference Vegetation Index (NDVI) is one of the most commonly used vegetation indices based on the reflectance of red and near-infrared light. It can be used to characterize crop growth stages, to evaluate crop density, and to predict crop yield (Rutkoski et al., 2016). In crops, including maize, wheat, sorghum, and barley, scientists have identified significant correlations between biomass and NDVI with some correlation coefficients above 0.70 (Chen et al., 2011). Values of NDVI, especially two to three weeks before and after heading have been found to be highly correlated with grain yield in wheat (Babar et al., 2006).

Another trait that can be used to evaluate crop status is CT. Crop CT is the surface temperature of the plant canopy and is related to the amount of transpiration that results in evaporative cooling. CT plays an important role in the observation of the crop water relationship which is a factor of crop yield, and CT has been shown to have potential for selecting heat and drought tolerant genotypes in stressed environments (Reynolds et al., 2009). Several factors including root length and biomass, stomatal conductance, number of stomata, metabolic activities, and photosynthate translocation are the important biological factors that result in variation in CT in different genotypes (Reynolds et al., 2012). (Mason et al., 2013) suggested that CT is a complex trait controlled by loci of small effects with most of the loci having pleotropic effects on traits like plant height and days to heading. Even though the exact mechanism of CT difference is unresolved, research has shown that the correlation between CT and grain yield in wheat is generally negative under heat stress environments providing selection strategies to identify heat tolerant lines (Amani et al., 1996; Gutierrez et al., 2010; Mason and Singh, 2014).

While CT can be easily measured using handheld infrared radiometers (Pask et al., 2012) and often has moderate heritability (Lopes et al., 2012), the application CT in breeding has been limited because some of the inconsistent nature of the CT measurements. Canopy temperature is impacted by a variety of environmental factors including solar radiation intensity, atmospheric temperature, humidity, soil moisture, and wind speed which can quickly change throughout the day (Reynolds et al., 2012). The complexities of CT measurements suggest it is important to determine how to effectively use CT to select the better yielding lines in large wheat breeding programs under heat stressed environments.

Both CT and NDVI can be measured multiple times throughout the growing season which gives a powerful approach to capture the temporal dynamics of the growing crop. Using just a single measurement to evaluate the lines in a breeding program neglects the temporal dynamics of plant growth and development (Crain et al., 2018). Incorporating a combination of multiple variables that show strong correlation between secondary traits and the primary trait can be used to develop precise inferences about crop phenotypes like grain yield prediction using secondary traits (Guo et al., 2014). While NDVI and CT have been advocated for plant selection, little work has been done on incorporating multiple measurements into selection decisions.

As precision phenotyping becomes more routine in breeding programs, new challenges include how to best utilize and translate this data into improved prediction models and selection strategies (Tester and Langridge, 2010). Our research objective was to evaluate how dense, temporal phenotypic measurements from proximal sensing of NDVI and CT as well as other agronomic traits could be used within the national plant breeding of Bangladesh programs to assess line performance in heat-stressed environments. Additionally, an emphasis was placed on statistical modeling that could account for highly correlated measurements of secondary traits.

Materials and Methods

Experimental design and field management

During the 2015-16, 2016-17, 2017-18, 2018-19, and 2019-20 growing seasons, different sets of 540 advanced lines from International Maize and Wheat Improvement Center (CIMMYT) were evaluated in Bangladesh. Each year, different sets of 540 lines from CIMMYT were evaluated as new heat tolerant material became available, and in addition to lines from CIMMYT there were seven different local checks including BARI Gom 26, or BARI Gom 30 which served as the benchmark check variety of Bangladesh.

All lines were evaluated in a high heat stress environment at the Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute (BARI), Jamalpur, Bangladesh (N 24.93, E 89.93, 23 masl). The climate of this region is hot and humid leading to overall heat-stressed environment, classified as ME5A according to the CIMMYT wheat mega-environment classification system (Rajaram et al., 1993).

To manage spatial variability, the lines were placed in multiple trials each growing season. Each trial consisted of 60 entries including 54 breeding lines and the six check varieties in two replications. Complete trials were planted within a given day each year with planting dates for each season of December 4-8, 2015; November 25-28, 2016; November 29-30, 2017; November 28, 2018; and December 05, 2019. The trials were arranged in an alpha-lattice design each with two replications for a total of 120 plots in each trial. Each replication was composed of 12 blocks with five entries randomly assigned to each block. The plots were composed of six rows of 4.17 m length and 20 cm row spacing for a total experimental plot size of 5 m^2. Plots were separated by a 40 cm alley. The 2015-16 season had a total of 10 trials. Subsequent years had a total of 11 trials, with the 11th trial representing second year testing of highest performing lines from the previous season

The Bangladesh Wheat Research Center's recommended agronomic practices were followed during the growing season. Fertilizer application consisted of 100:26:50:20:5:1 kg/ha of N:P:K:S:Zn:B respectively each year. Irrigation was applied as needed to prevent water deficit. In the 2015-16 growing season three irrigations were applied at tillering, heading, and grain filling, while from 2016-17 to 2019-20 two irrigations were applied at tillering and booting (Zadoks et al., 1974). Manual weeding was completed every season to keep the plots weed free. No pesticides were applied during the growing seasons.

Traits measurements

We considered grain yield as primary trait, CT and NDVI as sensor based secondary traits and all other traits as agronomic traits. Total grain of each of the plot was harvested, dried, weighed and then divided by the plot size (5 m^2) to get yield (kg / m^2) and then converted into metric tons per hectare. Throughout the growing season phenotypic data was recorded for agronomic traits including ground coverage (GrndCov), days to heading (DTHD), days to maturity (DAYSMT), plant height (PH), grains per spike (GRNSPK), severity of Helminthosporium leaf blight disease (HELSPSEV), number of spikes per unit area (SN), spikelets per spike (SPLN), spike length (SPKLNG), and thousand grain weight (TGW). Days to heading was recorded as the number of days to when 50% of total plants in a plot had extended spike from the leaf sheath. Days to maturity was recorded when 80% of the plants in a plot had peduncles that had turned from green to golden. Plant height was measured as the length from ground level to the apex of the spike excluding awn. The number of total heads per square meter (spike number; SN) was assessed by measuring the number of spikes counted from 3.5 meter long 20 cm spacing (0.7 m2) and converted the number into per square meter. Spike length on a representative spike within the plot was measured as the length from the base to the tip of a spike excluding awn.

The secondary traits of CT and NDVI data were collected 8, 14, 12, 13, and 15 different times during the 2015-16, 2016-17, 2017-18, 2018-19, and 2019-20 growing seasons, respectively. The measurements represented plant growth from tillering through senescence (Zadoks et al., 1974) with measurements taken between 11 am to 2 pm corresponding to solar noon on each day of observation. Canopy temperature was measured using a handheld infrared thermometer (IRT) (Apogee, Logan, UT, United States of America), that provided a high accuracy, non-contact surface temperature measurement from -30 °C to 65 °C with a precision of ±0.124 °C. IRT readings were taken at a 30° angle from the horizon for measurement and 70 cm above the crop canopy (Pask et al., 2012). The IRT functions at 0.6 hertz, but only the average canopy temperature was recorded for each measurement. Normalized difference vegetation index was collected using a GreenSeeker handheld sensor (Trimble Inc. Sunnyvale, CA, United States of America). The GreenSeeker was used by passing the sensor 75 cm over the crop canopy. Two-person teams were employed for CT and NDVI collection, with one person operating the instrument and the other person recording the data. It took approximately 3 hours with two teams (four people) to measure CT and NDVI of all plots. The data were recorded in the Field Book program (Rife and Poland, 2014).

Data analysis

All analysis were completed in R software (Team, 2017) using several packages including lme4 (Bates et al., 2015), leaps (Lumley, 2017), tidyverse (Wickham et al., 2019), glmnet (Friedman et al., 2001), plyr (Wickham, 2011), ggplot2 (Wickham, 2016), caret (Williams et al., 2018), PerformanceAnalytics (Peterson et al., 2014), and readr (Wickham et al., 2017).

Statistical analysis

A mixed model to account for the trial design was used to obtain the best linear unbiased estimators (BLUEs) for each genotype using the following model fit separately for each trial:

Equation 2.1. A mixed effects model for BLUEs

$$y_{ij} = \mu + g_i + r_j + b_{l(j)} + \varepsilon_{ij}$$

where, y_{ij} is the observed phenotypic response variable (GRYLD, CT, …, NDVI) for the i^{th} genotype, j^{th} replicate, μ is the overall mean of the individual trial, g_i is the fixed effect of i^{th} genotype (line) with i taking the values 1-60, r_j is the random effect of j^{th} replicate with j corresponding to 1 or 2 with a normal distribution $N(0,\sigma^2_r)$, b_l is the random effect of lth block, nested within replicate j, where l ranges from 1-12 distributed as $N(0,\sigma^2_l)$, and ε_{ij} is the residual effect for genotype *i* in replicate *j* with normal distribution $N(0,\sigma^2_e)$. Best linear unbiased estimators (BLUEs) were calculated for each site year individually.

To estimate heritability for each trial, a random term for genotype was used in (Equation 2.2), resulting in variance components to calculate the broad-sense heritability. Heritability was estimated using the following formula (Holland et al., 2003):

Equation 2.2. An equation for heritability

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_e^2}{r}}$$

Where σ_g^2 is genotypic variance and σ_e^2 is residual model variance and r is number of replications which is two. Heritability estimates were calculated for all agronomic traits during the growing season and for each of the time-points of NDVI and CT observations. In addition to calculating heritability on a trial basis, we estimated BLUPs and variance components across the full experiment each year for each trait using the following model:

Equation 2.3. A mixed effects model for BLUPs

$$y_{ijk} = \mu + t_k + g_{i(k)} + r_{j(k)} + b_{l(ij)} + \varepsilon_{ijk}$$

where, y_{ijk} is the phenotype of the trait of interest for i^{th} genotype, j^{th} replicate, k^{th} trial, μ is the overall mean of the population, t_l is the random effect of the l^{th} trial with a normal distribution N(0, σ^2_l), g_i is the random effect of i^{th} genotype (line) nested within trial with *i* taking the values 1-60 with a normal distribution $N(0,\sigma^2_i)$, r_j is the random effect of j^{th} replicate nested within trial with *j* corresponding to 1 or 2 with a normal distribution $N(0,\sigma^2_j)$, b_l is the random effect of l^{th} block, nested within trial *i* and replicate *j*, with *l* ranges from 1 to 12 distributed as N(0, ,σ^2_l), and ε_{ijk} is the residual effect for the i^{th} genotype j^{th} replicate in the k^{th} trial with normal distribution $N(0,\sigma^2_e)$.

Canopy Temperature Depression (CTD) depression was calculated as the subtraction of canopy temperature (CT) from the atmospheric maximum temperature (Ta) with the equation 2.4.

Equation 2.4. An equation to calculate canopy temperature depression

$$CTD = Ta - CT.$$

Where Ta is the daily maximum atmospheric temperature. The CTD were then used to calculate BLUEs. Correlations were calculated between BLUEs of individual observation day of CTD and grain yield.

Statistical models for grain yield prediction

Using the BLUEs for each trait, four different statistical models were used to predict grain yield using multiple measurements of NDVI, CT, and agronomic traits. The models included stepwise regression and three shrinkage regression models of ridge regression, least absolute shrinkage and selection operator (LASSO) regression and elastic net regression. In all models, we used all the secondary traits and agronomic traits collected from the field to predict grain yield. Stepwise regression performed forward selection followed by backward elimination (Friedman et al. 2001). Shrinkage models function by shrinking estimated effects towards zero. These models add a penalty that allows less contributing variables to have a coefficient close to or equal to zero. The tuning parameter lambda thus determines the amount of shrinkage. LASSO regression model performs L1 regularization (absolute value of the residual error term), and it can select variables by eliminate variables with a coefficient of zero. Ridge regression performs L2 regularization (squared value of residual error term) and the coefficients cannot be zero thus retaining all variables in the model. The penalty for the elasticNet regression is a combination of ridge regression and LASSO allowing for both variable shrinkage and feature selection (Friedman et al., 2001; James et al., 2013). The models were built in an iterative process, for each year we evaluated models with NDVI only, CT only, and all secondary and agronomic traits together.

For each model, a cross-validation approach was evaluated to determine predictive ability for yield using the trial structure of the CIMMYT trials. As related lines (e.g., full-sibs) are evaluated in the same trial, this approach prevents highly related, full- or half-sibling lines, from predicting their own performance. In the cross-validation scheme, all entries from ten (nine in 2015-16 and 2018-19 season) trials were used to fit the model, and prediction was completed on the 11th (10th in 2015-16 and 2018-19 season) trial. This process was repeated by dropping a single trial fitting the model and predicting the left-out trial until all entries had been predicted. The reported prediction accuracy was assessed as the correlation between the predicted value and the BLUEs for grain yield.

Data availability

All phenotypic data and code for analysis have been placed in the Dryad Digital Repository available at https://datadryad.org/stash/share/Vo_pfWVyVLHvF2tV8huoPuQLt2nN2X6ZYCI3XYnH0Vk.

Results

Broad sense heritability

We observed moderate to high broad-sense heritability (repeatability) for grain yield and other agronomic traits, across the five seasons from 2015-16 to 2019-20 when considering the entire experiment (all trials together) (Table 2.1) and as well as on an individual trial basis (Table A.1 to Table A.5). For agronomic traits including DTHD, DAYSMT, and PH, we observed a consistent and high heritability. The highest heritability was recorded from days to heading (H^2 = 0.97 followed by days to maturity (H^2 = 0.90) across the trials and growing seasons.

For secondary trait measurements, the sensor-based NDVI and CT had heritability ranging from low to high. The CT showed a narrower range of heritability compared to that of the heritability of NDVI (Figure 2.1), but CT heritability was almost always lower than NDVI heritability. The highest value of heritability was calculated as 0.56 for CT and that for NDVI was 0.74. We observed the values of heritability for both NDVI and CT were higher at grain filling stage (mid February to mid March) than the early growth stages (Figure 2.1).

Correlations among the measured traits

Phenotypic correlations were calculated for all measured agronomic traits considering all trials together to determine the relationship among them and GRYLD (Table 2.1). We also calculated the correlations between yield and other agronomic traits for individual trials (Table A.6 to Table A.10). Days to heading showed moderate but negative correlation to grain yield in all the seasons. Days to maturity also showed negative correlation in three of the five growing seasons. The highest correlation was observed between TGW and GRYLD ($r = 0.49$) followed by GRYLD and SN ($r = 0.41$) in 2017-18 season. The most consistent correlation to grain yield was observed for PH and TGW across the growing seasons

The correlation among the measured CTs at individual time points and GRYLD ranged widely with a trend of being strongly negative at the start of the season to positive correlation at the final measurement (Figure 2.2). The strongest correlations were recorded from CT measurement taken during the grain filling stage (mid-February to mid-March). The correlation between CT and GRYLD was more consistent in 2017-18 season and had the least consistency in 2015-16 season.

Generally, NDVI tended to show positive correlations with GRYLD at early to middle growth stages (Figure 2.2). Out of total 63 individual days of NDVI measurement at five growing seasons, 58 days showed significant correlation with GRYLD. The positive correlation, however, changed at the later crop growth stages of all the seasons, where the correlations between NDVI and GRYLD were negative and correlations between CT and GRYLD were positive.

There were strong correlations between multiple days of secondary trait measurements and the yield components across seasons (Figure A.1 to Figure A.5). It was not uncommon for correlation among days of NDVI to have correlations of 0.3. Correlations between days of CT were often not as highly correlated as multiple days of NDVI (Figures A.1 to Figure A.5).

The correlation between CTD and grain yield ranged from low to medium (Figure A.6). The CT showed negative correlation with grain yield while the CTD showed positive correlation with grain yield. At the end of the season the CTD had negative correlation that might be due to the late maturity of some lines which were still green and had lower grain yield.

Yield prediction using univariate model

Yield predictions were developed by implementing a prediction model tested for accuracy with a cross-fold validation strategy. Overall, using a single secondary or agronomic trait, the results were inconsistent with prediction accuracies ranging from zero to 0.59. The prediction accuracy of individual secondary traits varied greatly depending on the trait and the time of measurement (Figure 2.3), with traits measured around grain filling providing the highest values, while traits early or late in the growing season had inconsistent values.

Yield prediction using multivariate models

Using four different multivariate models, the accuracy of grain yield prediction was estimated by using a cross validation strategy where the accuracy was the correlation of the predicted value and the genotypic BLUE. Yield prediction accuracy of the models varied widely from 0.17 to 0.68 (Table 2.2). When using all traits as predictor variables, it was apparent that stepwise regression performed similar to shrinkage models, but the proportion of variance explained by the model was always substantially higher than other models. Stepwise regression was consistently the best among the models deployed with LASSO regression, ridge regression and ElasticNet regression performing similarly.

Difference in sensor based secondary trait selection

Grain yield prediction models were developed iteratively with two distinct secondary traits NDVI and CT, and other agronomic traits with prediction accuracy in the range of 0.17 to 0.45 for using CT only (Table 2.2). Using NDVI, the prediction accuracy was usually higher than using CT alone ranging from 0.32 to 0.58. When we incorporated both the NDVI and CT into the model, the prediction accuracy further increased with prediction ranging from 0.37 to 0.58. Incorporating all traits together resulted in the highest overall prediction accuracies ranging from 0.4 to 0.6 across the experiment years

Discussion

Phenotypic evaluation

The national priorities for wheat breeding programs in Bangladesh are focused on improving heat tolerance with early maturing varieties, improved yield and superior grain quality. Such breeding efforts necessitate selecting promising lines from large breeding trials. Precise phenotyping is the most important prerequisite to deciding which individuals should be selected. Observed heritability for the evaluated physiological high-throughput traits of NDVI and CT were consistent with previous literature (Reynolds et al., 1994). Most of the CT showed negative correlation while most days of NDVI observations showed positive correlation and as such should be useful parameters for selection of superior breeding lines (Babar et al., 2007;

Crain et al., 2017). Overall, the sensor-based traits had higher correlations than other agronomic traits and in the context of breeding are amendable to much higher throughput and rapid measurements. However, we also note that caution should be taken during CT and NDVI data collection as the weed population and irrigation management timing influence the data. Higher weed population increased NDVI values, and the higher transpiration after the irrigation lowers CT. The sensor based secondary traits like CT should be taken very carefully as it influenced by the weather conditions and finally it influences the CTD calculation. Such breeding trial management should be taken into consideration when using these proximal sensing measurements and developing prediction models and selection criteria.

Modeling yield prediction

We evaluated how measured traits could be used to predict grain yield through a variety of statistical models. We used a univariate model to predict grain yield using phenotypic data as our intention was to compare the univariate model to more complex multivariate prediction models. We observed that the univariate models had lower prediction accuracies than any of the multivariate models tested in this study. Using cross-fold validation, the multivariate stepwise model performed well, with the addition of more variables increasing the power of yield prediction. We found that stepwise regression was the best among the four multivariate models deployed in predicting grain yield using secondary traits in wheat.

Application to breeding programs

In a developing country like Bangladesh, genotyping facilities are not yet available. However, field-based phenotyping protocols are available, and these approaches can be implemented across national programs. Hence, within Bangladesh phenotypic modeling is directly tractable and applicable for implementation in the applied breeding programs for yield prediction and more tractable than modeling and selection based on genomic profiling. Our results indicate that large amounts of phenotypic data can be collected with the low-cost phenotyping tools. These methods should be approachable for any breeding program, allowing secondary traits data to predict the primary trait of interest and increase selection accuracy.

In these breeding trials we evaluated a large diversity of elite breeding germplasm that showed much promise in identifying superior performing candidate varieties for Bangladesh. Overall, there was a high proportion (24% - 57%) of the evaluated lines that outperformed the local check varieties BARI Gom 26 and BARI Gom 30 (Figure A.7). In addition, the average yield of selected entries (top 10% of evaluated lines) each year was one ton or more above the yield of the benchmark local checks. These observations and favorable selection results support the upward prospects of continued selection of heat-tolerant breeding materials and development of superior new candidate varieties for the supra-optimal temperatures found in Bangladesh. The combined use of more rapid selections with the proposed phenotyping tools and selection methods can further accelerated identification of these superior candidate varieties.

Our goal was to improve wheat yield prediction through using secondary traits and statistical models that could accommodate highly correlated variables. While we investigated models with secondary and agronomic data, sensor-based data of NDVI and CT can be measured easier than agronomic traits which can require more time and often cannot be measured until the end of the season. Supporting the value of these physiological sensor measurements in breeding, the yield prediction with only the sensor-based data showed prediction power almost as high as the prediction using all traits together. These sensor-based traits are easy to measure repeatedly during the season. This allows breeders to use sensor-based traits to predict grain yield with flexibility depending on the available equipment and to implement yield prediction on small observation plots. If a facility is limited NDVI could be used instead of CT for yield prediction. Regardless of the exact type of sensor-based measurement, breeders will have the ability to increase prediction power incorporating secondary traits. Breeders can use secondary trait measurements, obtained during the growing season, to increase selection accuracies prior to harvesting the plots and ensure that high yielding plots are harvested. This is of particular interest if these secondary traits can be measured on smaller plots at earlier generations in the breeding cycle enabling more intense selection prior to lines entering into replicated yield testing (Krause et al., 2020).

Conclusion

Overall, we found that proximal sensing including of NDVI and CT data were valuable in developing prediction models for yield. When multiple measurements were obtained throughout the growing season, multivariate prediction models were much more accurate than models using a single time measurement. Grain yield prediction was also improved for the incorporation of agronomic traits such as days to heading, days to maturity, and tiller numbers. While less tractable to measure the full suite of agronomic traits (e.g., spikelet number), the incorporation of the routine agronomic measurements into prediction models can be useful for predictions in the breeding program. In the future, if image-based measurement of the agronomic traits become tractable and high-throughput technology for breeding programs (Wang et al., 2019a; Wang et al., 2019b), these traits could be measured on large populations and incorporated into prediction models.

This work demonstrated that high prediction accuracy for grain yield can be obtained using the full combination of proximal sensing and agronomic traits with multivariate models. These traits can be measured on small (e.g., <1 m^2) plots that are used for early generations in the breeding program. Using these same prediction models, it could be possible to generate accurate predictions of grain yield at this stage, where current labor and time constraints prevent harvest assessment. Additionally, using new high-throughput phenotyping platforms and unmanned aerial vehicles that can capture NDVI and CT these measurements can potentially be expanded to tens-of-thousands of plots. By making predictions and more accurate selections much earlier in the breeding cycle, there is considerable potential to increase genetic gain, particularly for difficult and complex selection targets such as grain yield under heat stress.

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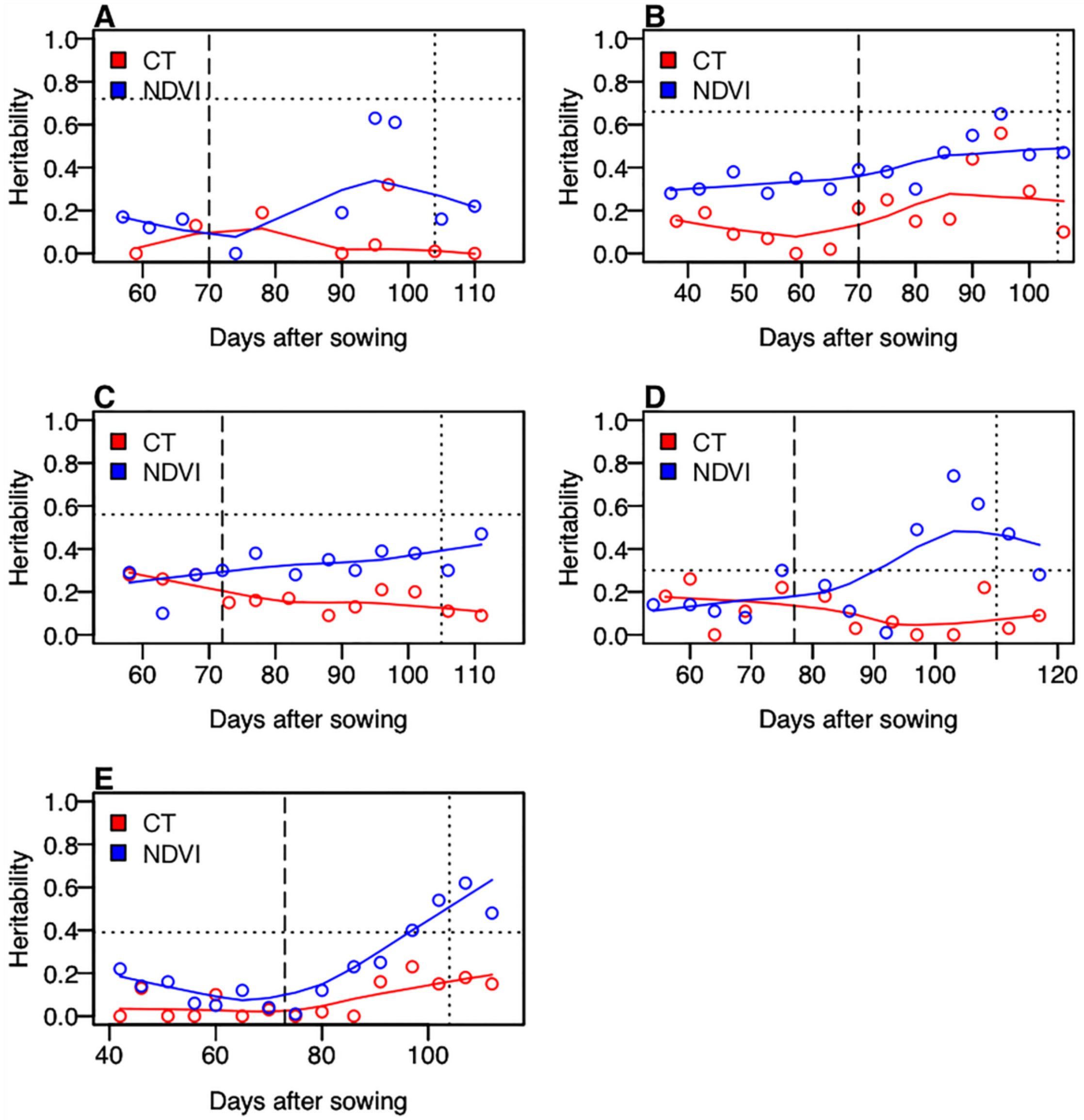

Figure 2.1. Broad sense heritability of normalized difference vegetation index (NDVI) and canopy temperature (CT) for days after seeding for five growing seasons. Each panel, A, B, C, D, and E represents the growing seasons 2015-16, 2016-17, 2017-18, 2018-19, and 2019-20 respectively. Horizontal dotted line represents the heritability of grain yield. Vertical dashed line indicates days to heading and dotted line is for days to physiological maturity.

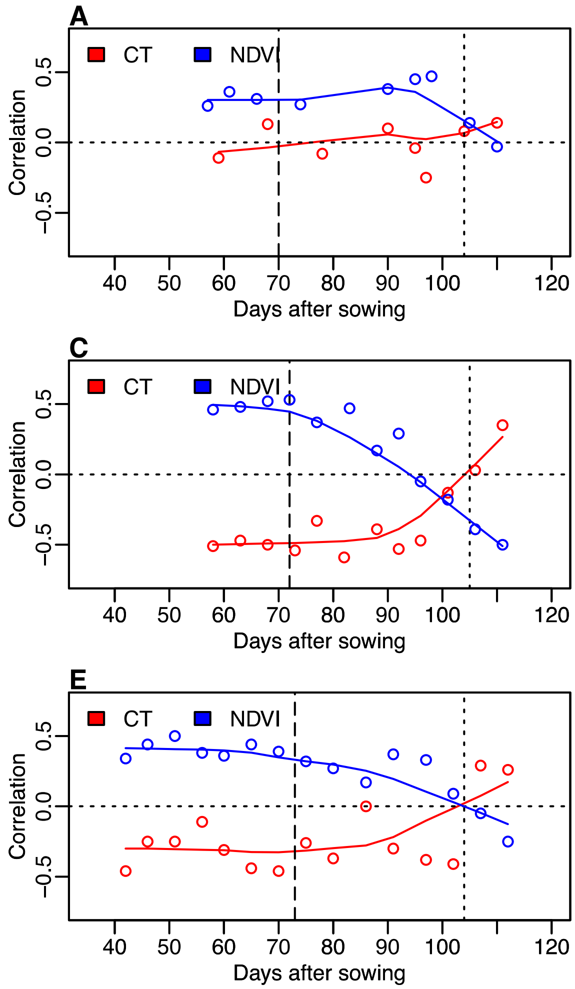

Figure 2.2. Correlation between grain yield and sensor based secondary traits of normalized difference vegetation index (NDVI) and canopy temperature (CT) for observation on days after seeding. Each panel, A, B, C, D, and E represents the growing seasons 2015-16, 2016-17, 2017-18, 2018-19, and 2019-20 respectively. Horizontal dotted line represents correlation value 0. Vertical dashed line indicates days to heading and vertical dotted line represents days to physiological maturity.

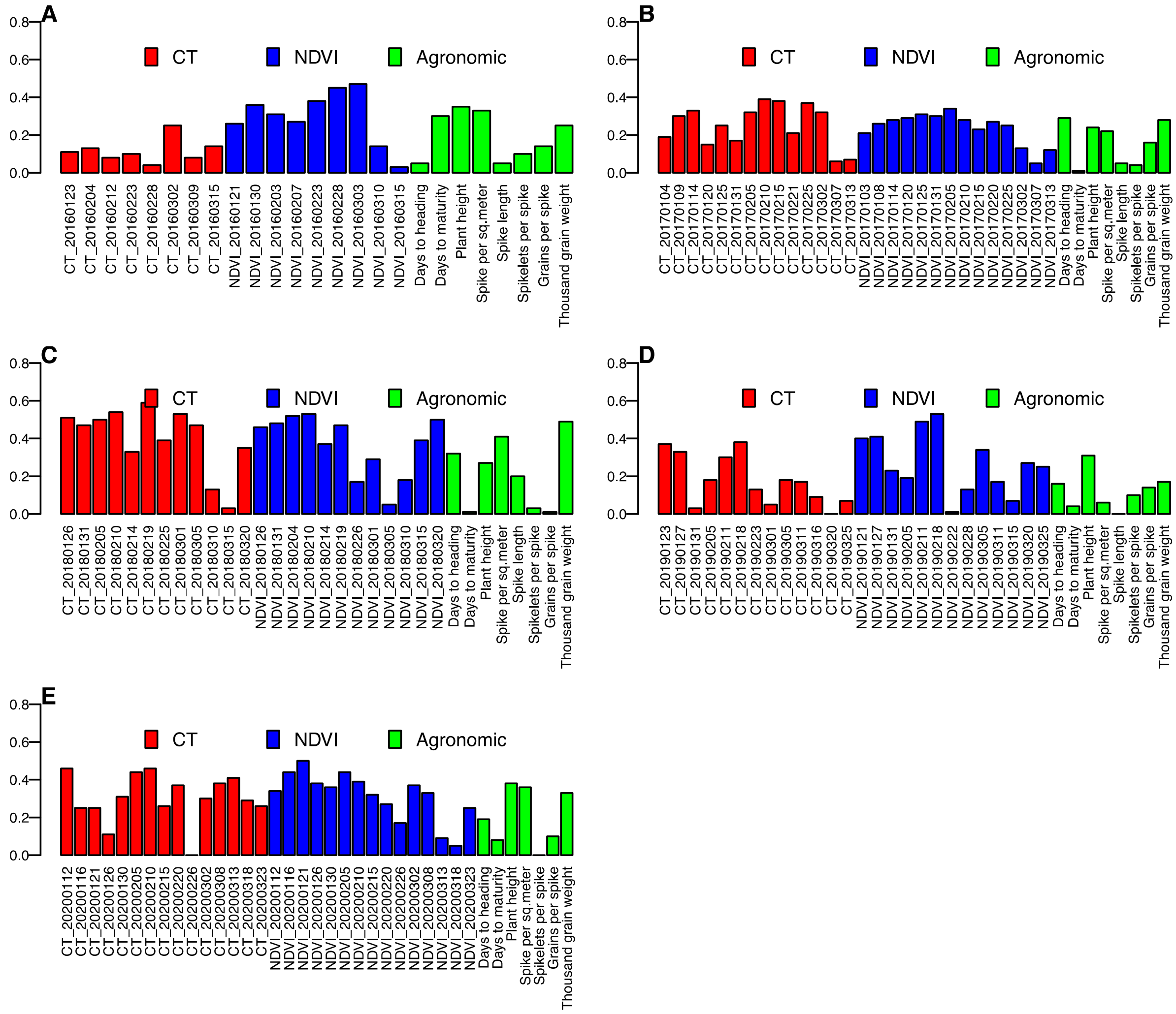

Figure 2.3. Correlation between predicted grain yield and observed grain yield for wheat grown in Bangladesh. Each panel represents one growing season where. A is for 2015-16, B is for 2016-17, C is for 2017-18, D is for 2018-19, and E is for 2019-20 season. Each prediction has been made by using a univariate model with one variable of phenotypic data, where CT is canopy temperature, NDVI is normalized difference vegetation index.

Table 2.1. Broad sense heritability of agronomic traits and correlation between agronomic traits and GRYLD for five growing seasons from 2015-16 to 2019-2020 for wheat grown in Bangladesh.

Traits	2015-16		2016-17		2017-18		2018-19		2019-20	
	H^2	*r*	H^2	*r*	H^2	*r*	H^2	*r*	H^2	*r*
Days to Heading	0.94	-0.05 ns	0.94	-0.29 ***	0.97	-0.32 ***	0.94	-0.16 ***	0.96	-0.19 ***
Days to Maturity	0.72	0.3 ***	0.9	-0.01 ns	0.9	-0.01 ns	0.88	-0.04 ns	0.87	0.08 *
Plant Height	0.51	0.35 ***	0.57	0.24 ***	0.35	0.27 ***	0.28	0.31 ***	0.44	0.38 ***
Number of Spikes per m^2	0.75	0.33 ***	0.03	0.22 ***	0.35	0.41 ***	0.05	0.06 ns	0.18	0.36 ***
Number of Spikelets	0.31	0.1 *	0.28	0.04 ns	0.19	-0.03 ns	0.29	-0.1 *	0.15	0 ns
Kernels per Spike	0.8	0.14 ***	0.36	0.16 ***	0.19	-0.01 ns	0.07	0.14 ***	0.15	0.1 *
Thousand Kernel Weight	0.47	0.25 ***	0.55	0.28 ***	0.61	0.49 ***	0.52	0.17 ***	0.41	0.33 ***
Grain Yield	0.72	-	0.66	-	0.56	-	0.30	-	0.39	-
Spike Length	0.42	0.05 ns	0.36	0.05 ns	0.3	0.2 ***	0.29	0 ns	-	-

H^2= Broad sense heritability, and *r* = Correlation between grain yield and agronomic trait

Table 2.2. Yield prediction accuracies for five wheat growing seasons from 2016 to 2020 in Jamalpur, Bangladesh using four different multivariate models. Yield predictions were based on cross fold validation where accuracy r is the correlation between the Best Linear Unbiased Estimator and the cross-fold validation predicted yield for each trial. R2 is the variance explained by the prediction model.

Yield predictors	Models	2015-16		2016-17		2017-18		2018-19		2019-20	
		r	R2	r	R2	r	R2	r	R2	r	R2
Canopy temperature (CT)	stepwise	0.17	0.07	0.45	0.26	0.34	0.31	0.39	0.14	0.23	0.25
	LASSO	0.17	0.06	0.44	0.24	0.33	0.28	0.39	0.1	0.22	0.23
	ridge	0.17	0.08	0.44	0.25	0.34	0.3	0.4	0.11	0.23	0.23
	elasticnet	0.17	0.08	0.44	0.25	0.34	0.3	0.4	0.11	0.23	0.23
Normalized Difference Vegetation Index (NDVI)	stepwise	0.35	0.2	0.34	0.1	0.43	0.36	0.58	0.36	0.42	0.33
	LASSO	0.35	0.19	0.32	0.08	0.42	0.35	0.58	0.35	0.42	0.32
	ridge	0.36	0.21	0.32	0.08	0.42	0.35	0.58	0.35	0.41	0.32
	elasticnet	0.36	0.21	0.32	0.08	0.42	0.35	0.58	0.35	0.42	0.33
NDVI & CT	stepwise	0.38	0.29	0.48	0.27	0.48	0.42	0.58	0.37	0.43	0.37
	LASSO	0.37	0.27	0.45	0.23	0.44	0.37	0.56	0.31	0.41	0.33
	ridge	0.38	0.28	0.47	0.25	0.44	0.38	0.56	0.32	0.42	0.33
	elasticnet	0.38	0.28	0.46	0.24	0.44	0.38	0.57	0.34	0.42	0.33
Agronomic†	stepwise	0.53	0.3	0.53	0.33	0.57	0.48	0.4	0.14	0.6	0.48
	LASSO	0.53	0.29	0.53	0.32	0.57	0.47	0.41	0.13	0.59	0.46
	ridge	0.53	0.3	0.53	0.32	0.57	0.48	0.41	0.13	0.6	0.47
	elasticnet	0.53	0.3	0.53	0.32	0.57	0.48	0.41	0.13	0.6	0.47
All Traits	stepwise	0.58	0.46	0.68	0.5	0.64	0.58	0.65	0.43	0.64	0.57
	LASSO	0.58	0.44	0.66	0.48	0.62	0.52	0.62	0.39	0.62	0.51
	ridge	0.58	0.44	0.67	0.49	0.62	0.52	0.62	0.39	0.61	0.51
	elasticnet	0.58	0.43	0.67	0.49	0.62	0.52	0.63	0.39	0.61	0.51

†Agronomic traits include days to heading, days to maturity, plant height, spikes per square meter, spike length, spikelets per spike, grains per spike, thousand grain weight, etc.

Chapter 3 - Genome-Wide Association Study of Grain Yield and Yield Components in CIMMYT Spring Wheat Lines Grown in Heat Stressed Environments of Bangladesh

Abbreviations:

GWAS, Genome Wide Association Study; SNP, Single Nucleotide Polymorphism; LD, Linkage Disequilibrium

Abstract

A genome wide association study was conducted for grain yield, yield components and other secondary traits in elite spring wheat germplasm grown in natural heat stressed environment in Bangladesh to identify genomic regions that control component traits and contribute to yield potential. A total of 2682 unique advanced wheat lines of F_6 from the CIMMYT bread wheat program were planted in cohorts of ~540 lines in five wheat growing seasons with measurement of crop phenology including plant height, days to heading, days to maturity, grain yield and yield component traits and proximal sensing data including normalized difference vegetation index (NDVI) and canopy temperature (CT). There was a broad range in heritability (H2=0 to 0.98), as well as genetic correlation with yield from strongly negative for CT (-0.59) to highly positive for component traits. To understand the genetic architecture of these traits, genome-wide association study (GWAS) was conducted using 39,912 SNPs from genotyping-by-sequencing. GWAS result were insignificant and variable for CT and NDVI supporting a highly polygenic architecture. In contrast, large effect loci associated with days to heading and days to maturity were found on chromosomes 5A, and 5B at the Vrn-A1 and Vrn-B1 loci and the frequency and impact of these alleles was observed to vary over successive

cohorts. For other traits including plant height, number of spike and number of spikelets, thousand grain weight some consistent associations were found. We able to find significant association in chromosome 3B and 4A for grain yield that mapped with loci identified for thousand grain weight. Overall, this study highlights the utility of secondary traits including sensor based NDVI and CT to identify chromosome regions that contribute to yield and stress tolerance in South Asian spring bread wheat and better understand the genetic architecture, particularly for heading date and maturity which are critical targets of selection to avoid extreme terminal heat stress.

Introduction

Wheat is one of the most important cereal food crops in the world. It is also one of the most important traded commodities in the world market (Curtis and Halford 2014). In parallel the demand of wheat is increasing day by day. Reflecting this, Bangladesh has both increasing demand and increasing wheat imports for the past decade. To develop a sustainable wheat supply that is meeting demand, it is estimated that 2% genetic gain in wheat yield is required to meet the predicted global demand (Lopes et al. 2012). Results of conventional breeding from hundreds of testing sites worldwide, however, showed a genetic gain of 0.6% for grain yield of spring wheat (Sharma et al. 2012). To accelerate wheat improvement, conventional breeding approach should be complemented with molecular approaches for a better understanding of the genetic basis of yield and implementing optimal selection strategies based on this knowledge.

The genetic basis of a complex trait like yield can be dissected by a combination of assessing the trait heritability, correlation to other traits, component traits and assessing the genetic architecture of the trait with genome wide association study (GWAS) and genomic predictions (Risch and Merikangas 1996). GWAS offers a high-resolution, cost effective way of identification of molecular markers and gene discovery from diverse population while QTL mapping is limited to mapping genomic regions with low resolution in bi-parental population. For GWAS, diverse populations are phenotyped for different traits, combined with whole-genome profiling for directly testing marker-trait associations on the unstructured panel (Zhu et al. 2008).

In the context of a breeding program, a collection of lines spanning the diversity of the whole program can capture more diversity and variants than would be found in a single bi-parental population. To facilitate molecular breeding and the strategic combination of traits in spring wheat, high-density polymorphic SNPs from 90K SNP array was used to detect molecular markers that were associated with yield and yield contributing traits (Sukumaran et al. 2015). The CIMMYT developed wheat association mapping initiative (WAMI) population for GWAS (Lopes et al. 2012) on which candidate gene association mapping was carried out for drought tolerance (Edae et al. 2014). With five known genes in five chromosomes the WAMI population facilitated locating functional markers for drought tolerance through association analysis (Lopes et al. 2015).

Days to heading is one of the most important traits in cereal crops (Kitagawa, Shimada, and Murai 2012) and the transition from vegetative stage to reproductive stage is a critical developmental phase which determines the adaptive capacity in both crop and wild cereals (Cockram et al. 2007). Heading time in wheat varies with environment changes and it indicates the life cycle duration which ultimately helps to maximize the yield potential in any environment (Seki et al. 2011). Vernalization requirement, photoperiod sensitivity and narrow-sense earliness are the three major genetic fctors which determines days to heading in wheat (Worland 1996). These various genetic factors play a major role in determined the relative maturity of wheat breeding lines and subsequently their specific adaptation to a target environment. For the extreme terminal heat stress environments and multiple cropping cycles found in Bangladesh, a very rapid growth and early heading period are key determinants of a successful variety to simultaneously avoid heat stress and fit within the narrow season between rice crops and the monsoon rains.

There are many important genetic factors impacting relative maturity in wheat. The VRN-1 and PHY-C genes affecting heading time in wheat are locate on the group 5 chromosomes (Wiebe et al. 2010, Tóth et al. 2003, Chen et al. 2014). A MADS-box transcription factor encoded by VRN-1 gene was found to be a regulator of vernalization requirement and heading date (Trevaskis et al. 2007). The concentration of VRN-1 must reach a threshold to trigger the transition from vegetative to reproductive stage (Loukoianov et al. 2005). The vernalization of winter cereals depends on the length of cold exposure of the VRN-1 allele (Trevaskis et al. 2006). The dominant mutations in the regulatory regions (promoter or intron1) of VRN-1 changes the winter habit into spring ones which does not require vernalization (Kiseleva et al. 2016). The level of transcription of VRN-1 and flowering date was determined by the variability of the first intron of VRN-1 (Shcherban et al. 2013). The homeologous loci in wheat have been identified via QTL analysis based on genetic linkage maps idendified some loci located on 5B chromosome which influence heading date (Bennett et al. 2012, Griffiths et al. 2009). There are some genetic maps based on SSR, RFLP, AFLP, RAPD, and DArT markers (Matthews 1999) which are not easily amenable to high-throughput genotyping. Now, SNPs are being used to construct high resolution genetic map and marker-trait association (Zhao et al. 2007, Shcherban, Efremova, and Salina 2012). (Kiseleva et al. 2016) identified 78 SNP markers in the pericentromeric region of 5B chromosome significantly associated with heading date variation. The completed wheat reference genome has placed each of the known determinants of flowering and maturity on a single physical map (Appels, Eversole, Stein, et al. 2018).

Heat stress reduces the average grain size and increases the proportion of small grains which downgrade the harvest at delivery. This is a serious constraint in many wheat producing areas around the world. In Australia and USA for example, the average yield loss due to heat stress is estimated as 10-15% (Wardlaw and Wrigley 1994). Number of grains decreases with the increase of floret sterility caused by heat stress at meiosis (Saini and Aspinall 1982). Terminal heat stress during grain filling is particularly detrimental to yield as heat stress at early grain filling reduces the grain size (Stone and Nicolas 1996). The unpredicted and sporadic natural heat stress and its cooccurrence with drought hampers breeding for heat tolerance by direct selection. In some environments with full irrigation, however, heat and drought stress are decoupled and the only limiting factor then become heat. This is the case in Bangladesh where abundant water supply can be provided throughout the growing season. Greater scientific knowledge about the physiological and genetic determinants of heat stress including marker trait association for stress tolerance could be helpful for devising more effective selection methods.

Heat stress reduces the chlorophyl content and the photosynthetic area. Hence the ability of a genotype to maintain stay green is considered as an advantage (Cossani and Reynolds 2012). Likewise, in the presence of sufficient moisture, increased transpiration will lead to a cooler canopy and improved photosynthesis. Starch biosynthetic capacity in grain is influenced by soluble starch synthase which is vulnerable to heat stress (Blum et al. 1994). Heat stress triggers ethylene production which accelerate the mutation of grain (Jenner 1994). Increased temperature cause moisture stress and favorable water status enables stomata opening which facilitate photosynthesis and evaporative cooling of plant tissue through transpiration. Lower canopy temperature is correlated with yield performance in many heat/drought stressed environments (Pinto et al. 2010).

To implement molecular breeding strategies, understanding the genetics and the genetic architecture of a given trait is important. The QTLs and the QTL co-localizations are powerful ways to identify the traits associated with heat tolerance yield components. There have a few studies to identify the significant associations between molecular markers and the secondary traits for heat tolerance breeding. QTL mapping is a key approach for understanding the genetic architecture of complex traits in plants (Holland 2007). However, QTL mapping using bi-parental populations explains only a small portion of the genetic architecture of a trait because of several limitations including evaluation of just two specific allele specific and population specificity (Edae et al. 2014). Association mapping approach, a more efficient approach to use diverse germplasm has overcome the limitation of bi-parental mapping population by utilizing diverse germplasm and it can detect QTL for many traits with high resolution in a single study (Sorrells and Yu 2009, Waugh et al. 2009, Ersoz, Yu, and Buckler 2009, Breseghello and Sorrells 2006). Association mapping has been used to identify QTL for disease resistance (Crossa et al. 2007, Yu et al. 2011, Adhikari et al. 2012, Kollers et al. 2013), end-use quality traits (Breseghello and Sorrells 2006, Zheng et al. 2009), Russian wheat aphid resistance (Peng et al. 2009), and yield and yield component traits (Maccaferri et al. 2010).

Genome-wide association study (GWAS) using high density markers and a population of diverse lines provides higher mapping resolution than conventional QTL mapping based on cross-derived segregating population, and enables one to provide or identify causal genes (Zhang et al. 2015). GWAS has been used to dissect the complex traits of some crops (e.g., maize and rice (Tian et al. 2011, Poland et al. 2011, Li et al. 2013, Huang et al. 2010). The genetic architecture of days to flowering, days to maturity, duration of flowering to maturity, and plant height in soybean was studied using GWAS with a total of 27, 6, 18, 27 loci were identified for the traits respectively (Zhang et al. 2015). The flowering time differences of maize inbreed lines were caused by the cumulative effects of many minor alleles with small effects but not by the major alleles with large effects (Buckler et al. 2009).

Due to the large genome size, GWAS has been limited in wheat from a lack of reference positions for anchoring variants. With the availability of wheat reference genome sequenced in hand (Appels, Eversole, Feuillet, et al. 2018), genotyping-by sequencing (GBS) approach to rapidly call genomewide variants becomes more powerful for GWAS studies being more efficient and appealing in studying and dissecting important secondary traits. The objective of this study was genome-wide scan for identifying genomic regions specially the single nucleotide polymorphisms (SNPs) associated with yield and yield related important secondary traits of interest in wheat.

Materials and Methods

Plant materials

We evaluated a total of 2682 genetically diverse advanced unique elite lines (F_6 and onward generations) of spring wheat from the CIMMYT bread wheat breeding program. These lines were selected for broad adaptation, superior performance and yield potential along with strong disease resistance. The lines were screened for heat tolerance targeting heat stressed environments in South Asia and we have further evaluated this germplasm in a severe heat stress environment in Bangladesh.

Field trials

The collected breeding lines were evaluated in five consecutive wheat growing seasons from 2015-16 to 2019-20 in Regional Agricultural Research Station, Bangladesh Agricultural Research Institute (BARI), Jamalpur, Bangladesh (N 24.9343175238333, E 89.932690164). The trials were planted in optimum timely sown condition in November in all four years while in the 2016 trials were sown in first week of December 2015. The trials were arranged in an alpha lattice design with two replications. Each experimental plot was 5 m^2 consisting of six rows at 20 cm row spacing in 2016 season and next four seasons were planted in 3.57 m long 7 rows plot. The experimental site is under natural heat stress in a hot, humid region. The Wheat Research Center (WRC) of BARI has its own fertilizer and irrigation recommendation which were followed to grow the crop. Fertilizer application consisted of 100:26:50:20:5:1 kg/ha of N:P:K:S:Zn:B respectively each year. Irrigation was applied to prevent water deficit with each year consisting of three irrigations at crop establishment, heading, and grain filling stages during 2015-16 growing season. Two irrigations were applied at crown root initiation and booting stages for rest of the four seasons.

Data were collected on ground coverage (BIOMASS), canopy temperature (CT) in several observation days during the growing seasons, normalized difference vegetation index (NDVI) were also measured in several measurement days, days to heading (DTHD), days to maturity (DAYSMT), plant height (PH), plant population (SN), spike length (SPKLNG), spikelets per spike (SPLN), grains per spike (GRNSPK), thousand grain weight (TGW), grain yield (GRNYLD), biomass weight (BM), harvest index (HI), etc. The CIMMYT wheat physiological phenotyping guide was followed for collecting crop phenology data (Pask et al. 2012).

Genotyping

DNA was extracted from leaf tissues of all the CIMMYT wheat lines when seedlings were two weeks old. The lines were then profiled and sequenced in an Illumina HiSeq2000 following the procedures described by (Poland et al. 2012). Single Nucleotide Polymorphisms (SNPs) were called through TASSEL GBSv2 pipeline considering IWGSC RefSeq v1.0 (cv. 'Chinese Spring') as reference genome (Appels, Eversole, Feuillet, et al. 2018). The called SNPs were filtered for percent missing data (< 40%), percent heterozygosity (< 10%), minor allele frequency (MAF) (< 1%). SNPs were further filtered by removing markers with more than 50% missing values. Beagle v4.1 was used to impute SNPs (Browning and Browning 2016). Finally, 39911 clean and curated SNPs scored on 535 wheat lines in crop season 2015-16, 589 lines in 2016-17, 599 lines in 2017-18, 532 lines in 2018-19, and 601 lines in 2019-20 were used for genome wide association study. We also pooled the lines all together from five years and after removing duplicated lines, resulted in 2682 total lines in the combined analysis.

Data recording and analyses

We used R programming for all remaining data analysis (R core team 2017). A mixed effects model was used to analyze the data. The total entries each year were divide into 10 trials consisting of 54 entries and six check varieties. The experimental design was alpha lattice with two replicates for each trial. The design was adjusted considering replications, sub-blocks within replication as random effects, and entry as random effect in the following model:

Equation 3.1. A mixed effects model for BLUEs

$$y_{ij} = \mu + g_i + r_j + b_{l(j)} + \varepsilon_{ij}$$

The model $(\mathrm{Y} \sim (1|\mathrm{Genotype}) + \mathrm{Rep} + (1|\mathrm{Rep{:}\,Subblock}))$ was implemented in the R environment with the 3.6.1 version, where y_{ij} is the phenotypic response (the secondary traits) for the i^{th} genotype in j^{th} replication, g_i is the genotype used as random term with i for 1-60 corresponding to the number of entries within a single trial, r_j is the random effect of replication with j takes value from 1 to 2 and b_l is the random effect of i^{th} block nested within j^{th} replicate with i taking the values 1-12, and e_{ij} is the residual effect for i^{th} genotype in j^{th} replication. Adjusted means were calculated for each trait of interest.

Broad-sense heritability was calculated with the variance components derived from the best linear unbiased predictions (BLUPs) using '*lme4*' (Bates et al. 2014) package. Heritability of all the traits were also estimated using the formula:

Equation 3.2. An equation for heritability

$$= \frac{\sigma_g^2}{\sigma_g^2+\frac{\sigma_e^2}{r}} \boldsymbol{H}^2 = \frac{\boldsymbol{\sigma_g^2}}{\boldsymbol{\sigma_g^2}+\frac{\boldsymbol{\sigma_e^2}}{\boldsymbol{r}}}$$

Where, H^2 is the broad sense heritability, σ^2_g is the genetic variance, σ^2_e is the error variance, and r is the number of replications, which in this experiment is equal to two.

Genome-wide association analysis

A total of 535, 589, 599, 532, and 601 lines were obtained from 2015-16, 2016-17, 2017-18, 2018-19, and 2019-20 seasons, respectively, for Best Linear Unbiased Estimators (BLUEs) with mean of the respective traits. As a part of structure analyses we conducted PCA, by default the command also created marker based kinship matrix (K) utilizing VanRaden method and generated association panel based clustering heat map (Lipka et al. 2012, Wang et al. 2017). A genome wide scans in GAPIT using 39912 markers with known positions, with respective trait means was conducted using the first five principal components to account for population structure as the fixed component and K matrix as the random component. The threshold for a marker to be significant is usually taken at p-value < 10^{-4}, considering the number of markers and the deviation of the observed F test statistics from the expected F test distribution

(Sukumaran et al. 2012). We also used a more stringent threshold with Bonferroni correction for multiple testing added to determine significance threshold at 5% level of significance divided by number of SNPs (*P*= 0.05/39912 =1.25*10E-6), i.e. a threshold of $-\log_{10}$ (1.25*10E-6) ≥ 5.9 is considered as significant, to restrict false SNP-traits association. The results obtained from GAPIT were evaluated in '*cm plot*' (https://github.com/YinLiLin/R-CMplot) to construct Manhattan plot.

Results and Discussion

Phenotypic variation and heritability estimate

The average yield of the tested lines ranged from 2.41 to 3.5 ton ha^{-1} in the different years (Figure 3.1). The highest average grain yield of the five growing seasons was recorded in 2020 growing season. As 2020 was a very cool year with below average temperature across the growing season, the high yield in this season shows the overall significant yield losses due to heat stress in any given year. Weather data for the five wheat growing seasons were presented in Appendix A (Table A.12 – Table A.16).

The observed phenotypes for all traits were normally distributed, supporting a polygenic genetic architecture of these traits in the breeding program germplasm. The heritability estimate ranged from low to high for different traits (Figure 2.1 and Table 2.1 of chapter 2). The highest heritability was recorded for days to heading followed by days to maturity.

Correlations among the traits

The correlation among the traits ranged from low to high (Figure 2.2 and Table 2.1of chapter 2 and Rahman et al., 2021 – a manuscript submitted to Frontiers in Plant Science). Among the phenology traits, days to heading showed negative correlation to yield across the years, which further supports the favorable performance of early maturity lines in this environment. Plant height and thousand grain weight showed moderate to high correlation with grain yield in most of the growing seasons. This observation further supports that these secondary traits are contributing to final yield and can be important targets of selection as well as contain additional importance for modeling and prediction in genomic selection.

For the high-throughput proximal sensing, we observed positive correlations for the vegetation index (NDVI) while canopy temperature (CT) had negative correlation to grain yield. This supports a hypothesis that improved photosynthesis (e.g. stay green) and increased transpiration resulting in lower canopy temperature contribute to improved yield potential (Reynolds et al., 2012). For these physiological traits, the highest correlations were obtained at grain filling stage of the crop growth. This further supports that this is critical developmental stage in heat stress environments. However, there was much variation from day to day as the correlations were inconsistent for sensor-based traits. The variable performance of the proximal sensing has been known as these measurements are greatly impacted by ambient conditions such as cloud cover. Thus, identification and selection of phenotyping dates with good conditions and moderate to high heritability of the sensor measurements *per se* are more predictive of final grain yield (Crain et al., 2017).

GWAS

Understanding of the genetic architecture of a trait is the key point for accurate selection and for combining desired allels. A total of 2682 lines were used to dissect the genetic architecture of nine key traits of bread wheat using GWAS. Many significant marker-trait associations were identified on the basis of p-values passing the corrected experimental threshold. The model using population structure and kinship (PC + K) matrix showed less deviation of the expected value from the observed values in the Q-Q plots, supporting that this was the best model. We ran GWAS with the data from five years and found loci significantly associated with the traits of interest (Figure 3.2 to Figure 3.5). A total of 39912 markers were used to identify the association. We also ran GWAS with data from individual year and got significant associations (Figure B.1 to Figure B.9).

Association for days to heading and maturity

Significant associations were found in chromosomes 5A and 5B for days to heading (Figure 3.2). Most significant associations were found in chromosome 5A. The highest consistent and significant association were recorded on Chr. 5A at 586.6 Mb. These positions are at the position of Vrn-A1, a strong determinant of days to heading. All the 13 significant SNPs on chromosome 5A showed positive effects while all the four significant SNPs on chromosome 5B showed negative effects. Most of the genomic regions associated with days to heading were also associated with days to maturity (Figure 3.2). Seven SNPs were found significantly associated with days to maturity in chromosomes 5A, and 5B. Among the significantly associated SNPs those on chromosome 5A showed positive effect while the only SNP on chromosome 5B had negative effect.

Interestingly, we observed a shift from Vrn-A1 to Vrn-B1 as the major effect for relative maturity across the cohorts observed here. In the early cohorts of germplasm (e.g. 2015 – 2017) the Vrn-A1 was a significant and large effect. In contrast, the latter cohorts from 2018 onward showed a larger effect for Vrn-B1.

Plant height, spikes number, and spikelets number

Two loci S5A_586600382 and S5A_586669400 on chromosome 5A were significantly associated with plant height (Figure 3.2). The spikes per sq. meter had association with two

SNPs S6B_674159322 and S6B_674380816 on chromosome 6B (Figure 3.3). Spikelets per spike had significant association in chromosome 4A at position S4A_699037779 (Figure 3.3).

Thousand grain weight and grain yield

Among nine significant SNPs seven were found on chromosome 3B and two were on chromosome 4D for thousand grain weight. The strongest association was found in S4A_699037779 on chromosome 3B (Figure 3.3). Grain yield is the most important and complex trait. We completed the GWAS for grain yield in five seasons and found significant associations with loci in chromosome 3B (S3B-5601689), and 4A (S4A-660920825) (Figure 3.3). The locus S4A-660920825 had negative effect while the locus S3B-5601689 had positive effect.

Canopy Temperature (CT)

It was observed that the sensor-based CT was controlled by many minor alleles. We found inconsistent associations for CT on all the chromosomes when data were pooled from five years (Figure 3.4). Some consistent associations were also found when individual year was considered.

Normalized Difference Vegetation Index (NDVI)

The sensor-based trait NDVI was found associated with the loci of almost all of the chromosomes (Figure 3.5). The association results were inconsistent and did not observe any association peak consistently across years. It indicates that the trait is controlled by many minor alleles and should have a highly polygenic architecture. Condorelli et al., (2018) also found association on several chromosomes for NDVI and drought adaptivity in durum wheat.

Conclusion

The development of high throughput phenotyping encouraging breeders to use traits associated with grain yield potential. Predictors of agronomic performance must be stable for various genetic background and target environment. To this end, we observed several important physiological parameters including flowering time, vegetation index and canopy temperature that were significantly associated with yield. The NDVI is the commonly used indicator of healthy wheat growth, biomass and grain yield of hot and humid mega environments (Reynolds, Pask, and Mullan 2012). The heritability and correlation of these secondary traits with grain yield were comparable with other research findings (Gizaw et al., 2016). The moderate heritability and genetic correlations of these traits obtained in this panel are a realistic estimate of the potential for secondary trait selection in the breeding program. We confirmed that flowering time is a very important factor for yield under these heat stress conditions and that selection for early maturity is giving superior yield. Further, we observed strong effects for the VRN-1 loci both on Chr. 5A and 5B in this materials. Finally, the increased NDVI and decreased CT, support the connection of a cool, green canopy giving higher grain yield potential. As such, the combination screening for increased NDVI and lower CT in early maturity germplasm is an ideal selection target for improved yield potential in Bangladesh.

The secondary traits that improve grain yield in stressed environment are important for sustainable yield. Many of the secondary traits studied in this study showed loci that passed Bonferroni threshold but few of them passed the near genome-wide significance threshold. It was seen that when we considered individual year, the loci that passed the genome-wide significance threshold were different when the pooled data from five years were considered (Supplementary figures 3-). Overall, we can consider that the lack of prominent associations for the secondary traits of NDVI and CT, support that these are highly polygenetic traits with a diffuse genetic architecture. As such, they are very suitable as selection criterial per se (e.g. using NDVI for selection), but will not be good molecular breeding targets apart from genomic prediction. In contrast, the strong effect of VRN-1 loci in the Bangladesh environments are very favorable for using marker assisted selection to select for the early alleles at this locus.

Overall, there is promise for use of genomics tools for wheat breeding in Bangladesh. However, these must be balanced with the pragmatic application of the most suitable tools. NDVI and CT for example can be directly assessed on the candidate lines and selections made without further complications of needed genotyping. As such these are powerful selection tools, particularly on early generation nurseries before extensive yield testing. In contrast, marker assisted selection for VRN-1 alleles can be achieved most directly through external sourcing and collaboration for high-throughput genotyping platforms. However, this must be balanced with the throughput and power of directly selecting for early heading types directly in the field.

Association mapping is a powerful tool to identify molecular markers for physiological and agronomic traits in wheat. Through GWAS we identified pleotropic chromosomal regions associated with different yield and yield contributing traits including physiological measurements in spring wheat germplasms developed in CIMMYT, Mexico. The sensor-based measurements for CT and NDVI had inconsistent associations with other agronomic traits. Chromosomal regions on 5A, 5B are strongly associated with days to heading, days to maturity, plant height and chromosomal regions on 3B are associated with thousand grain weight and grain yield. These associations on 5A, 5B, and 3B chromosomes can be important targets for marker-based breeding and will be further validated for use in breeding programs.

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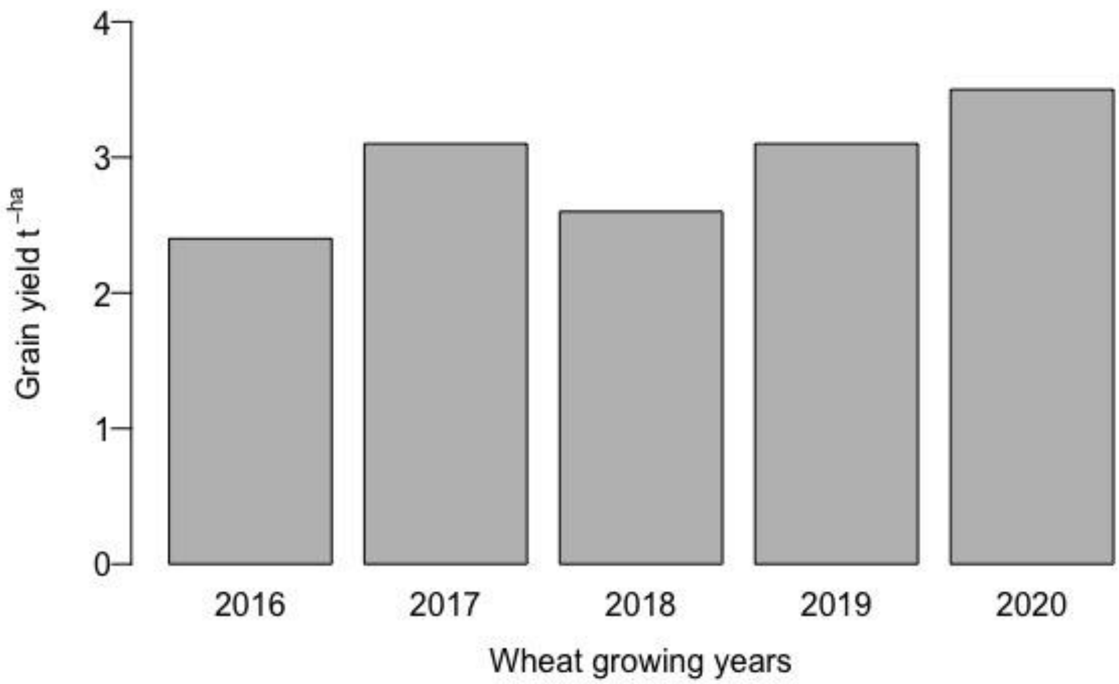

Figure 3.1. Average grain yield of wheat in the genomic selection trials at Regional Agricultural Research Station, BARI, Jamalpur, Bangladesh in five consecutive wheat growing seasons

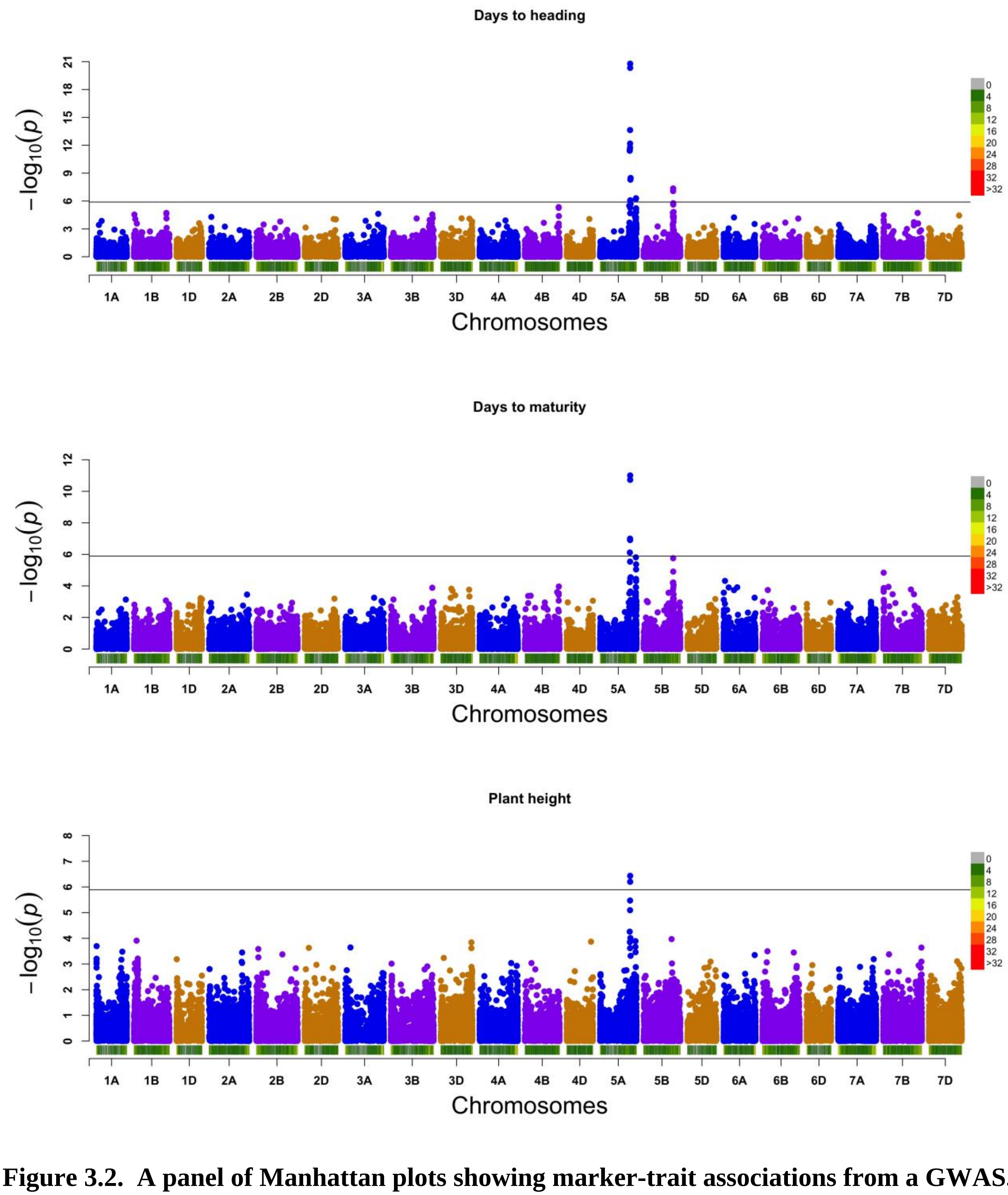

Figure 3.2. A panel of Manhattan plots showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.05 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

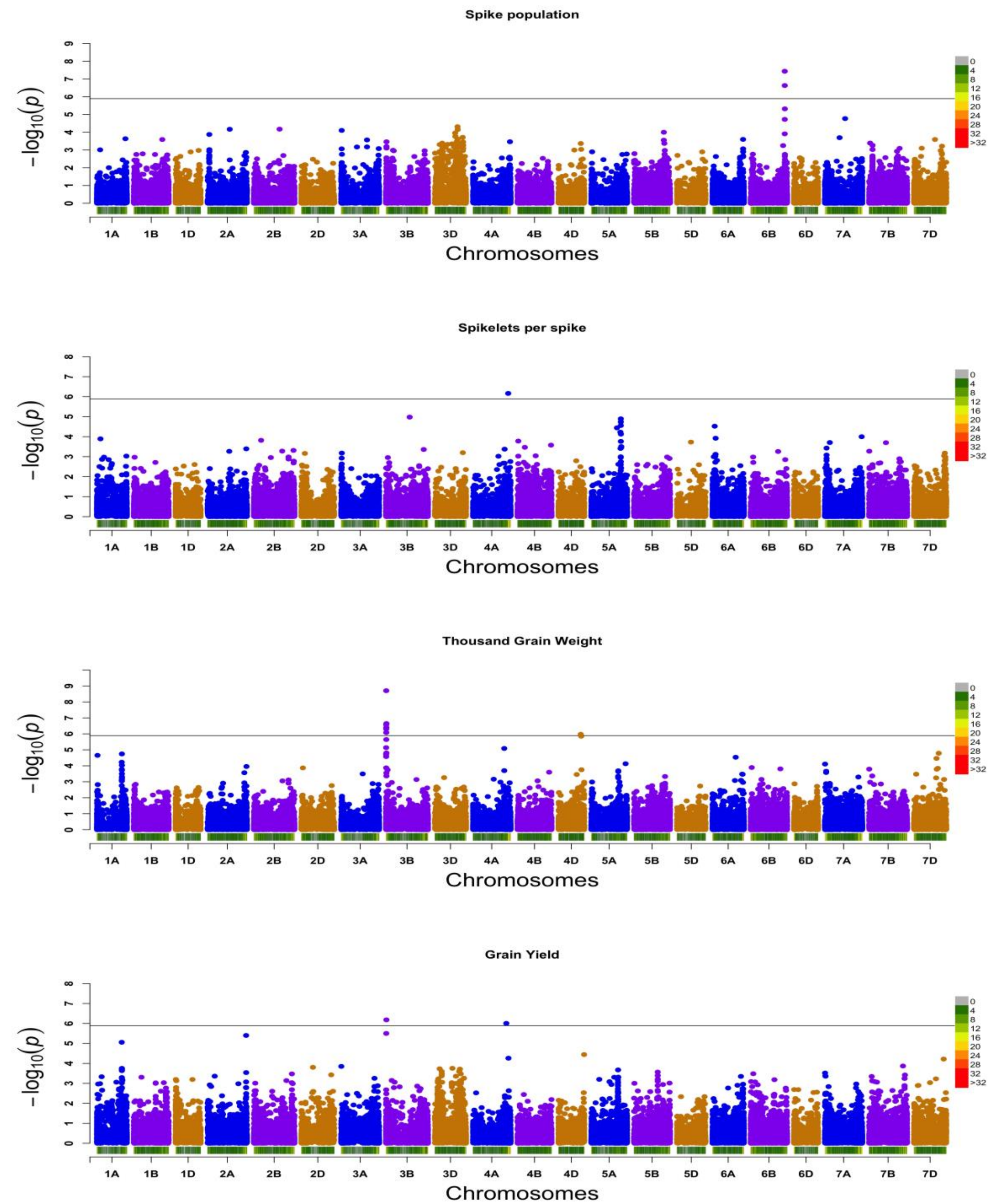

Figure 3.3. A panel of Manhattan plots showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.05 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

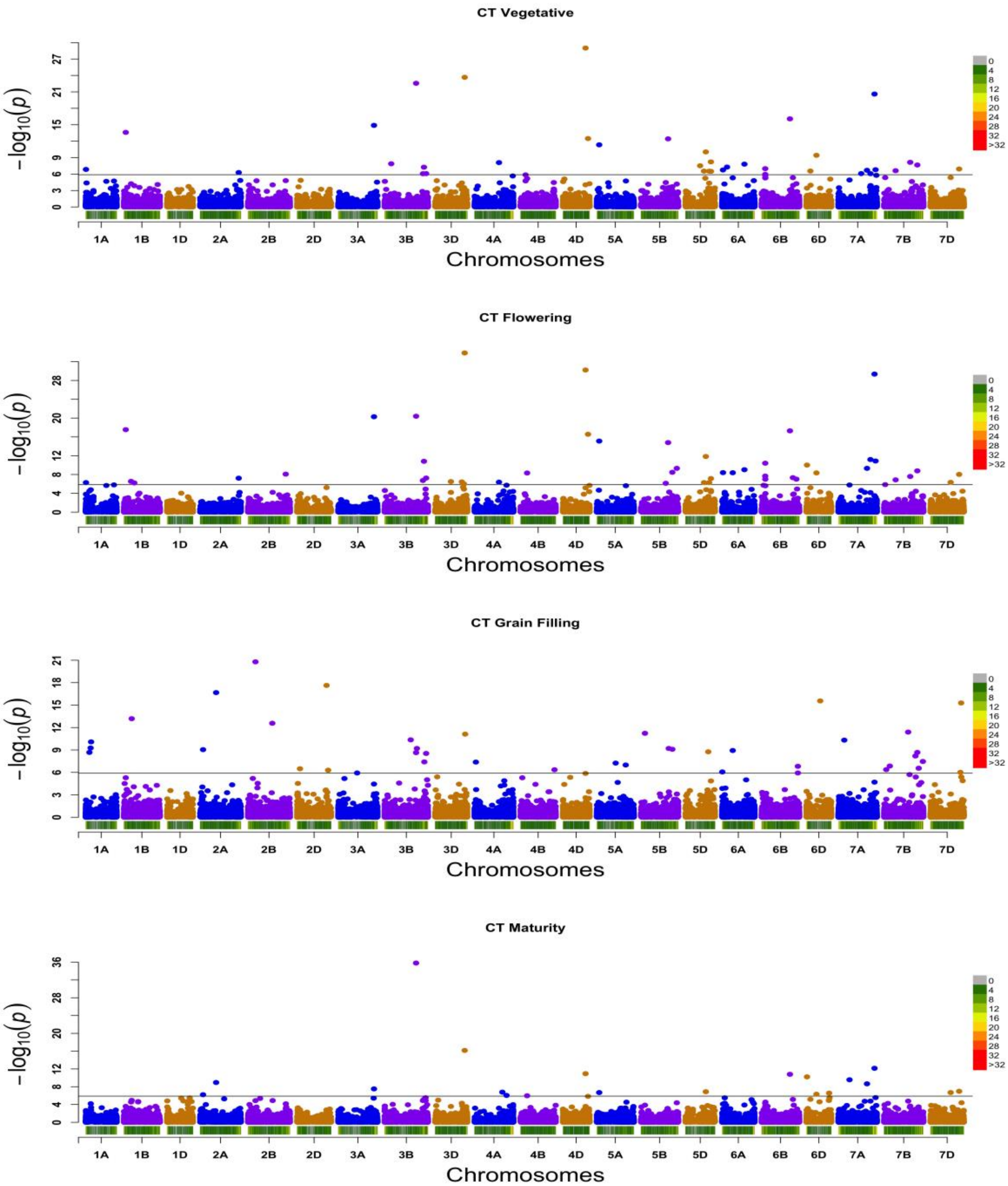

Figure 3.4. A panel of Manhattan plots showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.05 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

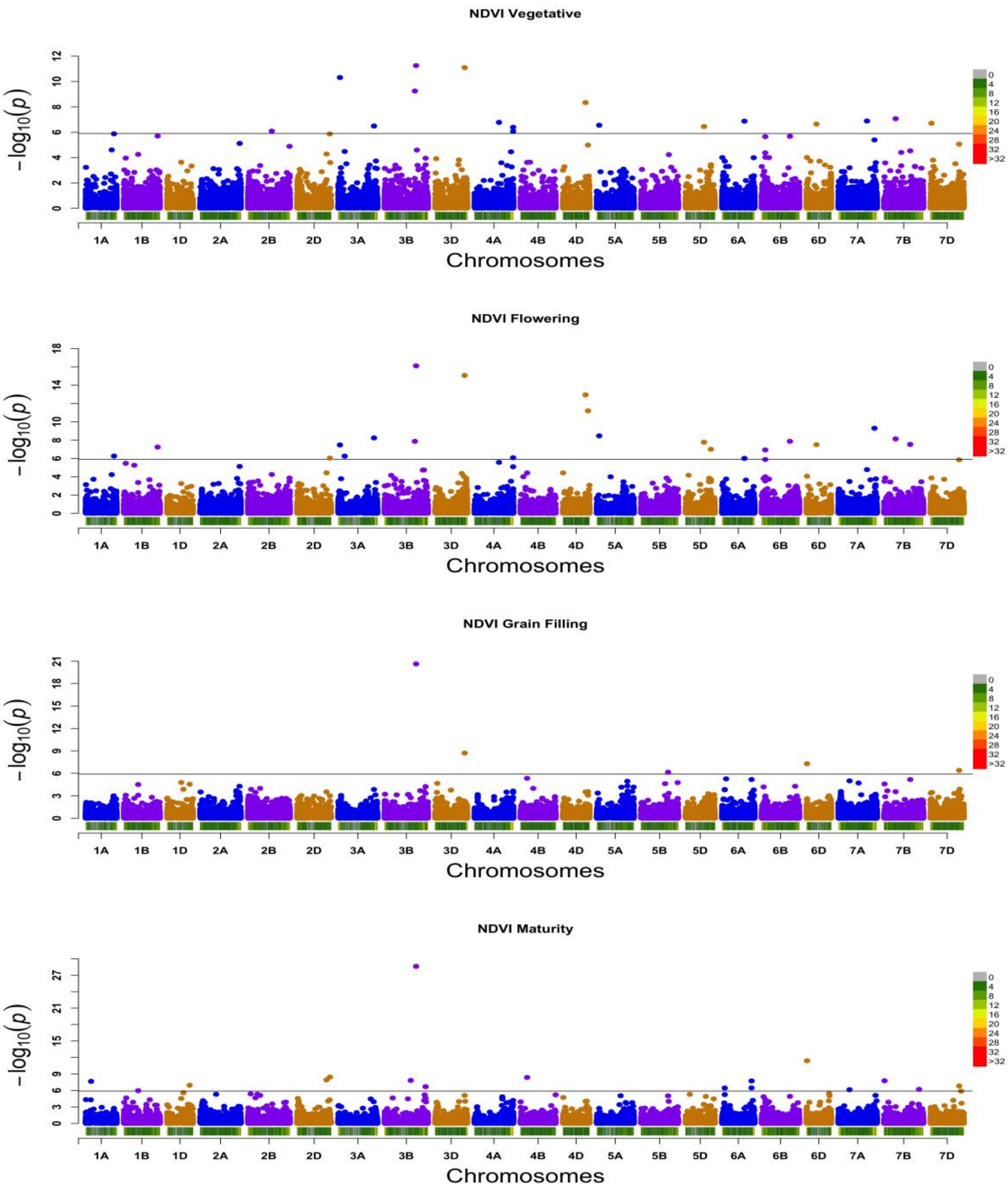

Figure 3.5. A panel of Manhattan plots showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.05 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

Chapter 4 - Development of Genomic Selection in South Asia Bread Wheat for Bangladesh Breeding Programs

Abbreviations

Genomic Selection, GS

Abstract

Genomic Selection (GS), a new breeding technology has been implemented in various species with considerable success in animal breeding. Researchers are trying to explore the potentiality of the GS to reshape the wheat breeding as many of them found several times higher genetic gain with GS. The GS is a new window for wheat breeding and the best strategy for its implication is still being determined with different strategies being optimal for different breeding programs. The objective of this study was to develop and train a model for genomic selection in wheat in South Asia. For this purpose, each year ~ 540 lines were collected from CIMMYT, Mexico for five consecutive years from 2015-16 to 2019-20 seasons. The yield trials were laid out as alpha lattice design with two replications in Regional Agricultural Research Station (RARS), Bangladesh Agricultural Research Institute (BARI), Jamalpur, Bangladesh and phenotyped using high density phenotyping platforms. The germplasms were genotyped using genotyping by sequencing (GBS). For GS across trials one of the ten trails was used as breeding population (BP) and rest of the trials were used as training population (TP). Similarly, data from one of the five years were used as BP and data from the remaining years were used as TP for GS across the years. With tenfold cross validation we conducted genomic selection and the selected entries were grown in the next season as eleventh trial. The prediction accuracy varied across trials and years for different traits where days to heading and days to maturity showed the highest

and most consistent accuracy than any other traits. Among the yield components thousand grain weight and grains per spike showed medium prediction accuracy. The most variable and inconsistent prediction of spike population could have led to low the prediction accuracy and low heritability of grain yield. However, we still obtained good prediction accuracy for grain yield that can be used to increase genetic gain through the implementing the proposed genomic selection model for Bangladesh.

Introduction

Identification of superior candidate lines for variety release is the main target of any breeding programs which exerts huge efforts to evaluate breeding lines across the locations. Collaborative programs have been taken in CIMMYT, Mexico with South Asia to deliver advanced wheat lines for releasing as varieties. Bread wheat is the staple food for millions of people globaly with a variety of food items including breads, noodles, cookies, cakes, pastries, etc. The human population is increasing exponentially and current projections predict a population of >9 billion by 2050 (Gerland et al. 2014). Improved crop varieties and advanced agronomic practices are greatly needed to have an intersection between the demand and supply of wheat to ensure food security in the coming decades. There is also demanded to produce high quality, more nutritious food to satisfy the sustainable development goal (SDG). Grain yield combined with improved agronomic performance and disease resistance is the primary focus of wheat breeding historically. Visual selection of lines for the targeted traits is the principle of conventional breeding program. Phenotyping a large number of plots is time consuming, costly, and require larger field. In addition, there is limitation for developing any wheat cultivars with good agronomic traits and disease resistance. Accurate good agronomic traits, disease resistance, and end-use quality prediction models would allow breeding programs to cull unacceptable lines or target specific lines earlier in the pipeline before time and resources are invested in lines that will not pass the final test.

With the recent development of inexpensive, high-density genetic markers, whole-genome marker profiles can now be obtained for every experimental line, making possible new analyses that rely on large amounts of genomic data including diversity studies and genomic selection (Poland and Rife 2012). Marker-assisted selection with previously identified significant markers has limited prediction power in the scope of breeding for quantitative complex traits like grain yield (Heffner, Jannink, and Sorrells 2011).

Genomic selection models, however, use high-density genotype data sets and simultaneously model all additive genetic variance. These models use entries with known phenotype and genotype to train a prediction model, and then predict traits in materials with only genotype information available. This approach was first introduced into animal breeding by (Meuwissen, Hayes, and Goddard 2001) demonstrating that ridge regression and Bayesian approaches could be used to model the total additive variance and predict breeding values. Their claim that attaining genome-wide marker profiles would become cheaper than phenotyping each individual is becoming a reality (Poland and Rife 2012). Taking all this into consideration, GS could serve as a way to predict grain yield and yield component phenotypes earlier in the pipeline before breeders have enough resources for testing and allow predictions of more individuals than would be possible to phenotype.

Genomic selection has been evaluated many times for wheat yield and disease resistance ((Arruda et al. 2015); (Crossa et al. 2010, Crossa et al. 2014); (Dawson et al. 2013); (Heffner, Sorrells, and Jannink 2009); (Poland, Endelman, et al. 2012); (Rutkoski, Heffner, and Sorrells 2011, Rutkoski et al. 2012, Rutkoski et al. 2014). Genomic selection was tested in soft wheat for end-use quality in a biparental population and a small breeding population (Heffner, Sorrells, and Jannink 2009, Heffner et al. 2011). These studies relied on cross-validation, rather than forward prediction approaches, to assess the prediction accuracy of the GS models. They did find processing and end-use quality traits to be more highly predictive than grain yield.

Genomic selection is a new breeding approach that utilizes total allelic effects across the genome to predict phenotypes and to select superior lines (Meuwissen, Hayes, and Goddard 2001). Breeders are using and investing the GS approach to reduce the breeding cycle (Heffner et al. 2010, Anderson, Maas, and Ozias-Akins 2009, Heffner, Sorrells, and Jannink 2009), and to increase selection intensity in a breeding program (Cros et al. 2015), (Battenfield et al. 2016) GS has two sets of population: 1) a population that has been both phenotyped and genotyped which is used to train the prediction model and 2) a population that has been only genotyped (Heffner, Sorrells, and Jannink 2009), (Heffner et al. 2010). A prediction model is then used to predict the traits of the second set of population to select the superior genotypes. Scientists used different designations each of the two population (Rincent et al. 2012), (Rutkoski et al. 2015), (Isidro et al. 2015). Here we will refer to the two populations as the training population (TP) and the breeding population (BP), respectively.

Phenotyping of a large population is a land, labor, and time-consuming endeavour and hence an optimal design for selecting a TP is a research topic of high interest to the breeders (Akdemir, Sanchez, and Jannink 2015), (Spindel et al. 2015), (Isidro et al. 2015). Researchers are still in search of the ideal training population as they have limited understanding of its characteristics. Two of the important factors of selecting an ideal TP are – population size and their relatedness. It has been shown that with the increasing size of the population, the prediction accuracy increases (Zhong et al. 2009). On the contrary, there are generally diminishing returns, that is if more lines are added to the training population, the gains in accuracy reduces (Asoro et al. 2011). The training population and breeding population must be interrelated with common alleles and markers to increase predictability. Results have been shown that if the training population and the breeding population are closely related the prediction accuracy increases (Hayes, Visscher, and Goddard 2009), (Long et al. 2011), (Pszczola et al. 2012), (Rutkoski et al. 2015). We are interested to implement the genomic selection model in the wheat breeding program in Bangladesh for yield and yield contributing traits that are regularly assessed by the conventional phenotyping approach. To this end, we have generated whole-genome profiles via genotyping-by-sequencing for SABWGPYT entries of last five years starting from 2016 to 2020. This genetic data was used as a TP for GS and evaluate prediction differences between yield trials. The objective of this study was to determine prediction accuracy of the GS model for the complex traits, assess the accuracy of prediction into the next year, and introduce GS for the bread wheat breeding program in South Asia especially in Bangladesh.

Materials and Methods

Germplasm

Wheat lines used in the training and testing the GS model were from advanced lines from CIMMYT, Mexico intended to develop heat tolerant varieties for South Asia. Each year new lines were tested and hence we received ~2700 lines for five years. The 540 materials were planted in lattice design in two replications with six checks including local check BARI Gom 26 for first three years and BARI Gom 30 for last two years. The lines were tested in the Regional

Agricultural Research Station, BARI, Jamalpur, Bangladesh. The GIS coordinate of the trial location is N 24.9343175238333, E 89.932690164.

Library construction and data processing

The GBS libraries were prepared following the protocol detailed by (Poland, Endelman, et al. 2012). Briefly, DNA was digested with two enzymes PstI and MspI and barcoded adapters were added to the ends of the fragments. Samples were then pooled at 192-plex, amplified, and sequenced on an Illumina HiSeq 2000. The SNPs were called using the approach of (Poland, Endelman, et al. 2012). SNPs having at least 1% minor allele frequency were kept and at least 30% of the data present across lines. For subsequent genomic prediction, genotype data from the lines grown in five seasons in Bangladesh were used.

Phenotypes

Phenotyping was done using handheld instruments for the five consecutive growing seasons. Sib and non-sib lines were included in the trials to increase variance among the lines for breeding selection. We used Infrared Thermometer (IRT) for canopy temperature measurement, green seeker for Normalized Differences Vegetation Index (NDVI) measurement, barcode scanner for taking plant height, USB scale connected with barcode scanner and fieldBook to measure thousand grain weight and plot weight, FieldBook for easy data processing. All the crop phenology data including days to heading, days to maturity, spike population, spikelets per spike, grains per spike, disease scoring for bipolaris leaf blight, rusts, etc.

Genotypes

From each line we collected leaf tissue and bulked them. DNA was extracted using CTAB protocol (Saghai-Maroof et al. 1984). For the purpose of genotyping by sequencing the extracted was quantified, normalized to 10 μL at 10 ng μL^{-1}, digested with two-enzymes *PstI* and

MspI, ligated with barcoded adapters, amplified, and then sequenced following the protocol of (Poland, Brown, et al. 2012). Sequences were trimmed to 64 bp, unique sequence tags were aligned, and single-nucleotide polymorphisms (SNPs) were recoded numerically as (−1, 0, 1) using TASSEL GBS v2 using Chinese spring wheat (IWGSC RefSeq v1.0) as reference genome (Appels et al. 2018). The MAF was ≥0.01. The SNPs were filtered using three methods inbreed coefficient, Fisher Exact Test, and Chi-Square test. SNPs with >70% missing data were also removed from further analysis. Beagle v4.1 was used to impute SNPs (Browning and Browning 2016). We got 39912 clean and curated SNPs scored on 2682 lines from five wheat growing seasons that were used in this study.

Analyses

The Best Linear Unbiased Estimates (BLUEs) were calculated using a mixed effects model (Equation 4.1).

Equation 4.1. A mixed effects model for BLUEs

$$y_{ij} = \mu + g_i + r_j + b_{l(j)} + \varepsilon_{ij}$$

where, y_{ij} is the observed phenotypic response variable (GRYLD, CT, …, NDVI) for the i^{th} genotype, j^{th} replicate, μ is the overall mean of the individual trial, g_i is the fixed effect of i^{th} genotype (line) with i taking the values 1-60, r_j is the random effect of j^{th} replicate with j corresponding to 1 or 2 with a normal distribution $N(0,\sigma^2_r)$, b_l is the random effect of lth block, nested within replicate j, where l ranges from 1-12 distributed as $N(0,\sigma^2_l)$, and ε_{ij} is the residual effect for genotype i in replicate j with normal distribution $N(0,\sigma^2_e)$. Best linear unbiased estimators (BLUEs) were calculated for each site year individually.

A realized additive relationship matrix (A) was constructed using the A.mat function in the rrBLUP package in R (Endelman 2011).

Results and Discussion

Materials and genotyping

Moving from phenotyping-based breeding to allele-based breeding, an entire genome profile was created from every one of the lines included in the yield trials and therefore an acknowledged relationship grid was determined. Here, we utilized genotyping by sequencing method with an inner arrangement-based pipeline to find 39912 SNP.

Phenotype Means, Heritability and Correlation

Phenotyping was carried out on ~2700 advanced lines for all phenologies including, yield and yield contributing traits. We calculated mean and standard errors for each trait within each year and observed phenotype distribution of all traits within all years followed an approximately normal distribution (Figure C.1, Table C.1). The estimated heritability of different traits was low to high ranging from 0 to 0.97 and the correlations of those traits to grain yield were low to medium ranging from -0.32 to 0.49 (Table 2.1 of chapter 1). Days to heading showed the highest heritability followed by days to maturity. Among the yield component traits, TGW showed the highest and consistent heritability. Most of the secondary traits showed significant correlation with grain yield (Table 2.1 in chapter 2). Plant height and thousand grain weight showed significant positive correlation across years while days to heading showed negative correlation across years. Thousand kernel weight had the highest and positive correlation with grain yield.

Genomic prediction

We used genome modeling approach to predict all the phenotypes including yield and yield contributing traits in the trials of last five years from 2016 to 2020. This genomic prediction was made in two ways – across trials and across years. For genomic prediction across trials within year, a training population was created using nine of the ten trials and the remaining trial was used as prediction set. Same way for the prediction across years one of the five years used as prediction set and rest of the four years was used to create training set. The genomic prediction accuracy assessed as the Pearson correlation between the calculated BLUPs and predicted values of the prediction set.

We observed differences in genomic predictability of the traits among the trials within year ranging from low to medium (Figure 4.1) while among the years the predictability was ranging from low to high (Figure 4.2). The highest and most consistent genomic prediction was observed for days to heading followed by days to maturity. Days to heading and days to maturity are two most important traits and early heading and maturity are important selection target for any breeding program for heat stressed environment like Bangladesh. Early days to heading and late maturity will facilitate long duration for grain filling. However, too late of maturity eventually will increase the chance to face the terminal heat stress. Thus, quick grain filling is important to escape the heat stress. So, early heading and maturity is the most important trait to select best lines in heat stressed environment.

The spike population, a very important yield component, showed mostly inconsistent and low prediction accuracy among the traits in both within and across years. The highly variable spike population and lower prediction accuracy for this trait could have led to lower prediction accuracy and low heritability for grain yield. The variable spike population is likely due to the large impact of early season tillering on this trait. With variable early season temperatures, there was a large variation in spike population across the years, introducing G×E and likely resulting in lower prediction accuracy.

The grains per spike is a yield component trait that showed most consistent and moderate prediction accuracy in both by year and by trial methods of calculation. The spikelets per spike had low genomic predictability but better consistent than spike population when considered by year.

Thousand grain weight (TGW) is directly correlated to grain yield and we found consistent and moderate genomic prediction for TGW. Heat stress affects on grain size and shape. Large and bold grain type is important selection target in heat stressed environment.

Plant height showed low predictability across years, but it showed consistent and moderate correlation to grain yield. The negative predictability of plant height in 2020 could have led to low and negative predictability of grain yield in 2020. Depending on the target environment, breeding in Bangladesh is generally focused on short plant types that maintain good grain yield. There is limited value for the wheat straw and therefore increased biomass is not an important selection target.

Grain yield is the most complex trait that had moderate prediction accuracy across years when the prediction was calculated across trials within year and it showed low prediction accuracy when predicting across years. We observed very low prediction accuracy (negative) for grain yield in 2020, which also corresponded to negative prediction accuracy for spike population (e.g. tillering). This growing season was marked by cool weather which led to a very high level of tillering, which much different than 'normal' years with higher temperatures and minimal tillering. The yield therefore in 2020 season was much higher than average, largely driven by the increased tillering. As these were unusual conditions for this environment in Bangladesh, the prediction of component trait (spike population) and yield were negative. However, in such a year, we still observed consistently high predictions for thousand grain weight and grains per spike, enabling positive selection for these other component traits.

Conclusions

Genomic selection is an approach that plant breeders and geneticists are trying to finetune its application in superior line selection around the world. In Bangladesh, wheat breeders are using conventional breeding approach to select lines from their breeding trials and nurseries but it takes long time to reach the final destination of releasing a new variety. If we can use advanced breeding methodologies like genomic selection it would complement the conventional breeding approach and it would reduce the land, labor, time and other resources. Here we have developed a prediction model that can be used for South Asia especially in Bangladesh to select promising wheat lines from large breeding trials. We applied this model last five years and selected several superior lines from the trials and those were better yielder than the national best check variety. Our target is to continue using this new breeding approach in Bangladesh to accelerate genetic gain in the national wheat breeding program in Bangladesh.

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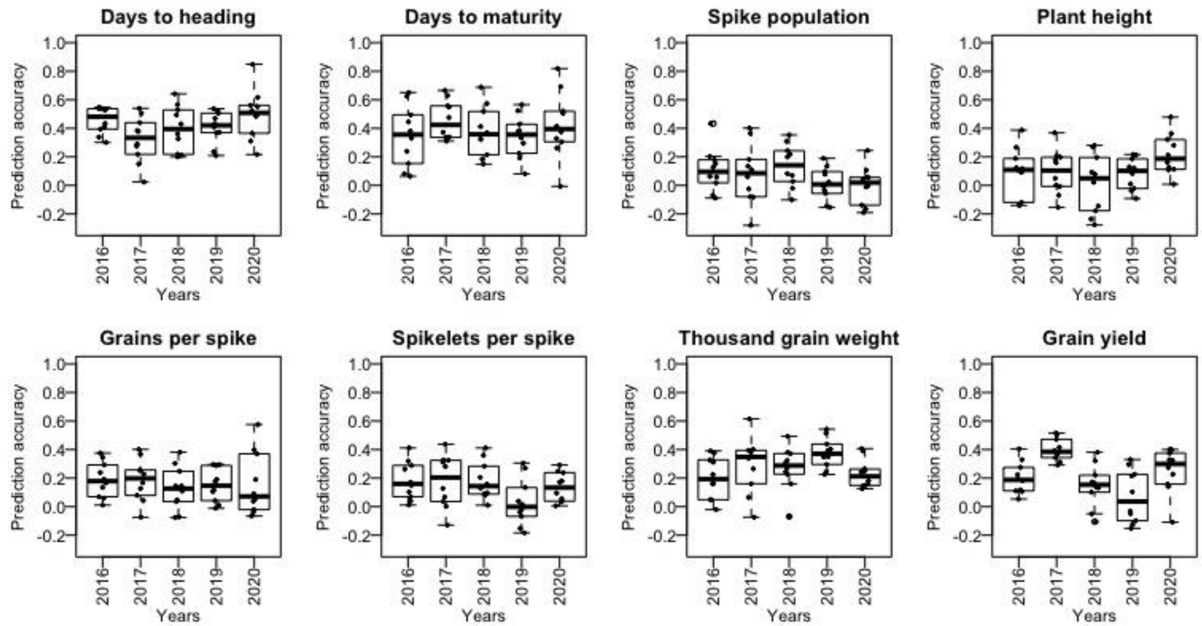

Figure 4.1. A panel boxplot of genomic prediction accuracy of different traits of wheat predicted across trials within year. The prediction accuracy was the correlation between predicted value and observed values of a trait. The predicted values were calculated by keeping the lines of one trial as breeding population and lines from rest of the trials as training population.

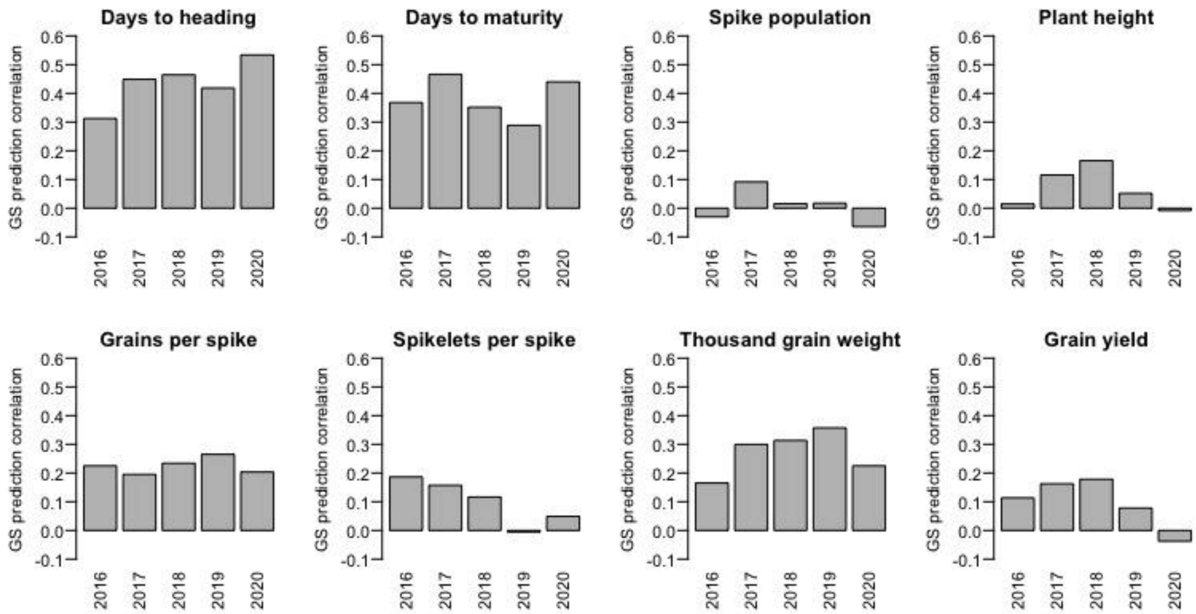

Figure 4.2. A panel plot of genomic prediction accuracy of different traits of wheat predicted across years. The prediction accuracy was the correlation between predicted value and observed values of a trait. The predicted values were calculated by keeping the set of lines of one year as breeding population and lines from rest of the years as training population.

Appendix A - Supplementary Material Chapter 2

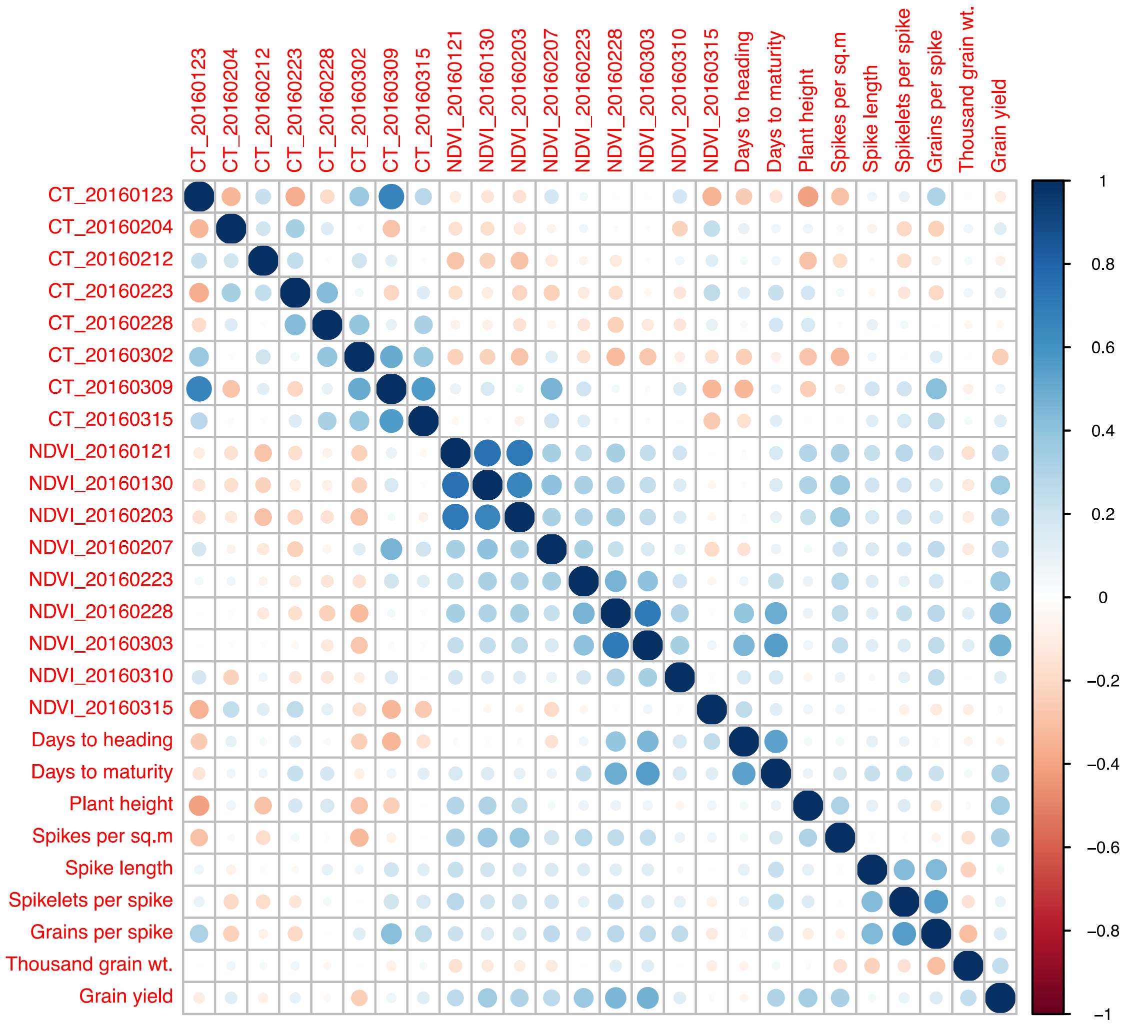

Figure A.1. Correlation matrix of all traits in 2015-16 season. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in Jamalpur, Bangladesh

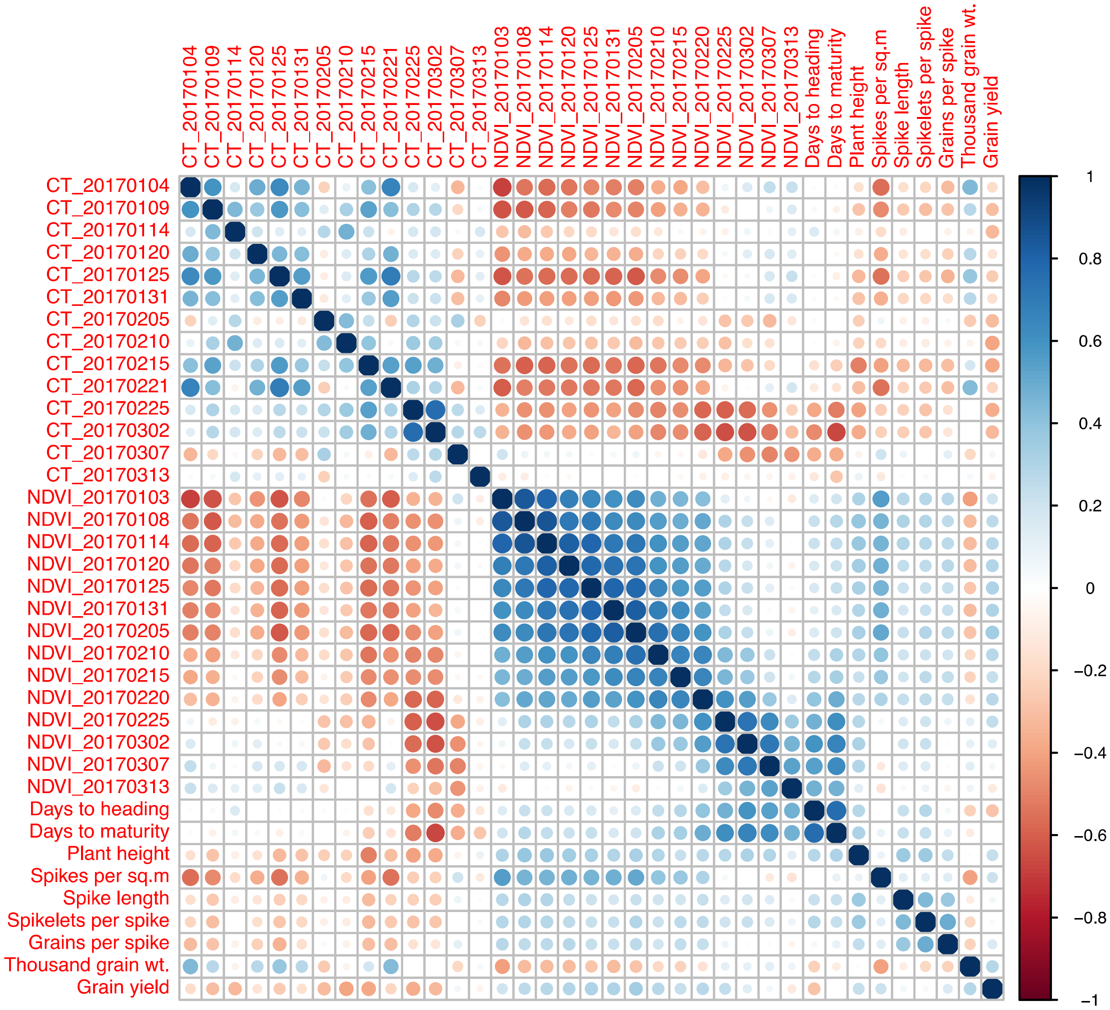

Figure A.2. Correlation matrix of all traits in 2016-17 season. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in Jamalpur, Bangladesh

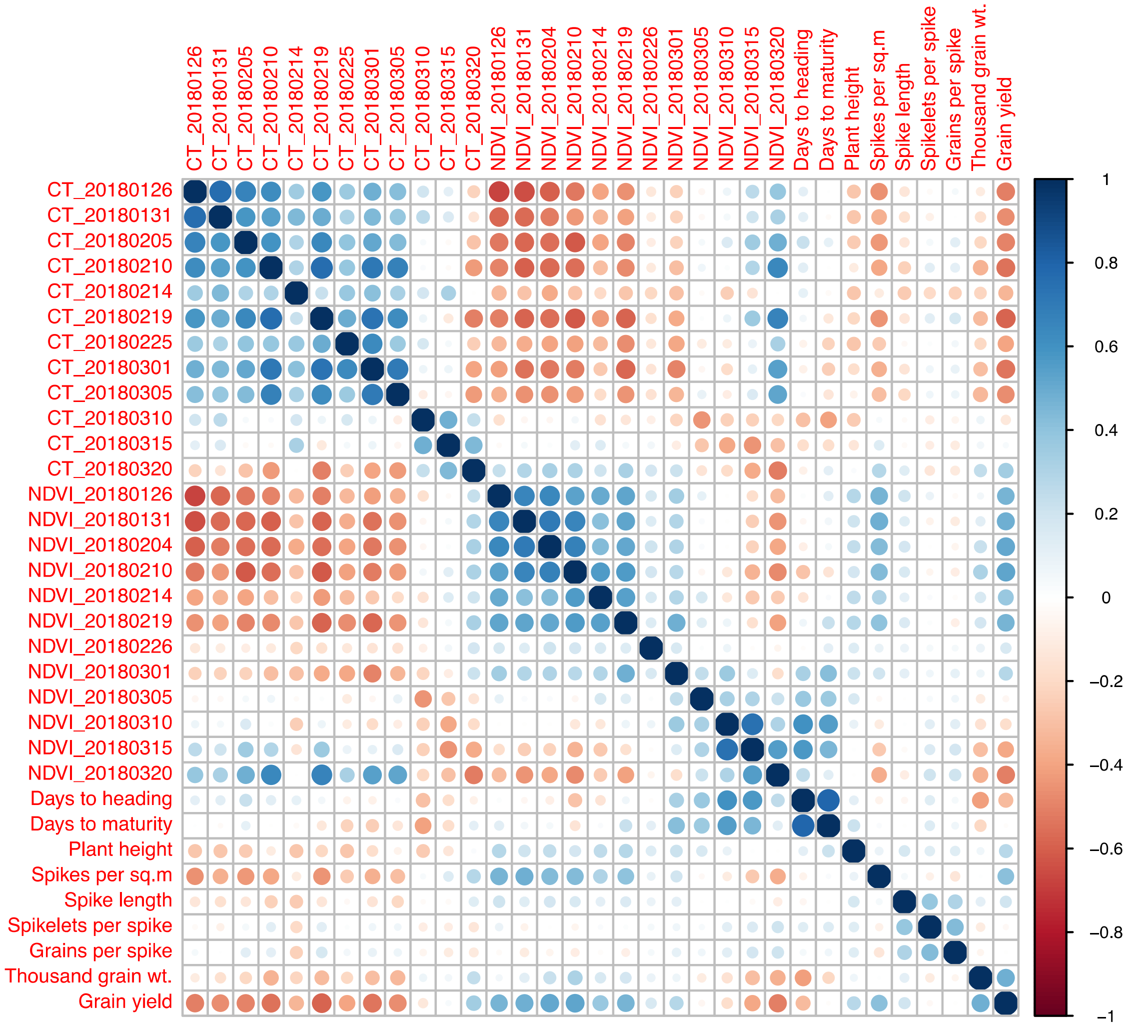

Figure A.3. Correlation matrix of all traits in 2017-18 season. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in Jamalpur, Bangladesh

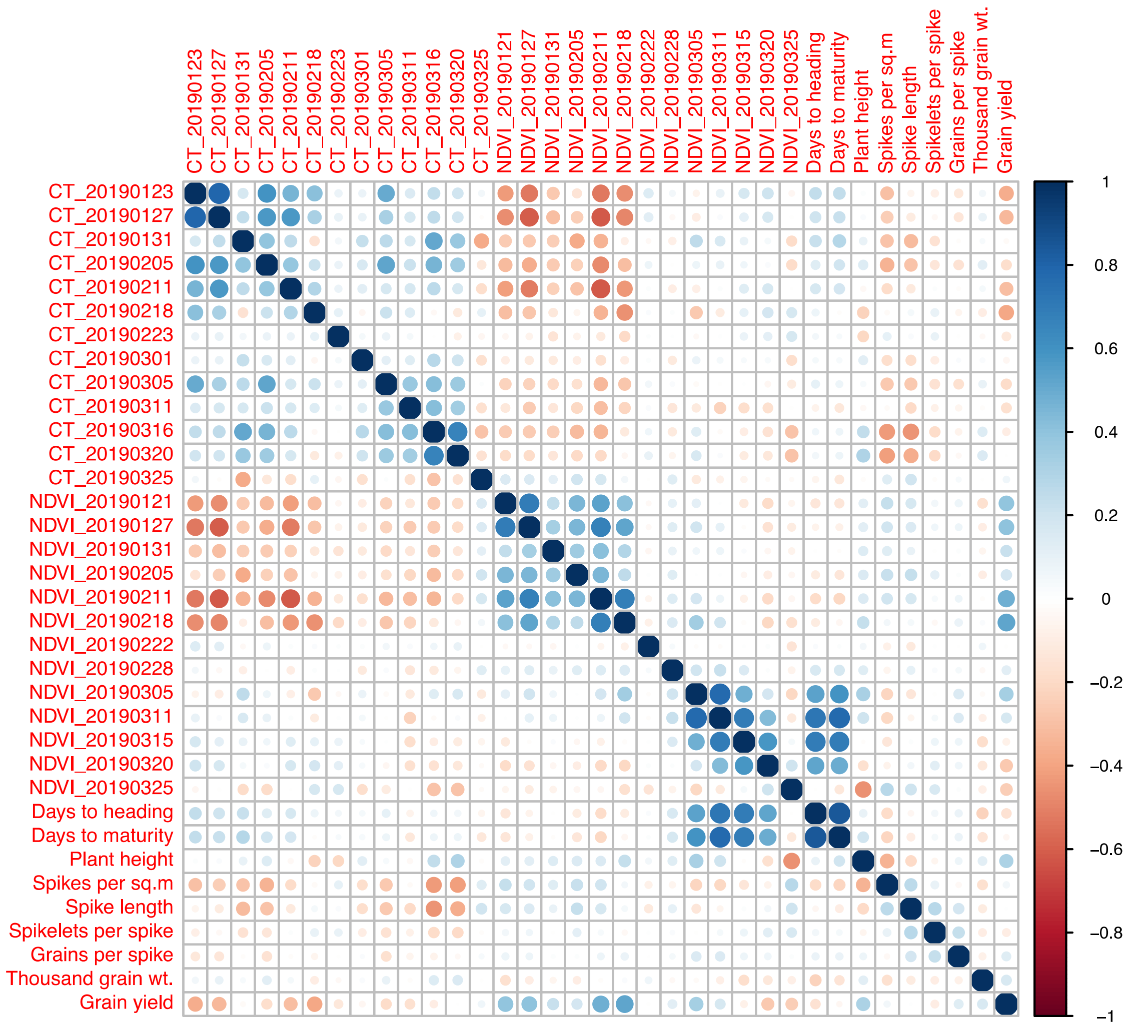

Figure A.4. Correlation matrix of all traits in 2018-19 season. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in Jamalpur, Bangladesh

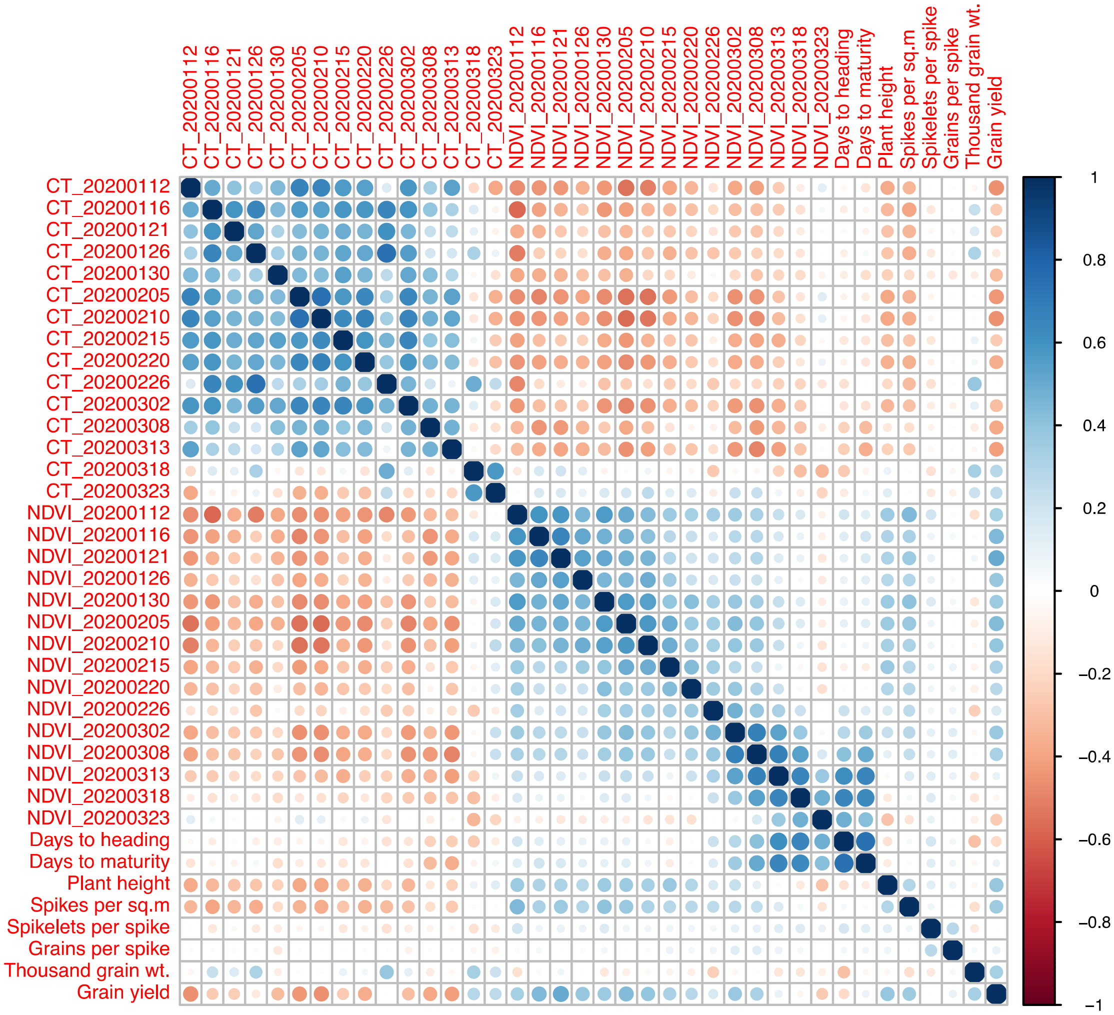

Figure A.5. Correlation matrix of all traits in 2019-20 season. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in Jamalpur, Bangladesh

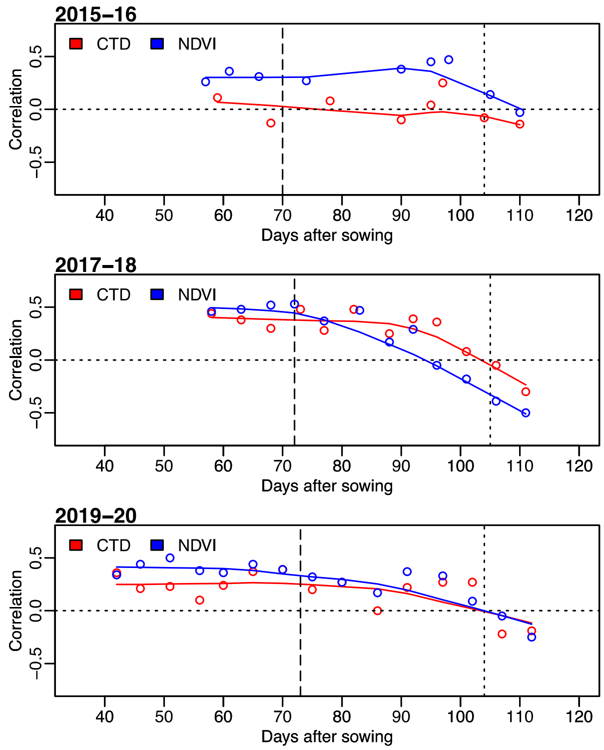

Figure A.6. Correlation of CTD at different observation days with grain yield in five wheat growing seasons.

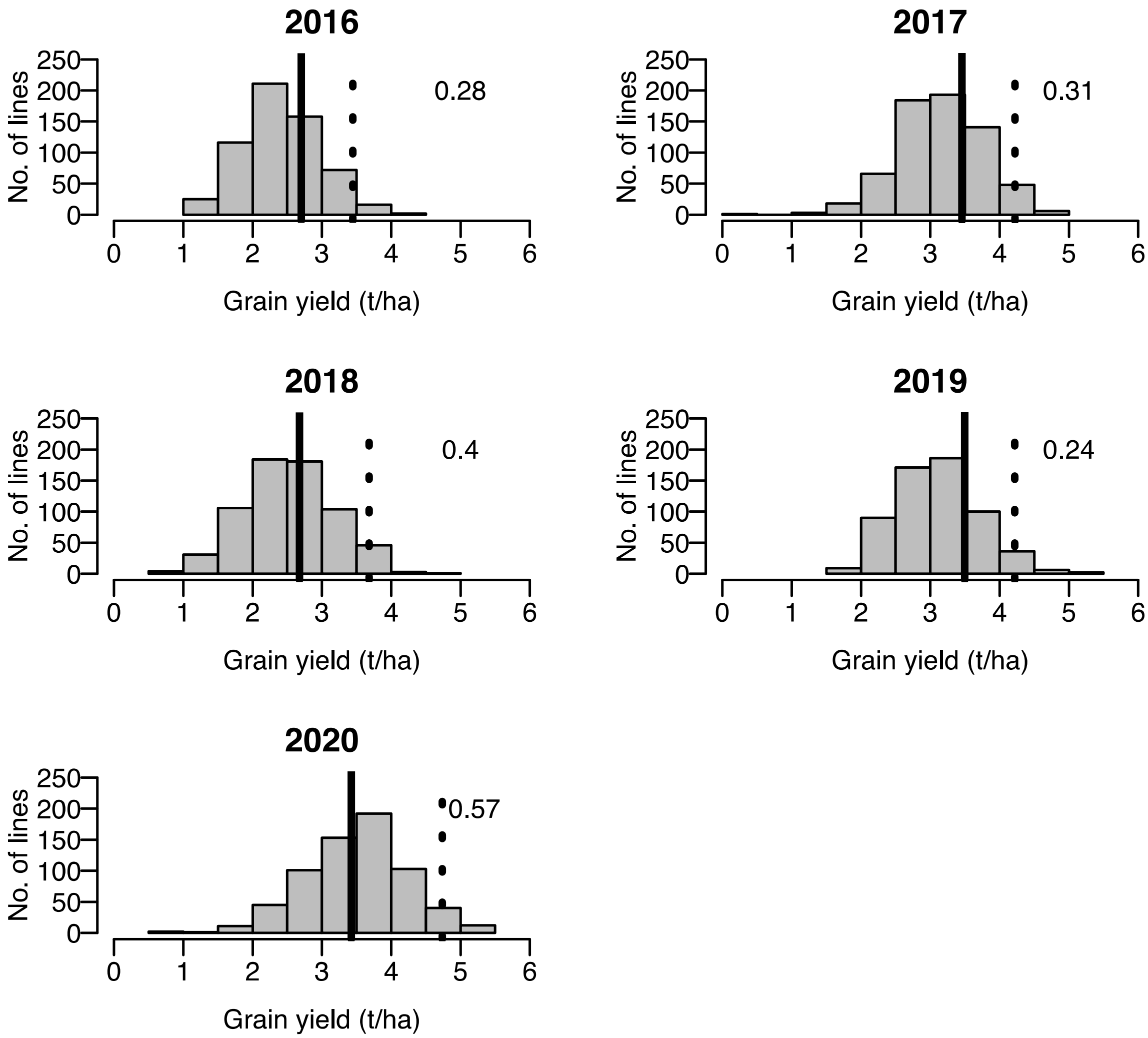

Figure A.7. A panel plot of the selected lines from genomic prediction trials in five wheat growing season. The solid line is the average grain yield of the national check variety in the trials, which was BARI Gom 26 as local check in 2016 - 2018 and BARI Gom 30 in 2019 and 2020 season. We selected 59 top yielder lines in each year. The dotted line is the average of the selected 59 lines from each year. The text number is the fraction (percentage) of the lines that superseded the yield of the check variety.

Table A.1. Broad see heritability for the 2015-16 growing season for phenotypic data. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in multiple yield trials (1-10) in Jamalpur, Bangladesh.

Traits	Trial_1	Trial_2	Trial_3	Trial_4	Trial_5	Trial_6	Trial_7	Trial_8	Trial_9	Trial_10
CT_20160123	0.00	0.00	0.00	0.07	0.00	0.10	0.00	0.00	0.16	0.00
CT_20160204	0.28	0.00	0.00	0.00	0.26	0.39	0.00	0.27	0.04	0.09
CT_20160212	0.00	0.10	0.21	0.33	0.23	0.26	0.27	0.20	0.15	0.00
CT_20160223	0.23	0.09	0.06	0.00	0.00	0.00	0.00	0.03	0.00	0.03
CT_20160228	0.09	0.00	0.20	0.12	0.00	0.43	0.00	0.00	0.00	0.12
CT_20160302	0.53	0.44	0.43	0.00	0.58	0.56	0.28	0.06	0.00	0.50
CT_20160309	0.21	0.00	0.12	0.00	0.21	0.22	0.00	0.17	0.00	0.19
CT_20160315	0.01	0.37	0.01	0.00	0.00	0.03	0.00	0.00	0.31	0.00
NDVI_20160121	0.19	0.00	0.42	0.00	0.00	0.11	0.00	0.33	0.41	0.34
NDVI_20160130	0.21	0.00	0.22	0.06	0.57	0.36	0.00	0.00	0.20	0.27
NDVI_20160203	0.00	0.00	0.21	0.08	0.00	0.48	0.00	0.46	0.00	0.64
NDVI_20160207	0.02	0.06	0.00	0.09	0.38	0.00	0.00	0.00	0.24	0.00
NDVI_20160223	0.49	0.14	0.06	0.29	0.00	0.00	0.40	0.26	0.21	0.00
NDVI_20160228	0.68	0.51	0.68	0.64	0.60	0.71	0.46	0.73	0.44	0.67
NDVI_20160303	0.79	0.60	0.65	0.74	0.76	0.62	0.17	0.64	0.54	0.36
NDVI_20160310	0.00	0.00	0.35	0.00	0.08	0.14	0.42	0.40	0.00	0.26
NDVI_20160315	0.22	0.40	0.00	0.28	0.35	0.30	0.00	0.30	0.12	0.30
Days to Heading	0.98	0.93	0.96	0.91	0.97	0.94	0.96	0.90	0.91	0.91
Days to Maturity	0.80	0.84	0.81	0.66	0.78	0.52	0.50	0.67	0.59	0.55
Plant Height	0.00	0.44	0.64	0.52	0.75	0.50	0.00	0.49	0.63	0.68
Spike Number	0.91	0.83	0.75	0.84	0.74	0.78	0.86	0.64	0.24	0.74
Spike length	0.36	0.38	0.36	0.43	0.42	0.32	0.21	0.68	0.26	0.54
Spikelets per spike	0.47	0.34	0.46	0.27	0.00	0.00	0.51	0.37	0.34	0.31
Grains per spike	0.86	0.71	0.84	0.84	0.82	0.84	0.92	0.74	0.70	0.77
Thousand grain wt.	0.61	0.40	0.42	0.51	0.38	0.62	0.68	0.00	0.38	0.35
Grain yield	0.73	0.58	0.78	0.76	0.65	0.64	0.40	0.73	0.90	0.78

Table A.2. Broad see heritability for the 2016-17 growing season for phenotypic data. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in multiple yield trials (1-11) in Jamalpur, Bangladesh.

Traits	Trial_1	Trial_2	Trial_3	Trial_4	Trial_5	Trial_6	Trial_7	Trial_8	Trial_9	Trial_10	Trial_11
CT_20170104	0.19	0.00	0.23	0.00	0.00	0.00	0.33	0.19	0.45	0.26	0.04
CT_20170109	0.10	0.23	0.12	0.38	0.17	0.15	0.16	0.45	0.28	0.01	0.00
CT_20170114	0.00	0.00	0.00	0.01	0.18	0.04	0.00	0.29	0.12	0.11	0.00
CT_20170120	0.07	0.11	0.27	0.42	0.10	0.00	0.33	0.00	0.00	0.31	0.00
CT_20170125	0.00	0.00	0.14	0.10	0.41	0.00	0.06	0.17	0.00	0.00	0.00
CT_20170131	0.00	0.00	0.00	0.00	0.52	0.00	0.00	0.00	0.15	0.00	0.13
CT_20170205	0.52	0.05	0.36	0.40	0.29	0.13	0.04	0.01	0.00	0.26	0.07
CT_20170210	0.05	0.00	0.24	0.17	0.24	0.11	0.23	0.34	0.18	0.56	0.15
CT_20170215	0.42	0.32	0.00	0.00	0.00	0.12	0.25	0.00	0.00	0.40	0.35
CT_20170221	0.31	0.30	0.32	0.42	0.00	0.00	0.53	0.35	0.00	0.24	0.00
CT_20170225	0.54	0.60	0.31	0.49	0.69	0.00	0.63	0.49	0.09	0.37	0.43
CT_20170302	0.61	0.60	0.64	0.73	0.62	0.72	0.69	0.43	0.25	0.55	0.39
CT_20170307	0.14	0.00	0.61	0.55	0.00	0.83	0.77	0.48	0.51	0.56	0.13
CT_20170313	0.00	0.05	0.09	0.19	0.00	0.48	0.00	0.00	0.00	0.00	0.32
NDVI_20170103	0.41	0.12	0.58	0.24	0.48	0.09	0.32	0.39	0.13	0.11	0.18
NDVI_20170108	0.66	0.40	0.63	0.41	0.45	0.08	0.44	0.59	0.00	0.09	0.15
NDVI_20170114	0.52	0.72	0.00	0.51	0.32	0.58	0.46	0.44	0.06	0.30	0.34
NDVI_20170120	0.52	0.56	0.20	0.65	0.18	0.47	0.42	0.21	0.00	0.35	0.28
NDVI_20170125	0.51	0.46	0.34	0.49	0.30	0.55	0.53	0.47	0.20	0.08	0.33
NDVI_20170131	0.36	0.44	0.12	0.53	0.03	0.58	0.29	0.38	0.29	0.00	0.35
NDVI_20170205	0.51	0.51	0.47	0.44	0.06	0.65	0.69	0.38	0.26	0.41	0.27
NDVI_20170210	0.54	0.25	0.53	0.40	0.60	0.52	0.42	0.44	0.00	0.09	0.20
NDVI_20170215	0.58	0.00	0.25	0.07	0.39	0.29	0.22	0.56	0.01	0.35	0.55
NDVI_20170220	0.72	0.03	0.64	0.54	0.65	0.63	0.59	0.49	0.00	0.33	0.30
NDVI_20170225	0.73	0.61	0.42	0.61	0.68	0.65	0.65	0.34	0.06	0.30	0.40
NDVI_20170302	0.77	0.64	0.80	0.50	0.35	0.66	0.78	0.76	0.43	0.74	0.59
NDVI_20170307	0.41	0.50	0.09	0.56	0.40	0.78	0.68	0.23	0.61	0.44	0.25
NDVI_20170313	0.29	0.13	0.43	0.00	0.41	0.79	0.47	0.59	0.63	0.55	0.15
Days to Heading	0.96	0.92	0.95	0.96	0.95	0.96	0.95	0.95	0.88	0.94	0.93
Days to Maturity	0.87	0.86	0.95	0.90	0.95	0.94	0.91	0.89	0.86	0.89	0.84
Plant Height	0.46	0.39	0.06	0.59	0.79	0.57	0.79	0.33	0.38	0.46	0.69
Spike Number	0.09	0.28	0.23	0.21	0.00	0.00	0.00	0.45	0.00	0.00	0.00
Spike length	0.14	0.36	0.03	0.54	0.44	0.29	0.00	0.25	0.38	0.37	0.76

Spikelets per spike	0.14	0.28	0.00	0.28	0.12	0.36	0.48	0.00	0.39	0.60	0.30
Grains per spike	0.53	0.10	0.26	0.32	0.48	0.36	0.44	0.14	0.30	0.44	0.34
Thousand grain wt.	0.69	0.72	0.20	0.70	0.74	0.05	0.75	0.85	0.00	0.53	0.72
Grain yield	0.78	0.71	0.74	0.76	0.75	0.73	0.59	0.70	0.53	0.53	0.20

Table A.3. Broad see heritability for the 2017-18 growing season for phenotypic data. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in multiple yield trials (1-11) in Jamalpur, Bangladesh.

Traits	Trial_1	Trial_2	Trial_3	Trial_4	Trial_5	Trial_6	Trial_7	Trial_8	Trial_9	Trial_10	Trial_11
CT_20180126	0.33	0.00	0.00	0.19	0.18	0.37	0.00	0.42	0.00	0.17	0.65
CT_20180131	0.39	0.00	0.22	0.20	0.00	0.55	0.00	0.36	0.05	0.12	0.45
CT_20180205	0.35	0.09	0.00	0.21	0.19	0.39	0.13	0.25	0.00	0.35	0.56
CT_20180210	0.39	0.00	0.00	0.33	0.08	0.32	0.00	0.00	0.00	0.29	0.41
CT_20180214	0.33	0.00	0.15	0.20	0.00	0.29	0.00	0.24	0.17	0.29	0.03
CT_20180219	0.31	0.00	0.13	0.40	0.00	0.26	0.00	0.14	0.00	0.27	0.46
CT_20180225	0.03	0.53	0.00	0.03	0.55	0.17	0.00	0.29	0.20	0.00	0.00
CT_20180301	0.12	0.35	0.00	0.18	0.60	0.14	0.00	0.03	0.17	0.26	0.00
CT_20180305	0.00	0.06	0.00	0.19	0.23	0.14	0.52	0.46	0.10	0.27	0.34
CT_20180310	0.02	0.00	0.13	0.57	0.65	0.32	0.00	0.13	0.20	0.25	0.00
CT_20180315	0.00	0.27	0.12	0.55	0.39	0.00	0.00	0.26	0.00	0.00	0.36
CT_20180320	0.21	0.33	0.29	0.44	0.00	0.17	0.00	0.00	0.00	0.46	0.00
NDVI_20180126	0.41	0.37	0.00	0.26	0.34	0.27	0.26	0.17	0.24	0.30	0.60
NDVI_20180131	0.20	0.27	0.17	0.43	0.16	0.00	0.16	0.00	0.01	0.13	0.70
NDVI_20180204	0.21	0.25	0.30	0.31	0.58	0.09	0.02	0.00	0.33	0.00	0.34
NDVI_20180210	0.41	0.38	0.50	0.58	0.65	0.14	0.32	0.00	0.00	0.32	0.00
NDVI_20180214	0.26	0.59	0.37	0.41	0.49	0.04	0.22	0.26	0.41	0.19	0.16
NDVI_20180219	0.48	0.52	0.26	0.00	0.00	0.49	0.00	0.00	0.10	0.00	0.23
NDVI_20180226	0.13	0.00	0.00	0.05	0.00	0.30	0.35	0.06	0.00	0.00	0.00
NDVI_20180301	0.33	0.68	0.45	0.47	0.78	0.46	0.00	0.16	0.20	0.00	0.14
NDVI_20180305	0.37	0.78	0.27	0.17	0.00	0.74	0.44	0.05	0.05	0.67	0.10
NDVI_20180310	0.43	0.68	0.52	0.72	0.71	0.70	0.66	0.80	0.61	0.71	0.56
NDVI_20180315	0.27	0.72	0.66	0.21	0.57	0.78	0.81	0.80	0.63	0.86	0.63
NDVI_20180320	0.00	0.29	0.14	0.00	0.40	0.44	0.26	0.55	0.00	0.15	0.30
Days to Heading	0.95	0.98	0.93	0.97	0.98	0.97	0.98	0.95	0.96	0.97	0.94
Days to Maturity	0.86	0.96	0.87	0.85	0.94	0.90	0.92	0.84	0.77	0.91	0.86
Plant Height	0.41	0.45	0.17	0.57	0.26	0.30	0.39	0.07	0.52	0.34	0.24
Spike Number	0.41	0.64	0.00	0.12	0.30	0.19	0.11	0.50	0.27	0.35	0.54
Spike length	0.12	0.45	0.44	0.37	0.49	0.26	0.42	0.46	0.00	0.46	0.11

Spikelets per spike	0.20	0.32	0.12	0.34	0.12	0.37	0.00	0.50	0.00	0.37	0.00
Grains per spike	0.00	0.51	0.36	0.41	0.00	0.41	0.00	0.14	0.00	0.10	0.31
Thousand grain wt.	0.39	0.71	0.65	0.54	0.75	0.68	0.64	0.50	0.52	0.70	0.63
Grain yield	0.28	0.63	0.68	0.80	0.71	0.54	0.67	0.08	0.33	0.71	0.66

Table A.4. Broad see heritability for the 2018-19 growing season for phenotypic data. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in multiple yield trials (1-10) in Jamalpur, Bangladesh.

Traits	Trial_1	Trial_2	Trial_3	Trial_4	Trial_5	Trial_6	Trial_7	Trial_8	Trial_9	Trial_10
CT_20190123	0.10	0.28	0.11	0.00	0.00	0.22	0.42	0.13	0.22	0.16
CT_20190127	0.12	0.01	0.31	0.29	0.15	0.03	0.34	0.33	0.34	0.16
CT_20190131	0.10	0.34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
CT_20190205	0.05	0.01	0.00	0.02	0.30	0.00	0.15	0.24	0.26	0.00
CT_20190211	0.00	0.44	0.22	0.28	0.00	0.38	0.28	0.21	0.32	0.05
CT_20190218	0.00	0.29	0.05	0.00	0.34	0.55	0.25	0.30	0.00	0.28
CT_20190223	0.00	0.00	0.00	0.00	0.00	0.20	0.04	0.00	0.00	0.00
CT_20190301	0.18	0.47	0.34	0.00	0.00	0.00	0.00	0.14	0.03	0.11
CT_20190305	0.00	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.01	0.00
CT_20190311	0.00	0.15	0.33	0.11	0.00	0.22	0.00	0.00	0.00	0.43
CT_20190316	0.00	0.06	0.09	0.00	0.37	0.19	0.34	0.05	0.47	0.48
CT_20190320	0.00	0.02	0.08	0.53	0.28	0.00	0.00	0.00	0.29	0.00
CT_20190325	0.27	0.18	0.00	0.19	0.37	0.36	0.00	0.03	0.00	0.00
NDVI_20190121	0.29	0.30	0.24	0.11	0.24	0.00	0.00	0.02	0.28	0.20
NDVI_20190127	0.29	0.16	0.23	0.08	0.30	0.30	0.29	0.01	0.00	0.22
NDVI_20190131	0.05	0.21	0.10	0.00	0.15	0.00	0.00	0.29	0.22	0.63
NDVI_20190205	0.04	0.12	0.14	0.06	0.00	0.38	0.11	0.04	0.00	0.00
NDVI_20190211	0.00	0.43	0.41	0.00	0.17	0.34	0.45	0.19	0.31	0.39
NDVI_20190218	0.16	0.22	0.46	0.00	0.14	0.05	0.00	0.26	0.27	0.54
NDVI_20190222	0.00	0.12	0.27	0.40	0.11	0.03	0.21	0.22	0.00	0.00
NDVI_20190228	0.44	0.07	0.01	0.00	0.00	0.00	0.00	0.00	0.17	0.15
NDVI_20190305	0.58	0.86	0.45	0.43	0.50	0.05	0.56	0.29	0.35	0.44
NDVI_20190311	0.81	0.82	0.78	0.55	0.75	0.84	0.76	0.69	0.56	0.73
NDVI_20190315	0.73	0.77	0.74	0.69	0.37	0.50	0.40	0.54	0.60	0.69
NDVI_20190320	0.26	0.06	0.44	0.40	0.68	0.49	0.66	0.65	0.01	0.77
NDVI_20190325	0.19	0.11	0.29	0.19	0.66	0.14	0.18	0.20	0.05	0.17
Days to Heading	0.94	0.97	0.94	0.91	0.97	0.94	0.86	0.92	0.90	0.95
Days to Maturity	0.89	0.88	0.93	0.88	0.89	0.87	0.82	0.91	0.85	0.91
Plant Height	0.37	0.11	0.24	0.47	0.61	0.00	0.00	0.32	0.34	0.01

Spike Number	0.06	0.00	0.17	0.00	0.10	0.11	0.28	0.00	0.09	0.16
Spike length	0.00	0.54	0.57	0.49	0.00	0.21	0.07	0.33	0.18	0.00
Spikelets per spike	0.15	0.00	0.42	0.00	0.09	0.41	0.00	0.00	0.00	0.26
Grains per spike	0.00	0.00	0.00	0.00	0.15	0.34	0.07	0.00	0.41	0.41
Thousand grain wt.	0.59	0.55	0.35	0.59	0.17	0.57	0.47	0.48	0.68	0.60
Grain yield	0.24	0.00	0.45	0.01	0.35	0.48	0.61	0.04	0.41	0.36

Table A.5. Broad see heritability for the 2019-20 growing season for phenotypic data. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in multiple yield trials (1-11) in Jamalpur, Bangladesh.

Traits	Trial_1	Trial_2	Trial_3	Trial_4	Trial_5	Trial_6	Trial_7	Trial_8	Trial_9	Trial_10	Trial_11
CT_20200112	0.00	0.00	0.31	0.00	0.00	0.00	0.00	0.00	0.17	0.34	0.01
CT_20200116	0.15	0.30	0.14	0.00	0.22	0.00	0.11	0.07	0.00	0.30	0.27
CT_20200121	0.00	0.16	0.19	0.01	0.00	0.00	0.32	0.36	0.03	0.31	0.00
CT_20200126	0.12	0.24	0.10	0.00	0.00	0.20	0.00	0.01	0.11	0.20	0.00
CT_20200130	0.01	0.30	0.27	0.35	0.00	0.00	0.22	0.00	0.00	0.08	0.32
CT_20200205	0.00	0.05	0.30	0.07	0.18	0.00	0.00	0.00	0.00	0.29	0.00
CT_20200210	0.20	0.00	0.44	0.03	0.00	0.00	0.07	0.00	0.00	0.00	0.23
CT_20200215	0.00	0.00	0.43	0.15	0.00	0.00	0.00	0.17	0.00	0.00	0.22
CT_20200220	0.40	0.00	0.26	0.07	0.00	0.07	0.00	0.00	0.12	0.20	0.02
CT_20200226	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.12
CT_20200302	0.54	0.12	0.11	0.20	0.00	0.00	0.00	0.30	0.00	0.14	0.08
CT_20200308	0.00	0.00	0.42	0.44	0.24	0.40	0.00	0.40	0.02	0.17	0.00
CT_20200313	0.05	0.04	0.28	0.12	0.00	0.37	0.19	0.29	0.37	0.04	0.00
CT_20200318	0.00	0.00	0.05	0.22	0.16	0.27	0.18	0.51	0.14	0.33	0.00
CT_20200323	0.00	0.00	0.30	0.33	0.00	0.45	0.41	0.00	0.00	0.28	0.11
NDVI_20200112	0.07	0.00	0.26	0.07	0.00	0.32	0.00	0.22	0.47	0.35	0.30
NDVI_20200116	0.27	0.24	0.41	0.16	0.00	0.13	0.13	0.06	0.25	0.00	0.26
NDVI_20200121	0.00	0.19	0.28	0.35	0.00	0.07	0.16	0.30	0.20	0.00	0.40
NDVI_20200126	0.12	0.37	0.06	0.00	0.06	0.19	0.14	0.00	0.09	0.00	0.14
NDVI_20200130	0.10	0.18	0.20	0.06	0.18	0.39	0.00	0.00	0.24	0.00	0.00
NDVI_20200205	0.00	0.21	0.00	0.22	0.37	0.13	0.00	0.22	0.24	0.03	0.16
NDVI_20200210	0.23	0.17	0.01	0.00	0.02	0.00	0.00	0.00	0.16	0.27	0.00
NDVI_20200215	0.00	0.05	0.00	0.41	0.12	0.00	0.09	0.06	0.20	0.00	0.00
NDVI_20200220	0.00	0.47	0.00	0.00	0.05	0.22	0.00	0.01	0.36	0.23	0.00
NDVI_20200226	0.10	0.21	0.17	0.00	0.00	0.33	0.00	0.22	0.53	0.48	0.17
NDVI_20200302	0.10	0.30	0.29	0.00	0.09	0.43	0.40	0.56	0.35	0.48	0.07
NDVI_20200308	0.41	0.28	0.46	0.06	0.20	0.47	0.50	0.53	0.64	0.52	0.29
NDVI_20200313	0.59	0.42	0.67	0.62	0.57	0.36	0.69	0.53	0.54	0.65	0.10
NDVI_20200318	0.21	0.70	0.58	0.75	0.64	0.65	0.84	0.82	0.57	0.51	0.18
NDVI_20200323	0.18	0.28	0.39	0.57	0.34	0.38	0.81	0.70	0.33	0.53	0.00

GrndCov_20200112	0.30	0.23	0.17	0.27	0.00	0.32	0.00	0.12	0.00	0.00	0.58
GrndCov_20200206	0.07	0.18	0.15	0.00	0.23	0.00	0.00	0.56	0.33	0.00	0.44
DLA_Feb26	0.45	0.64	0.74	0.71	0.37	0.32	0.49	0.56	0.71	0.47	0.29
DLA_Mar09	0.27	0.40	0.56	0.43	0.69	0.46	0.66	0.55	0.45	0.66	0.69
Days to Heading	0.97	0.94	0.95	0.97	0.95	0.93	0.96	0.96	0.96	0.96	0.95
Days to Maturity	0.88	0.81	0.92	0.95	0.90	0.88	0.86	0.87	0.86	0.83	0.57
Plant Height	0.22	0.35	0.76	0.37	0.47	0.25	0.53	0.52	0.52	0.25	0.47
Spike Number	0.29	0.00	0.01	0.20	0.30	0.62	0.13	0.00	0.40	0.00	0.00
Spikelets per spike	0.20	0.00	0.37	0.02	0.28	0.00	0.45	0.31	0.00	0.22	0.17
Grains per spike	0.23	0.06	0.06	0.28	0.00	0.40	0.01	0.24	0.00	0.23	0.21
Thousand grain wt.	0.48	0.22	0.33	0.58	0.57	0.65	0.37	0.53	0.68	0.00	0.32
Grain yield	0.09	0.18	0.37	0.67	0.09	0.34	0.70	0.51	0.45	0.12	0.17

Table A.6. Correlation between grain yield and phenotypic traits for the 2015-16 season. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in multiple yield trials (1-10) in Jamalpur, Bangladesh

Traits	Trial_1	Trial_2	Trial_3	Trial_4	Trial_5	Trial_6	Trial_7	Trial_8	Trial_9	Trial_10
CT_20160123	0.06	0.00	-0.21	-0.15	-0.16	0.00	0.00	0.12	0.12	0.12
CT_20160204	-0.03	-0.27 *	0.05	0.02	-0.04	0.07	-0.33 *	0.17	-0.04	0.14
CT_20160212	-0.25	0.05	0.04	-0.19	-0.13	0.15	-0.23	0.26 *	-0.10	-0.29 *
CT_20160223	0.09	-0.16	-0.29 *	-0.03	0.13	0.15	0.11	0.08	-0.06	-0.09
CT_20160228	-0.20	-0.22	-0.39 **	0.11	-0.25	-0.18	-0.02	0.09	0.04	-0.12
CT_20160302	-0.54 ***	-0.50 ***	-0.58 ***	-0.33 *	-0.46 ***	-0.35 **	-0.13	-0.04	0.00	-0.08
CT_20160309	-0.14	-0.21	-0.17	-0.21	-0.31 *	-0.04	-0.20	-0.26 *	-0.39 **	-0.23
CT_20160315	-0.02	0.21	-0.10	0.00	-0.15	0.01	-0.02	-0.09	-0.12	0.05
NDVI_20160121	0.24	0.33 **	0.23	-0.05	0.43 ***	-0.28 *	0.51 ***	0.03	0.29 *	0.16
NDVI_20160130	0.20	0.28 *	0.15	0.02	0.35 **	-0.16	0.31 *	-0.09	0.28 *	0.17
NDVI_20160203	0.32 *	0.29 *	0.24	-0.06	0.13	-0.36 **	0.29 *	0.03	0.26 *	0.58 ***
NDVI_20160207	0.23	-0.18	0.27 *	0.13	0.06	-0.02	0.33 *	-0.13	-0.10	-0.23
NDVI_20160223	0.47 ***	0.06	0.18	-0.24	-0.10	-0.02	0.02	-0.13	0.02	0.00
NDVI_20160228	0.30 *	0.46 ***	0.49 ***	0.38 **	0.28 *	0.34 **	0.58 ***	0.02	0.28 *	0.37 **
NDVI_20160303	0.34 **	0.40 **	0.49 ***	0.45 ***	0.48 ***	0.54 ***	0.45 ***	0.07	0.44 ***	0.17
NDVI_20160310	0.07	0.13	0.09	0.11	0.04	0.28 *	0.34 **	0.00	0.21	0.16
NDVI_20160315	-0.12	0.03	-0.05	-0.03	-0.21	0.14	-0.07	0.19	-0.04	0.07
Days to Heading	0.04	-0.03	0.04	-0.09	-0.24	0.00	0.05	0.06	-0.05	-0.21
Days to Maturity	0.28 *	0.29 *	0.29 *	0.26 *	0.19	0.34 **	0.28 *	0.04	0.37 **	0.00
Plant Height	0.13	0.19	0.27 *	0.33 *	0.30 *	0.46 ***	0.10	0.03	0.32 *	0.45 ***
Spike Number	-0.03	0.19	0.21	-0.03	0.16	0.03	0.4 **	-0.37 **	-0.04	0.48 ***
Spike length	-0.08	-0.22	-0.28 *	-0.21	0.19	-0.03	0.07	-0.01	0.12	0.01
Spikelets per spike	0.02	0.06	-0.03	0.05	0.28 *	0.33 **	0.03	0.00	0.22	0.09
Grains per spike	0.11	0.02	0.22	0.16	0.08	0.04	0.10	0.06	0.22	0.11
Thousand grain wt.	0.44 ***	0.47 ***	0.51 ***	0.42 ***	0.43 ***	0.45 ***	0.33 **	0.03	0.25	0.33 **

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the <0.001 probability level.

Table A.7. Correlation between grain yield and phenotypic traits for the 2016-17 season. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in multiple yield trials (1-11) in Jamalpur, Bangladesh

Traits	Trial_1	Trial_2	Trial_3	Trial_4	Trial_5	Trial_6	Trial_7	Trial_8	Trial_9	Trial_10	Trial_11
CT_20170104	0.05	-0.30 *	0.16	-0.39 **	-0.32 *	-0.18	-0.02	0.05	-0.29 *	-0.36 **	-0.35 **
CT_20170109	-0.05	0.01	-0.02	-0.35 **	-0.51 ***	-0.15	-0.12	0.21	-0.32 *	-0.33 **	-0.26 *
CT_20170114	-0.02	-0.20	-0.17	-0.29 *	-0.52 ***	-0.21	-0.26 *	0.03	-0.56 ***	-0.45 ***	-0.31 *
CT_20170120	0.05	-0.17	-0.04	-0.41 ***	-0.38 **	0.02	-0.09	0.05	-0.15	-0.12	-0.30 *
CT_20170125	-0.13	-0.06	-0.25	-0.34 **	-0.61 ***	-0.03	-0.12	-0.14	-0.33 **	-0.35 **	-0.08
CT_20170131	-0.11	-0.07	-0.07	-0.09	-0.33 **	-0.10	-0.18	0.13	-0.10	-0.22	-0.21
CT_20170205	-0.26 *	-0.13	-0.39 **	-0.34 **	-0.58 ***	-0.31 *	-0.27 *	-0.31 *	-0.53 ***	-0.52 ***	-0.36 **
CT_20170210	-0.32 *	-0.32 *	-0.13	-0.33 *	-0.62 ***	-0.30 *	-0.15	0.06	-0.38 **	-0.53 ***	-0.28 *
CT_20170215	-0.03	-0.31 *	-0.30 *	-0.25	-0.42 ***	-0.16	-0.15	-0.13	-0.38 **	-0.56 ***	-0.50 ***
CT_20170221	-0.28 *	-0.34 **	-0.36 **	-0.26 *	-0.63 ***	-0.12	-0.20	-0.14	-0.19	-0.47 ***	-0.46 ***
CT_20170225	-0.40 **	-0.31 *	0.05	-0.34 **	-0.45 ***	-0.10	-0.42 ***	-0.11	-0.31 *	-0.53 ***	-0.38 **
CT_20170302	-0.44 ***	-0.26 *	0.04	-0.32 *	-0.37 **	-0.19	-0.36 **	-0.03	-0.17	-0.31 *	-0.30 *
CT_20170307	0.10	-0.4 **	0.16	-0.04	-0.17	0.06	-0.02	0.08	0.02	-0.20	0.00
CT_20170313	-0.28 *	-0.29 *	-0.15	-0.05	-0.11	-0.01	0.01	0.14	0.21	-0.28 *	-0.18
NDVI_20170103	-0.03	0.07	-0.20	0.29 *	0.29 *	0.00	-0.23	-0.21	0.39 **	0.33 *	0.28 *
NDVI_20170108	0.00	0.23	-0.21	0.20	0.30 *	-0.02	-0.28 *	-0.11	0.48 ***	0.40 **	0.40 **
NDVI_20170114	0.00	0.32 *	-0.09	0.25	0.30 *	0.06	-0.14	-0.17	0.50 ***	0.44 ***	0.39 **
NDVI_20170120	0.08	0.23	0.01	0.26 *	0.35 **	0.26 *	-0.15	0.06	0.55 ***	0.47 ***	0.31 *
NDVI_20170125	0.09	0.30 *	-0.09	0.28 *	0.43 ***	0.12	0.11	-0.04	0.55 ***	0.46 ***	0.38 **
NDVI_20170131	-0.07	0.25	0.21	0.29 *	0.41 **	0.23	0.20	0.06	0.62 ***	0.54 ***	0.33 *
NDVI_20170205	0.17	0.23	0.22	0.28 *	0.43 ***	0.25	0.26 *	0.00	0.64 ***	0.56 ***	0.45 ***
NDVI_20170210	-0.01	0.35 **	-0.02	0.15	0.40 **	0.21	0.30 *	-0.07	0.36 **	0.36 **	0.29 *
NDVI_20170215	0.04	0.12	-0.21	-0.09	0.3 *	0.22	0.35 **	0.01	0.52 ***	0.56 ***	0.16
NDVI_20170220	0.12	0.36 **	-0.03	0.25	0.46 ***	0.00	0.30 *	0.10	0.22	0.43 ***	0.03
NDVI_20170225	0.42 ***	0.36 **	-0.16	0.33 **	0.21	0.10	0.30 *	0.03	0.22	0.28 *	0.07
NDVI_20170302	0.3 *	0.18	-0.21	-0.05	-0.10	-0.02	0.28 *	0.12	0.06	0.27 *	0.13
NDVI_20170307	0.07	0.10	-0.35 **	-0.09	-0.11	-0.18	0.08	0.20	-0.04	0.23	0.13
NDVI_20170313	-0.12	0.07	-0.35 **	-0.04	-0.22	-0.23	-0.10	-0.21	-0.26 *	0.14	0.14
Days to Heading	-0.31 *	-0.18	-0.57 ***	-0.25	-0.21	-0.61 ***	-0.28 *	-0.38 **	-0.26 *	-0.11	0.04

Days to Maturity	0.13	0.01	-0.41 **	0.08	-0.11	-0.22	0.08	-0.06	-0.14	0.08	0.01
Plant Height	0.18	0.12	-0.12	0.14	0.29 *	0.15	-0.01	0.18	0.44 ***	0.32 *	0.29 *
Spike Number	0.31 *	0.14	0.02	0.22	0.22	0.02	-0.11	-0.19	0.31 *	0.48 ***	0.35 **
Spike length	-0.01	-0.11	-0.27 *	-0.16	-0.13	-0.23	0.04	-0.12	0.11	0.14	0.11
Spikelets per spike	-0.01	-0.3 *	-0.24	-0.26 *	-0.07	-0.15	0.14	-0.07	0.01	0.09	0.31 *
Grains per spike	0.17	-0.04	-0.02	0.05	0.15	0.08	0.23	-0.13	0.33 *	0.21	0.22
Thousand grain wt.	0.56 ***	0.45 ***	0.53 ***	0.31 *	0.53 ***	0.51 ***	0.48 ***	0.48 ***	0.29 *	0.09	-0.03

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the <0.001 probability level.

Table A.8. Correlation between grain yield and phenotypic traits for the 2017-18 season. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in multiple yield trials (1-11) in Jamalpur, Bangladesh

Traits	Trial_1	Trial_2	Trial_3	Trial_4	Trial_5	Trial_6	Trial_7	Trial_8	Trial_9	Trial_10	Trial_11
CT_20180126	-0.37 **	-0.16	-0.41 **	-0.44 ***	-0.18	-0.57 ***	0.06	-0.29 *	0.08	-0.4 **	-0.51 ***
CT_20180131	-0.36 **	-0.03	-0.38 **	-0.51 ***	-0.06	-0.46 ***	-0.12	-0.06	0.01	-0.48 ***	-0.50 ***
CT_20180205	-0.31 *	-0.22	-0.32 *	-0.30 *	-0.14	-0.6 ***	-0.32 *	-0.40 **	-0.12	-0.45 ***	-0.58 ***
CT_20180210	-0.37 **	-0.28 *	-0.53 ***	-0.32 *	-0.07	-0.73 ***	-0.20	-0.29 *	-0.02	-0.23	-0.50 ***
CT_20180214	-0.43 ***	-0.30 *	-0.39 **	-0.41 **	-0.25	-0.61 ***	-0.28 *	-0.48 ***	-0.07	-0.43 ***	0.00
CT_20180219	-0.36 **	-0.39 **	-0.46 ***	-0.17	0.06	-0.59 ***	-0.15	-0.28 *	0.01	-0.39 **	-0.36 **
CT_20180225	-0.26 *	-0.12	-0.23	-0.21	-0.10	-0.64 ***	0.09	-0.24	-0.03	-0.10	-0.09
CT_20180301	-0.32 *	-0.17	-0.25	-0.19	-0.03	-0.45 ***	-0.11	-0.41 **	-0.30 *	-0.22	-0.28 *
CT_20180305	-0.26 *	-0.20	-0.05	-0.09	-0.12	-0.28 *	0.04	-0.37 **	0.06	-0.12	0.02
CT_20180310	-0.30 *	0.04	-0.32 *	-0.10	0.04	-0.42 ***	0.21	-0.01	0.02	-0.18	-0.17
CT_20180315	-0.24	-0.08	-0.23	0.12	-0.03	-0.12	0.16	0.01	0.13	-0.01	-0.19
CT_20180320	-0.09	-0.25	-0.28 *	0.11	0.06	0.24	0.28 *	0.06	-0.25	0.03	-0.17
NDVI_20180126	0.28 *	0.08	0.50 ***	0.43 ***	0.12	0.17	0.04	0.26 *	-0.07	0.28 *	0.56 ***
NDVI_20180131	0.28 *	0.21	0.38 **	0.42 ***	0.02	0.38 **	0.08	-0.06	-0.1	0.22	0.63 ***
NDVI_20180204	0.27 *	0.19	0.35 **	0.37 **	0.30 *	0.21	0.20	0.23	0.00	0.08	0.59 ***
NDVI_20180210	0.32 *	0.37 **	0.35 **	0.44 ***	0.35 **	0.62 ***	0.22	0.35 **	0.22	0.41 **	0.38 **
NDVI_20180214	0.42 ***	0.27 *	0.36 **	0.21	0.14	0.61 ***	0.29 *	0.27 *	-0.16	0.41 **	0.17
NDVI_20180219	0.34 **	0.00	0.35 **	0.02	0.14	0.58 ***	-0.09	0.47 ***	0.15	0.34 **	0.35 **
NDVI_20180226	0.06	0.09	0.03	-0.23	0.16	0.37 **	0.06	-0.15	-0.07	-0.24	0.19
NDVI_20180301	0.16	0.02	0.24	-0.04	-0.08	0.36 **	0.13	0.15	0.27 *	0.04	-0.03
NDVI_20180305	0.03	-0.06	0.07	0.03	0.19	0.10	0.05	0.08	-0.14	-0.24	0.04
NDVI_20180310	-0.28 *	-0.32 *	-0.03	-0.23	-0.24	-0.18	-0.09	-0.12	-0.18	-0.29 *	-0.03
NDVI_20180315	-0.35 **	-0.32 *	-0.13	-0.23	-0.23	-0.38 **	-0.29 *	-0.27 *	-0.22	-0.26 *	-0.35 **
NDVI_20180320	-0.20	-0.23	-0.29 *	0.01	-0.21	-0.49 ***	-0.25	-0.37 **	-0.26 *	-0.32 *	-0.48 ***
Days to Heading	-0.33 **	-0.45 ***	-0.31 *	-0.42 ***	-0.40 **	-0.5 ***	-0.36 **	-0.23	-0.45 ***	-0.40 **	-0.22
Days to Maturity	-0.19	-0.34 **	-0.08	-0.33 *	-0.25	-0.24	-0.14	-0.12	-0.29 *	-0.16	-0.12
Plant Height	0.31 *	0.12	0.53 ***	0.34 **	0.16	0.33 **	0.19	0.29 *	0.16	-0.13	0.19

Spike Number	0.42 ***	0.09	0.42 ***	0.42 ***	-0.01	0.22	0.09	0.06	0.39 **	0.39 **	0.53 ***
Spike length	0.18	-0.14	0.04	0.13	-0.28 *	0.18	0.15	0.37 **	0.23	0.05	-0.18
Spikelets per spike	0.05	-0.20	0.09	-0.18	-0.11	0.03	0.14	0.19	0.08	0.14	-0.06
Grains per spike	-0.10	0.19	-0.01	0.22	0.31 *	-0.06	0.07	0.33 *	0.00	0.09	-0.05
Thousand grain wt.	0.26 *	0.61 ***	0.45 ***	0.17	0.5 ***	0.39 **	0.45 ***	0.29 *	0.05	0.20	0.28 *

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the <0.001 probability level.

Table A.9. Correlation between grain yield and phenotypic traits for the 2018-19 season. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in multiple yield trials (1-10) in Jamalpur, Bangladesh

Traits	Trial_1	Trial_2	Trial_3	Trial_4	Trial_5	Trial_6	Trial_7	Trial_8	Trial_9	Trial_10
CT_20190123	-0.21	-0.24	-0.68 ***	-0.47 ***	-0.22	-0.40 **	-0.49 ***	-0.63 ***	-0.52 ***	-0.54 ***
CT_20190127	-0.26 *	-0.37 **	-0.76 ***	-0.45 ***	-0.37 **	-0.36 **	-0.62 ***	-0.68 ***	-0.53 ***	-0.54 ***
CT_20190131	-0.27 *	0.01	-0.30 *	-0.15	-0.16	-0.27 *	-0.18	0.09	-0.08	-0.08
CT_20190205	-0.15	0.02	-0.46 ***	0.10	-0.23	-0.08	-0.28 *	-0.48 ***	-0.50 ***	-0.30 *
CT_20190211	0.15	-0.14	-0.24	0.14	-0.37 **	-0.51 ***	-0.45 ***	-0.55 ***	-0.52 ***	-0.66 ***
CT_20190218	-0.22	0.03	-0.21	-0.11	0.10	-0.6 ***	-0.43 ***	-0.55 ***	-0.63 ***	-0.65 ***
CT_20190223	-0.07	-0.45 ***	0.04	0.02	-0.11	-0.33 *	0.18	-0.04	-0.11	0.11
CT_20190301	-0.13	-0.29 *	0.03	0.02	0.15	-0.31 *	0.30 *	0.13	-0.21	0.17
CT_20190305	-0.28 *	-0.22	-0.19	-0.29 *	-0.17	-0.31 *	-0.4 **	-0.36 **	-0.33 *	-0.53 ***
CT_20190311	0.00	-0.16	0.18	-0.44 ***	0.09	-0.27 *	-0.25	-0.60 ***	-0.06	-0.27 *
CT_20190316	0.10	-0.26 *	-0.24	-0.5 ***	-0.25	-0.01	-0.25	-0.32 *	-0.27 *	-0.22
CT_20190320	0.23	0.08	-0.02	-0.29 *	-0.08	0.01	0.04	-0.11	-0.24	-0.31 *
CT_20190325	-0.14	-0.20	0.42 ***	0.17	0.32 *	0.14	0.10	0.17	0.05	-0.06
NDVI_20190121	0.35 **	0.13	0.66 ***	0.57 ***	0.25	0.34 **	0.32 *	0.59 ***	0.47 ***	0.51 ***
NDVI_20190127	0.25	0.13	0.57 ***	0.56 ***	0.33 **	0.43 ***	0.43 ***	0.65 ***	0.47 ***	0.47 ***
NDVI_20190131	0.29 *	0.30 *	0.24	0.36 **	0.18	0.25	0.25	0.05	-0.01	0.24
NDVI_20190205	0.28 *	0.24	-0.01	0.45 ***	0.12	0.31 *	0.34 **	0.43 ***	0.38 **	0.18
NDVI_20190211	0.41 **	0.21	0.69 ***	0.46 ***	0.29 *	0.53 ***	0.55 ***	0.75 ***	0.62 ***	0.53 ***
NDVI_20190218	0.21	0.37 **	0.69 ***	0.4 **	0.04	0.56 ***	0.40 **	0.74 ***	0.68 ***	0.52 ***
NDVI_20190222	0.12	0.04	-0.02	-0.24	0.09	-0.3 *	0.13	-0.01	0.06	-0.06
NDVI_20190228	0.07	0.18	0.30 *	0.23	-0.10	-0.02	0.08	-0.10	0.05	0.35 **
NDVI_20190305	0.03	0.24	0.38 **	0.39 **	0.25	0.14	0.06	0.58 ***	0.38 **	0.29 *
NDVI_20190311	-0.15	0.18	0.06	0.28 *	0.10	0.06	-0.01	0.28 *	0.07	0.17
NDVI_20190315	-0.19	-0.11	-0.12	-0.10	-0.12	-0.15	-0.25	0.07	-0.10	-0.04
NDVI_20190320	-0.34 **	-0.30 *	-0.37 **	-0.24	-0.22	-0.29 *	-0.43 ***	-0.07	-0.24	-0.16
NDVI_20190325	-0.32 *	-0.09	-0.52 ***	-0.23	-0.24	0.20	-0.09	-0.23	-0.28 *	-0.15
Days to Heading	-0.33 *	-0.05	-0.37 **	-0.10	-0.08	-0.20	-0.37 **	-0.26 *	-0.35 **	-0.20
Days to Maturity	-0.24	0.10	-0.22	0.04	0.03	-0.12	-0.36 **	-0.18	-0.31 *	-0.12
Plant Height	0.42 ***	0.35 **	0.5 ***	0.47 ***	0.26 *	0.20	0.06	0.23	0.02	0.01
Spike Number	0.16	0.13	0.43 ***	0.08	0.23	0.33 *	0.39 **	0.06	0.48 ***	0.17
Spike length	-0.04	-0.04	0.05	0.25	-0.09	0.09	0.03	0.19	0.46 ***	0.12

Spikelets per spike	-0.01	-0.23	-0.12	0.10	-0.02	-0.26 *	-0.31 *	0.14	0.16	-0.12
Grains per spike	-0.01	0.19	0.33 *	0.14	0.26 *	-0.01	0.23	0.06	0.21	-0.04
Thousand grain wt.	0.27 *	0.21	0.07	0.14	0.11	0.37 **	0.03	0.02	0.09	0.01

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the <0.001 probability level.

Table A.10. Correlation between grain yield and phenotypic traits for the 2019-20 season. Data include normalized difference vegetation index (NDVI) and canopy temperature (CT) measured at multiple times across the growing season (date of measurement) and agronomic traits for wheat grown in multiple yield trials (1-11) in Jamalpur, Bangladesh

Traits	Trial_1	Trial_2	Trial_3	Trial_4	Trial_5	Trial_6	Trial_7	Trial_8	Trial_9	Trial_10	Trial_11
CT_20200112	-0.44 ***	0.01	-0.01	-0.11	-0.18	-0.07	-0.15	-0.19	-0.23	-0.23	-0.63 ***
CT_20200116	-0.11	-0.08	-0.24	-0.05	0.09	-0.09	-0.32 *	-0.21	-0.15	-0.03	-0.44 ***
CT_20200121	-0.28 *	0.05	-0.12	0.05	-0.20	-0.05	-0.13	-0.09	-0.17	-0.18	-0.48 ***
CT_20200126	-0.13	-0.15	-0.40 **	-0.25	0.12	0.10	-0.17	0.13	-0.20	-0.16	-0.46 ***
CT_20200130	-0.44 ***	-0.12	-0.07	-0.35 **	0.13	0.04	0.08	0.00	-0.22	0.12	-0.24
CT_20200205	-0.12	-0.29 *	-0.35 **	-0.43 ***	-0.28 *	-0.15	0.06	0.10	-0.06	-0.18	-0.31 *
CT_20200210	-0.44 ***	-0.53 ***	-0.42 ***	-0.49 ***	-0.22	-0.06	0.08	0.00	-0.22	-0.26 *	-0.34 **
CT_20200215	-0.08	0.00	-0.13	-0.25	-0.12	-0.04	0.22	-0.23	-0.31 *	-0.12	-0.13
CT_20200220	-0.07	-0.27 *	-0.24	-0.29 *	0.05	0.06	0.02	-0.17	-0.13	-0.27 *	-0.23
CT_20200226	-0.24	-0.12	-0.27 *	-0.20	-0.04	0.08	0.06	0.06	-0.21	-0.22	-0.26 *
CT_20200302	-0.42 ***	-0.29 *	-0.29 *	-0.32 *	-0.19	0.11	-0.01	-0.17	-0.24	-0.28 *	-0.36 **
CT_20200308	-0.18	-0.13	-0.09	-0.36 **	-0.17	0.15	0.07	-0.05	-0.40 **	-0.28 *	-0.36 **
CT_20200313	-0.21	0.07	-0.29 *	-0.17	-0.04	-0.11	-0.03	-0.08	-0.47 ***	-0.28 *	-0.42 ***
CT_20200318	0.08	-0.32 *	-0.22	-0.14	-0.02	-0.28 *	0.25	-0.03	-0.33 *	-0.17	-0.09
CT_20200323	0.23	-0.21	-0.11	-0.16	0.05	0.00	0.18	0.08	-0.20	-0.14	-0.18
NDVI_20200112	0.41 **	0.25	0.35 **	0.27 *	0.15	0.07	0.32 *	0.32 *	0.28 *	-0.01	0.56 ***

NDVI_20200116	0.16	0.22	0.18	0.08	0.14	0.07	0.04	0.29 *	0.10	0.14	0.65 ***
NDVI_20200121	0.37 **	0.30 *	0.50 ***	0.29 *	0.05	-0.07	0.21	0.55 ***	0.26 *	0.08	0.64 ***
NDVI_20200126	0.38 **	0.06	0.21	0.38 **	0.18	-0.11	0.05	0.30 *	0.14	0.18	0.38 **
NDVI_20200130	0.40 **	0.45 ***	0.33 *	0.35 **	0.21	0.03	0.25	0.31 *	0.26 *	0.22	0.65 ***
NDVI_20200205	0.45 ***	0.5 ***	0.29 *	0.31 *	0.45 ***	0.07	0.11	0.27 *	0.16	0.40 **	0.45 ***
NDVI_20200210	0.38 **	0.32 *	0.40 **	0.41 **	0.06	0.05	0.20	0.29 *	0.13	0.36 **	0.17
NDVI_20200215	0.24	0.35 **	0.28 *	0.68 ***	0.36 **	-0.11	0.06	0.16	0.32 *	0.37 **	0.29 *
NDVI_20200220	0.41 **	0.25	0.18	0.53 ***	0.21	0.01	0.42 ***	0.14	0.06	0.18	0.45 ***
NDVI_20200226	0.24	0.33 **	0.36 **	0.41 **	0.28 *	-0.22	0.35 **	0.35 **	0.25	0.37 **	0.57 ***
NDVI_20200302	0.14	0.42 ***	0.31 *	0.37 **	0.32 *	-0.29 *	0.38 **	0.37 **	0.32 *	0.34 **	0.31 *
NDVI_20200308	0.02	0.45 ***	0.13	0.19	0.13	-0.07	0.03	0.23	0.26 *	0.45 ***	0.23
NDVI_20200313	0.00	0.05	0.08	-0.13	0.00	-0.11	-0.34 **	0.10	0.14	0.19	0.07
NDVI_20200318	-0.19	-0.06	-0.05	-0.09	-0.04	-0.21	-0.49 ***	-0.03	-0.11	0.16	0.21
NDVI_20200323	-0.33 *	-0.09	-0.33 **	-0.20	0.03	0.11	-0.60 ***	-0.10	-0.02	0.07	-0.43 ***
GrndCov_20200112	0.28 *	0.16	0.45 ***	0.39 **	0.43 ***	0.02	0.12	0.44 ***	0.41 **	0.25	0.72 ***
GrndCov_20200206	0.29 *	0.24	0.25	0.45 ***	0.46 ***	0.14	0.07	0.39 **	0.42 ***	0.17	0.73 ***
DLA_Feb26	-0.22	-0.11	-0.20	-0.22	0.07	-0.24	0.38 **	0.09	-0.05	-0.31 *	-0.05
DLA_Mar09	0.14	-0.26 *	-0.02	-0.28 *	-0.02	-0.22	0.27 *	0.09	-0.06	-0.03	0.19

Days to Heading	-0.19	-0.03	-0.20	-0.41 **	-0.27 *	-0.46 ***	-0.62 ***	-0.03	-0.25	-0.08	0.12
Days to Maturity	0.07	0.11	-0.01	-0.32 *	-0.15	-0.03	-0.43 ***	0.08	0.06	0.08	0.20
Plant Height	0.42 ***	0.29 *	0.47 ***	0.34 **	0.20	0.19	0.44 ***	0.18	0.32 *	0.07	0.33 **
Spike Number	0.19	0.29 *	0.11	0.44 ***	0.25 *	0.15	0.37 **	0.44 ***	0.44 ***	0.25	0.63 ***
Spikelets per spike	0.21	0.20	0.21	-0.10	0.04	0.03	-0.09	-0.06	-0.07	-0.12	0.23
Grains per spike	0.06	0.35 **	0.31 *	0.21	0.14	0.01	0.11	0.12	-0.10	0.34 **	0.05
Thousand grain wt.	0.19	0.24	0.44 ***	0.42 ***	0.28 *	0.51 ***	0.33 *	0.16	0.28 *	0.07	-0.09

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the <0.001 probability level.

Table A.11. Selected lines from each year.

year	trial	plot	gid	DTHD	DAYSMT	PH	SN	SPLN	GRNSPK	TGW	GRYLD
2016	5	entry5051	7046872	68.5	104.9	109.3	307.9	16.1	43.1	45.3	4.40
2016	9	entry9040	7175897	73.6	105.6	96.1	325.0	18.4	49.1	34.7	4.03
2016	5	entry5001	7171325	62.2	103.0	99.5	204.2	18.5	55.9	37.8	3.91
2016	6	entry6028	7174167	67.1	105.6	103.7	353.0	19.1	38.9	43.8	3.86
2016	5	entry5041	7173767	72.0	106.9	107.4	353.2	15.2	42.2	34.3	3.78
2016	9	entry9030	7175853	69.4	103.6	101.6	318.0	19.8	47.6	33.9	3.73
2016	5	entry5050	7173922	70.3	104.0	92.9	312.8	16.1	40.9	36.3	3.70
2016	4	entry4012	7177875	71.7	107.9	98.2	288.3	17.0	56.0	31.7	3.65
2016	5	entry5037	7173722	70.9	105.4	105.2	291.0	17.2	40.1	41.9	3.64
2016	5	entry5003	7171329	71.2	106.5	106.0	276.2	15.3	48.1	37.7	3.63
2016	5	entry5054	6333158	65.3	106.0	99.7	352.0	16.0	45.7	33.4	3.62
2016	9	entry9025	7175837	71.1	103.5	102.2	263.0	21.3	61.7	31.4	3.61
2016	5	entry5030	7173536	71.5	106.4	107.0	320.5	16.7	46.7	31.9	3.58
2016	3	entry3052	7177666	73.1	107.5	102.8	215.9	17.9	48.2	41.7	3.56
2016	5	entry5031	7046418	71.5	107.3	93.2	335.3	14.3	38.9	33.4	3.54
2016	5	entry5048	7173851	69.9	104.5	106.1	340.5	16.3	45.8	34.6	3.51
2016	6	entry6024	7047297	65.5	103.7	105.1	277.8	16.0	47.6	38.3	3.51
2016	6	entry6031	7174267	74.1	106.0	93.1	282.8	18.4	43.1	41.9	3.51
2016	5	entry5059	7047125	67.5	102.5	108.3	289.0	16.8	47.7	33.5	3.50
2016	5	entry5002	0	70.4	106.1	106.9	274.5	15.7	46.7	47.9	3.49
2016	6	entry6006	7174105	68.9	105.8	101.1	281.8	18.1	41.2	46.0	3.48
2016	6	entry6016	7174130	68.4	106.0	98.5	238.8	19.3	49.7	37.8	3.48
2016	4	entry4046	7171133	75.7	107.4	96.0	299.9	16.7	51.8	38.7	3.47
2016	6	entry6032	7174269	70.5	105.7	100.7	259.7	18.1	48.1	39.0	3.47
2016	9	entry9048	7048441	69.3	104.0	85.9	271.0	15.3	51.0	36.0	3.46
2016	5	entry5027	7046390	68.7	106.4	100.2	325.9	16.4	44.4	31.9	3.43
2016	5	entry5038	7173723	71.4	107.6	92.1	240.1	16.2	44.2	46.0	3.43
2016	5	entry5043	7173803	67.8	106.0	98.6	290.3	19.6	50.4	38.1	3.43
2016	5	entry5055	7047009	68.2	101.9	106.2	309.4	14.1	44.5	35.9	3.43
2016	5	entry5019	7173500	66.8	100.6	104.1	312.5	17.0	39.3	38.5	3.41
2016	6	entry6046	7174321	73.1	105.6	107.8	296.3	17.9	51.1	29.8	3.40
2016	5	entry5028	7173528	69.6	105.9	100.9	351.1	17.5	42.3	33.8	3.38
2016	9	entry9001	7047937	69.4	102.6	103.8	249.0	17.3	49.3	36.9	3.38
2016	5	entry5020	7173511	66.8	100.6	98.1	324.0	16.4	42.8	43.0	3.37
2016	5	entry5023	7173521	73.9	107.6	92.0	279.3	16.2	47.2	35.2	3.37
2016	6	entry6035	7174271	74.4	106.0	105.1	381.5	18.3	43.1	39.0	3.35
2016	9	entry9002	0	69.0	105.2	103.3	261.5	18.6	53.9	44.2	3.35
2016	5	entry5015	7173488	70.5	106.4	106.3	265.5	16.3	43.9	29.4	3.34
2016	5	entry5033	5398530	76.7	107.8	109.8	337.4	15.8	37.4	35.0	3.34
2016	4	entry4051	7046099	75.3	108.0	98.4	312.6	20.1	52.7	31.0	3.33
2016	9	entry9029	7175852	69.4	102.6	99.8	268.5	16.4	45.5	28.7	3.33
2016	4	entry4020	7170548	74.1	107.9	105.5	293.5	17.1	44.0	36.3	3.32
2016	6	entry6015	7047225	67.5	105.8	105.2	297.8	16.5	46.8	31.5	3.32
2016	6	entry6002	0	70.8	106.0	101.5	364.3	16.7	46.3	45.7	3.31
2016	5	entry5005	7173426	70.9	103.6	104.9	345.5	12.9	34.0	30.4	3.28
2016	5	entry5053	7174031	71.3	106.4	104.7	272.2	15.5	46.8	36.7	3.28
2016	9	entry9020	7175794	69.5	103.4	96.4	315.5	16.9	55.8	33.7	3.27
2016	3	entry3051	7177664	71.2	104.0	98.4	240.1	13.4	34.8	40.8	3.24
2016	5	entry5016	7173494	69.4	103.2	103.2	289.0	16.5	46.1	33.0	3.24
2016	5	entry5022	7173519	70.1	105.2	96.4	266.6	14.6	47.1	46.9	3.22
2016	5	entry5029	7173534	71.4	106.0	107.3	275.4	16.8	48.9	28.5	3.22
2016	4	entry4009	7177872	72.9	107.1	96.7	271.7	17.8	47.4	37.9	3.21
2016	6	entry6029	7174195	74.5	105.0	100.8	256.7	17.1	50.2	21.8	3.21
2016	6	entry6053	7174342	70.0	106.1	95.5	338.6	16.7	48.4	32.7	3.21
2016	6	entry6056	7174347	69.6	105.8	105.0	245.8	17.1	48.4	34.0	3.20

2016	5	entry5014	7173484	72.0	106.9	99.8	355.0	16.6	48.8	30.7	3.19
2016	3	entry3055	7177673	73.4	107.4	97.8	231.3	15.4	43.0	39.9	3.18
2016	4	entry4041	7171098	70.3	106.3	94.3	312.7	17.3	50.3	35.8	3.18
2016	6	entry6050	7174332	71.5	106.2	103.3	221.2	19.0	53.0	32.2	3.18
2016	4	entry4060	7046215	66.2	104.2	92.3	232.4	16.7	53.0	37.7	3.17
2017	1	entry1051	7399180	67.4	111.5	101.0	440.1	15.7	43.6	35.8	4.69
2017	2	entry2049	7399623	69.4	110.3	103.9	346.2	17.0	51.2	34.2	4.64
2017	3	entry3059	7400239	68.2	111.9	98.5	328.3	17.7	56.1	35.6	4.60
2017	2	entry2046	7399616	69.8	110.4	97.8	414.9	16.1	44.6	35.8	4.56
2017	3	entry3036	7399947	61.4	109.1	99.0	407.6	15.9	53.6	37.8	4.56
2017	9	entry9048	7398471	66.5	107.0	101.8	370.0	15.1	41.4	37.7	4.55
2017	2	entry2041	7399609	61.7	104.2	102.9	325.9	13.7	47.5	43.0	4.48
2017	7	entry7002	0	62.5	108.5	105.6	372.0	17.3	41.2	46.6	4.46
2017	7	entry7026	6175067	67.2	110.0	108.6	252.2	19.2	58.1	39.5	4.46
2017	4	entry4045	7400456	65.3	108.7	102.0	437.4	18.5	65.9	32.5	4.45
2017	3	entry3035	7399946	67.5	104.8	97.5	336.7	16.9	52.8	40.4	4.43
2017	7	entry7016	7311105	68.1	109.5	101.9	367.1	17.6	54.4	40.2	4.40
2017	3	entry3026	6175067	67.9	109.4	104.0	293.9	20.8	52.5	40.1	4.39
2017	9	entry9029	7398428	67.5	107.5	109.5	247.1	18.5	52.2	45.2	4.37
2017	8	entry8032	7397762	68.0	107.8	104.7	297.3	16.8	57.7	46.7	4.30
2017	11	entry11037	7174105	69.9	109.0	98.7	250.7	19.1	43.1	50.0	4.30
2017	7	entry7042	7397189	75.9	112.5	120.6	280.2	19.9	46.6	36.3	4.29
2017	5	entry5040	7396142	70.0	112.3	107.2	365.5	18.5	56.8	41.8	4.29
2017	7	entry7008	7310918	70.8	112.0	102.2	245.7	17.6	55.8	44.7	4.28
2017	6	entry6041	7396710	68.9	109.5	105.6	328.4	18.2	46.3	39.0	4.27
2017	8	entry8002	0	65.4	109.6	109.5	324.0	17.8	47.4	44.3	4.26
2017	3	entry3042	7400069	63.0	106.2	106.0	319.2	17.2	44.3	42.2	4.23
2017	3	entry3050	7400194	62.8	103.8	99.0	412.1	17.3	40.9	40.4	4.23
2017	8	entry8004	7397520	63.0	109.2	105.0	383.3	20.4	61.4	40.5	4.22
2017	4	entry4020	7400308	61.0	107.2	96.0	364.3	17.0	50.1	34.7	4.21
2017	5	entry5050	7396176	70.0	111.7	101.9	309.4	16.6	46.7	42.4	4.21
2017	7	entry7020	7396931	69.0	110.0	103.8	391.6	19.9	55.7	37.1	4.21
2017	11	entry11051	7175837	72.1	109.3	112.6	267.7	19.3	47.9	42.0	4.20
2017	5	entry5036	7396135	68.5	109.9	96.7	343.9	17.0	39.3	40.6	4.20
2017	8	entry8053	7398192	68.0	110.7	108.9	304.2	19.3	49.1	35.9	4.20
2017	4	entry4044	7400453	66.4	107.6	103.0	273.6	18.0	57.1	32.5	4.19
2017	1	entry1050	7399179	66.0	108.1	101.0	432.1	17.6	48.3	36.6	4.19
2017	7	entry7033	6332122	69.3	111.0	105.0	324.0	19.4	52.0	41.3	4.18
2017	4	entry4056	7400488	57.3	105.3	89.5	379.2	15.6	43.4	38.7	4.16
2017	4	entry4022	7400313	59.0	106.1	99.5	388.7	16.8	46.6	40.9	4.15
2017	3	entry3038	7399956	60.7	107.0	103.5	427.6	18.8	55.6	35.0	4.15
2017	2	entry2006	7399442	69.5	109.8	101.6	395.9	16.7	54.4	35.6	4.14
2017	8	entry8056	7398211	70.3	110.3	110.0	316.4	20.3	56.3	43.9	4.14
2017	7	entry7005	7310902	71.0	109.0	99.6	299.0	21.6	60.4	35.8	4.12
2017	2	entry2048	7399621	67.7	109.9	99.6	437.1	15.9	43.7	35.1	4.12
2017	3	entry3037	7399950	68.5	107.8	94.5	370.1	18.5	56.0	35.8	4.12
2017	8	entry8038	7397893	67.5	106.0	106.3	306.7	18.8	52.9	39.1	4.11
2017	3	entry3016	7399823	70.6	111.4	99.0	298.6	18.6	59.5	39.0	4.10
2017	3	entry3057	7313697	72.8	113.6	101.5	327.2	18.2	40.0	40.4	4.10
2017	9	entry9012	7398326	69.5	110.5	108.5	357.3	18.8	53.4	39.2	4.10
2017	7	entry7036	7311300	74.3	111.0	112.4	314.7	21.0	57.5	36.2	4.09
2017	4	entry4019	7400306	62.5	107.7	102.5	285.3	16.3	47.5	36.7	4.08
2017	10	entry10005	7400872	65.7	108.5	93.6	339.7	18.5	46.1	46.0	4.07
2017	9	entry9003	7398243	63.5	104.0	107.1	315.2	17.0	48.3	40.3	4.05
2017	8	entry8043	7398014	67.5	108.9	101.4	334.3	19.3	48.2	38.5	4.04
2017	8	entry8001	7397501	62.5	107.6	106.6	308.4	18.0	46.4	37.9	4.04
2017	7	entry7041	7397183	70.6	116.0	105.1	315.8	18.5	51.1	40.0	4.02
2017	7	entry7021	7311134	68.7	110.0	106.3	282.3	21.4	69.2	38.4	4.02
2017	7	entry7015	7311059	64.4	108.5	103.9	309.4	15.7	41.0	43.0	4.01

2017	4	entry4026	6175067	65.7	111.2	101.0	338.0	18.3	56.5	38.4	4.00
2017	9	entry9031	7398433	67.0	108.5	110.8	345.5	19.7	49.8	40.4	4.00
2017	4	entry4050	7400466	70.4	111.1	98.5	365.5	19.5	57.7	33.8	3.99
2017	2	entry2045	7399615	67.9	108.8	97.4	389.2	17.2	51.0	32.2	3.99
2017	10	entry10039	6341870	75.3	114.0	95.8	312.5	16.0	47.3	39.8	3.99
2017	9	entry9005	7398254	71.0	109.0	106.4	256.9	18.1	51.2	43.8	3.98
2018	10	entry10001	7631563	67.0	107.5	82.0	371.6	16.8	46.3	42.9	4.78
2018	4	entry4042	7626450	68.4	111.2	98.5	328.1	15.8	50.6	40.8	4.24
2018	4	entry4044	7626460	64.5	104.8	89.9	290.2	15.7	49.8	41.3	4.06
2018	9	entry9016	7631006	77.4	111.5	94.5	347.6	19.4	57.8	44.0	4.06
2018	8	entry8031	7629982	77.2	113.5	95.3	254.1	18.4	57.4	45.3	3.96
2018	9	entry9033	6332122	73.3	111.0	89.5	266.7	16.7	46.4	45.5	3.91
2018	8	entry8039	6341870	77.1	112.9	88.4	243.1	19.3	59.2	43.5	3.89
2018	10	entry10041	7632416	72.1	108.4	86.0	229.5	18.2	48.5	45.3	3.86
2018	10	entry10016	7631846	68.5	108.8	90.3	240.0	18.7	41.0	51.2	3.85
2018	8	entry8060	7630530	73.5	111.0	87.4	256.9	17.0	41.4	48.5	3.85
2018	8	entry8046	7630341	77.3	112.5	100.2	283.2	23.2	63.6	43.5	3.80
2018	9	entry9046	7631434	73.2	110.5	91.0	378.5	20.4	57.8	45.0	3.80
2018	3	entry3045	7626319	69.6	109.2	98.3	299.0	13.9	49.4	50.8	3.80
2018	10	entry10009	7631640	76.5	110.1	86.7	296.6	17.6	52.9	44.9	3.79
2018	8	entry8016	7629772	75.7	111.5	91.0	299.0	18.3	57.6	47.5	3.79
2018	6	entry6005	7627721	74.1	111.0	95.2	230.5	20.6	56.3	42.8	3.77
2018	9	entry9006	7630704	78.1	111.5	86.5	303.7	19.8	45.2	35.3	3.74
2018	6	entry6015	7627903	67.3	110.5	90.3	212.5	17.3	57.6	43.5	3.74
2018	8	entry8040	7630179	70.5	112.0	92.5	264.9	17.8	52.7	50.3	3.72
2018	10	entry10048	7632462	70.5	110.9	91.4	232.3	17.6	47.6	45.2	3.72
2018	10	entry10005	7631608	79.0	113.6	87.5	248.9	17.1	41.1	40.9	3.71
2018	4	entry4050	7626569	80.5	114.6	97.6	239.4	19.3	51.0	37.7	3.70
2018	4	entry4052	7626572	70.9	111.8	97.3	217.4	19.9	55.0	46.8	3.68
2018	10	entry10026	6175067	72.4	111.2	88.3	244.6	19.5	51.4	48.6	3.67
2018	8	entry8032	7630037	77.3	112.5	98.6	297.2	16.4	58.9	43.3	3.67
2018	3	entry3005	7626072	77.1	112.6	94.2	243.5	22.6	59.1	40.8	3.66
2018	9	entry9030	7631193	69.9	109.0	92.7	278.7	18.9	45.4	44.5	3.66
2018	9	entry9018	7631063	72.7	111.5	90.1	220.9	18.0	49.0	41.5	3.65
2018	4	entry4031	7626416	75.4	110.6	91.6	194.2	19.2	49.8	47.5	3.65
2018	9	entry9009	7630834	69.5	110.0	89.3	320.2	18.1	47.7	44.5	3.64
2018	8	entry8007	7629741	69.0	110.5	87.8	287.0	16.9	45.4	45.0	3.63
2018	8	entry8030	7629913	76.3	112.4	89.4	278.0	17.1	47.2	40.3	3.62
2018	10	entry10006	7631614	80.0	115.9	88.5	228.3	15.6	39.9	41.7	3.62
2018	8	entry8011	7629752	70.6	109.4	86.0	239.8	15.8	51.8	45.3	3.62
2018	8	entry8015	7629766	72.2	110.5	91.4	306.3	19.9	46.9	47.3	3.60
2018	8	entry8035	7630047	76.3	111.0	92.3	236.8	18.9	55.6	51.0	3.60
2018	8	entry8005	7629732	71.8	109.0	84.7	270.2	18.2	45.8	45.5	3.59
2018	9	entry9015	7630977	71.7	111.5	87.7	265.8	17.7	50.9	41.8	3.59
2018	4	entry4043	7626451	75.4	109.7	91.2	301.4	14.5	39.8	38.6	3.59
2018	10	entry10043	7632444	70.0	108.1	88.9	272.0	20.3	57.4	39.7	3.58
2018	11	entry11029	7399601	78.1	111.0	90.8	314.5	15.4	41.1	45.4	3.57
2018	7	entry7031	7629318	75.2	111.5	95.5	284.0	17.8	52.9	41.8	3.56
2018	3	entry3041	7626305	77.5	113.1	96.4	223.0	19.4	55.3	50.5	3.56
2018	6	entry6032	7628201	73.3	109.5	90.7	188.6	19.1	53.7	46.5	3.55
2018	8	entry8057	7630477	76.2	111.0	87.6	265.2	17.6	46.2	47.8	3.54
2018	3	entry3015	7626094	71.5	108.9	93.7	269.0	20.6	46.4	43.5	3.52
2018	10	entry10004	7631568	72.5	109.0	87.9	360.0	19.8	45.1	39.5	3.51
2018	9	entry9008	7630830	69.4	108.5	85.6	302.6	14.5	46.8	45.8	3.51
2018	10	entry10045	7632450	69.0	108.7	88.5	237.2	17.0	43.1	51.9	3.50
2018	10	entry10003	7631566	75.5	111.5	84.5	242.6	16.6	42.9	43.7	3.50
2018	10	entry10023	7631994	68.5	106.4	84.2	264.9	19.2	51.5	48.2	3.50
2018	8	entry8048	7630352	77.1	112.0	96.7	299.3	22.4	55.0	42.5	3.50
2018	8	entry8026	6175067	71.9	110.0	89.8	245.5	19.6	55.0	46.3	3.50

2018	4	entry4060	7626689	77.8	112.7	92.6	316.7	17.2	51.2	45.5	3.49
2018	10	entry10029	7632194	71.9	106.8	83.6	267.7	18.7	42.1	39.9	3.47
2018	9	entry9048	7631438	73.3	110.0	94.2	271.5	18.0	53.8	43.5	3.47
2018	8	entry8038	7630077	75.6	111.9	90.4	343.1	18.8	56.6	39.3	3.46
2018	7	entry7009	7628883	70.6	106.0	88.3	208.5	18.7	44.0	51.4	3.46
2018	10	entry10010	7631716	77.6	111.3	93.8	269.7	18.3	54.0	39.3	3.45
2018	8	entry8047	7630348	75.7	112.0	93.9	266.6	21.2	61.3	45.0	3.44
2019	9	entry9055	8053864	79.5	116.3	93.9	169.0	20.6	52.1	47.8	5.25
2019	8	entry8035	8052004	75.4	116.0	99.1	156.0	18.5	49.2	40.1	5.16
2019	3	entry3020	8051113	73.8	113.9	105.0	251.9	16.4	52.5	40.9	4.97
2019	3	entry3058	8044990	74.8	115.2	98.5	182.5	20.0	53.3	42.3	4.94
2019	10	entry10034	8054309	80.3	118.0	93.0	149.0	16.2	36.5	44.0	4.88
2019	8	entry8025	8051868	72.8	106.0	97.4	148.5	17.5	49.9	48.0	4.62
2019	5	entry5028	8047924	74.9	113.0	100.3	169.0	17.5	60.7	48.4	4.59
2019	3	entry3034	8051237	81.8	118.2	95.5	193.0	18.1	55.0	43.4	4.57
2019	4	entry4039	6681676	72.6	112.7	105.8	163.6	18.3	49.3	46.8	4.41
2019	8	entry8007	8051474	77.2	117.1	105.7	176.5	21.5	47.4	41.3	4.40
2019	8	entry8009	8051496	80.4	118.6	100.3	166.5	17.6	43.7	42.5	4.39
2019	10	entry10026	6341870	79.4	118.0	90.3	144.1	20.2	56.8	44.8	4.37
2019	3	entry3039	6681676	71.0	113.0	96.0	172.4	15.3	53.3	44.1	4.36
2019	10	entry10046	8054553	70.8	107.0	95.6	158.1	17.0	41.5	49.6	4.36
2019	3	entry3047	8044862	78.9	116.7	101.0	185.9	19.7	58.5	42.9	4.36
2019	3	entry3050	8044907	74.4	112.6	87.5	203.6	16.1	50.6	41.2	4.35
2019	9	entry9045	8053288	78.0	114.6	98.2	194.0	17.1	41.2	46.4	4.32
2019	10	entry10060	8055317	79.9	117.0	97.3	163.5	19.5	56.0	44.6	4.30
2019	4	entry4017	6175067	74.2	111.6	107.3	149.8	19.9	60.0	48.6	4.28
2019	10	entry10039	6681676	72.4	114.0	97.8	136.2	16.0	43.7	42.5	4.27
2019	10	entry10005	8054147	79.8	118.0	102.9	141.2	16.0	48.1	43.3	4.26
2019	8	entry8003	8051443	74.6	114.9	101.3	160.5	19.3	48.8	49.0	4.26
2019	9	entry9002	7890127	66.5	108.4	89.2	162.0	17.3	47.7	52.8	4.26
2019	8	entry8002	7890127	66.0	111.0	93.8	154.0	19.0	45.5	49.0	4.25
2019	3	entry3048	8044864	80.2	117.8	89.5	207.7	18.7	55.0	38.1	4.24
2019	1	entry1015	8048881	80.0	115.7	86.0	175.2	21.1	51.2	37.3	4.23
2019	10	entry10035	8054321	79.5	115.0	95.7	169.3	18.3	49.0	49.8	4.18
2019	7	entry7039	6681676	73.7	115.1	100.5	154.6	16.7	46.0	41.7	4.16
2019	3	entry3011	8051039	78.3	115.5	96.0	105.2	18.6	64.8	41.2	4.14
2019	4	entry4056	8046775	76.6	114.4	101.2	168.3	15.7	49.0	53.4	4.13
2019	7	entry7049	8059712	72.0	108.1	90.0	161.1	16.9	37.8	44.0	4.13
2019	10	entry10036	8054354	73.6	109.5	91.4	171.6	19.4	54.3	46.6	4.12
2019	4	entry4055	8046733	75.8	111.4	98.5	136.1	16.0	45.4	50.6	4.11
2019	9	entry9057	8053942	76.5	114.1	94.1	155.0	19.0	42.8	51.0	4.08
2019	10	entry10014	8054192	81.6	118.0	87.8	168.8	19.3	47.4	45.1	4.08
2019	3	entry3017	6175067	75.1	112.5	101.5	161.9	20.0	61.0	47.2	4.07
2019	7	entry7011	8058242	81.1	118.0	102.5	166.2	18.7	61.2	45.8	4.07
2019	10	entry10043	8054496	75.7	113.5	91.0	161.0	17.4	47.3	44.1	4.06
2019	7	entry7022	8059025	75.3	114.5	100.0	171.3	14.7	56.2	39.5	4.05
2019	7	entry7038	8059211	77.2	112.1	103.5	154.6	18.8	52.8	47.6	4.04
2019	8	entry8026	6341870	80.0	119.0	96.6	136.0	17.2	55.2	45.0	4.03
2019	3	entry3040	8044697	76.6	115.0	94.0	198.5	20.9	66.0	48.1	4.03
2019	3	entry3010	8051036	77.7	113.7	100.0	175.7	17.8	52.8	42.4	4.03
2019	8	entry8010	8051507	81.2	118.4	105.8	136.5	19.5	47.4	38.8	4.03
2019	7	entry7005	8058152	74.0	112.0	88.0	161.4	18.2	52.8	49.9	4.00
2019	8	entry8023	8051812	75.8	114.5	98.9	153.5	19.9	46.3	42.5	4.00
2019	3	entry3051	8044909	76.8	113.2	98.5	182.4	19.7	50.5	45.9	3.99
2019	6	entry6032	8057419	80.7	118.1	97.3	190.6	15.0	58.8	45.1	3.99
2019	4	entry4046	8046693	82.6	119.0	102.9	152.1	17.7	34.9	40.8	3.99
2019	2	entry2026	6341870	77.0	118.2	92.6	258.6	18.6	61.3	49.1	3.94
2019	10	entry10031	8054238	81.3	118.5	101.0	158.5	19.5	64.6	47.3	3.94
2019	10	entry10025	8054214	81.5	118.5	90.4	152.6	17.0	56.8	42.5	3.93

2019	5	entry5016	8047605	73.9	117.0	95.3	195.8	18.3	51.8	42.6	3.92
2019	5	entry5032	8048083	78.7	115.7	101.5	156.7	19.9	52.8	48.3	3.91
2019	8	entry8030	8051926	74.4	113.1	102.8	162.5	18.7	45.8	50.0	3.90
2019	8	entry8001	8051423	81.1	118.9	96.3	146.5	21.2	43.8	42.3	3.88
2019	8	entry8016	8051574	78.2	116.9	94.2	163.5	18.4	52.2	45.3	3.87
2019	3	entry3054	7175970	74.6	110.7	103.5	153.9	20.1	62.2	43.3	3.86
2019	7	entry7052	8059717	75.2	117.0	98.0	175.0	17.9	48.6	49.4	3.85
2019	3	entry3056	8044987	77.4	116.8	99.0	159.9	21.7	50.9	48.2	3.85
2020	3	entry3054	7398245	70.9	108.0	107.3	344.6	21.2	66.8	52.4	5.33
2020	5	entry5031	8244791	73.0	108.4	97.8	322.9	16.8	51.7	50.3	5.16
2020	4	entry4046	8243776	68.0	107.3	99.3	424.5	16.5	62.1	45.4	5.16
2020	9	entry9025	8241671	70.0	108.4	98.0	354.5	16.3	42.8	44.6	5.11
2020	8	entry8031	8240862	71.6	112.3	92.5	346.9	18.5	57.0	49.5	5.10
2020	10	entry10025	8242637	72.3	114.7	96.5	363.2	17.2	53.4	54.4	5.10
2020	9	entry9011	8241417	69.0	113.3	95.6	372.5	17.2	42.0	52.0	5.08
2020	10	entry10002	304660	62.0	110.1	91.0	351.8	16.9	56.3	50.0	5.06
2020	7	entry7001	8247612	70.8	112.5	96.4	303.4	17.9	53.5	54.9	5.05
2020	7	entry7012	8239328	72.8	111.0	98.4	412.5	23.3	60.4	37.8	5.01
2020	9	entry9041	8241804	70.9	112.6	99.5	300.0	15.9	55.0	54.9	5.01
2020	9	entry9028	8241729	70.5	113.3	96.6	326.5	18.4	48.4	48.0	5.01
2020	9	entry9055	8242209	71.0	111.2	98.1	422.5	16.6	46.0	47.3	4.98
2020	7	entry7026	6175067	70.3	111.5	98.3	378.9	18.6	54.0	44.0	4.96
2020	7	entry7039	7400769	72.5	112.5	90.9	357.0	18.4	58.2	57.3	4.92
2020	4	entry4043	8243589	70.5	105.0	95.2	384.5	15.8	51.0	46.3	4.86
2020	8	entry8041	8241091	70.9	110.5	98.0	337.7	21.1	53.0	51.3	4.83
2020	8	entry8046	8241137	73.6	111.0	97.1	400.9	16.7	51.5	48.8	4.83
2020	9	entry9018	8241577	71.5	110.6	97.2	356.0	17.1	51.5	46.2	4.80
2020	3	entry3026	6175067	70.4	111.0	103.0	357.9	18.4	60.7	46.0	4.80
2020	10	entry10054	7398245	70.1	112.3	101.5	299.6	18.7	54.6	56.9	4.80
2020	10	entry10021	8242598	72.2	114.2	96.0	336.8	16.0	60.0	44.0	4.79
2020	9	entry9045	8241952	74.9	115.8	91.6	366.5	19.4	46.5	50.7	4.78
2020	9	entry9040	8241794	71.0	112.1	87.6	389.0	17.4	47.5	49.9	4.77
2020	8	entry8003	8240217	78.1	114.2	98.9	384.7	17.9	50.0	44.8	4.75
2020	9	entry9046	8241963	68.5	112.6	100.3	356.0	20.0	47.0	51.7	4.73
2020	9	entry9005	8241241	71.0	110.1	100.5	310.0	16.5	44.0	56.7	4.73
2020	8	entry8044	8241122	74.7	112.7	96.4	336.4	19.3	46.5	47.8	4.72
2020	8	entry8039	7400769	72.0	113.0	88.9	426.8	15.4	45.5	56.0	4.72
2020	9	entry9056	8242211	70.9	112.5	97.7	353.5	16.5	42.5	49.7	4.67
2020	4	entry4045	8243716	71.0	108.3	86.6	307.0	16.9	50.3	45.5	4.66
2020	11	entry11007	8048669	66.8	107.0	95.3	276.4	18.2	40.0	54.0	4.64
2020	9	entry9054	7398245	70.4	111.3	99.2	356.0	18.5	58.5	48.8	4.63
2020	9	entry9023	8241656	71.0	110.9	100.5	410.0	17.6	49.5	47.6	4.63
2020	9	entry9052	8242164	71.0	112.0	95.9	448.5	17.0	46.5	50.1	4.62
2020	6	entry6026	6175067	71.0	110.0	99.2	398.3	16.0	48.0	59.3	4.62
2020	10	entry10016	8242520	74.4	113.5	89.5	360.9	17.5	59.0	49.6	4.61
2020	8	entry8010	8240449	69.4	111.2	92.6	389.4	19.3	50.0	39.5	4.61
2020	10	entry10005	8242376	71.5	112.6	96.0	318.8	18.6	61.0	39.7	4.60
2020	7	entry7010	8239306	65.4	109.0	100.6	356.2	17.8	47.9	52.3	4.60
2020	8	entry8032	8240864	71.3	109.2	97.5	361.2	20.5	50.7	44.0	4.57
2020	11	entry11055	8054321	75.0	113.0	95.9	304.6	17.7	49.1	44.3	4.57
2020	4	entry4054	7398245	67.6	108.4	101.0	371.5	19.8	51.5	55.3	4.56
2020	8	entry8030	8240840	72.6	111.9	98.9	358.7	17.9	52.0	43.5	4.56
2020	7	entry7008	8239262	70.5	112.0	92.0	359.9	17.2	47.2	49.8	4.54
2020	8	entry8018	8240574	75.7	112.9	96.5	347.8	20.1	54.0	51.0	4.54
2020	6	entry6002	304660	62.8	106.5	92.1	363.3	17.5	46.6	47.7	4.53
2020	8	entry8040	8241055	71.9	111.5	95.1	430.0	18.7	54.5	46.3	4.52
2020	9	entry9010	8241408	72.0	113.4	95.2	294.5	20.0	60.5	45.0	4.52
2020	8	entry8025	8240790	71.4	107.2	99.5	384.9	15.4	52.0	44.3	4.51
2020	6	entry6030	8246525	70.7	110.0	100.3	416.2	16.2	46.0	40.8	4.51

2020	8	entry8036	8240873	68.4	109.6	93.1	376.6	15.8	54.3	50.0	4.51
2020	4	entry4037	8243467	71.0	110.4	98.7	424.5	18.9	45.6	40.2	4.50
2020	7	entry7005	8239230	72.0	112.0	89.2	378.5	16.1	52.5	64.1	4.50
2020	6	entry6039	7400769	72.5	114.5	97.3	420.6	18.5	48.7	45.5	4.49
2020	6	entry6005	8245477	70.8	110.5	95.1	359.8	18.5	46.5	39.2	4.49
2020	10	entry10009	8242393	73.7	112.0	91.0	332.1	16.7	58.0	48.9	4.48
2020	4	entry4044	8243684	81.5	115.6	92.6	370.0	19.0	56.4	42.0	4.48
2020	8	entry8007	8240287	72.5	113.3	97.4	344.0	18.7	43.7	41.3	4.47
2020	11	entry11023	8046597	70.5	110.0	88.7	376.6	18.3	49.1	50.3	4.46

Table A.12. Weather data during wheat growing season 2015-16 in Jamalpur, Bangladesh

November'2015								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	31	21	26	77	71	84	77.3	
2	29	21	25	84	61	84	76.3	
3	29	21	25	70	64	84	72.7	
4	29	21	25	70	61	84	71.7	
5	29	21	25	77	63	84	74.7	
6	32	18	25	77	65	84	75.3	
7	32	18	25	77	71	84	77.3	
8	31	18	24.5	69	59	77	68.3	
9	32	19	25.5	76	65	77	72.7	
10	32	18	25	77	58	77	70.7	
11	30	18	24	77	58	70	68.3	
12	30	18	24	70	58	70	66.0	
13	30	18	24	70	58	70	66.0	
14	31	18	24.5	73	84	70	75.7	
15	30	20	25	84	84	70	79.3	
1st fortnight	**30.47**	**19.20**	**24.83**	**75.20**	**65.33**	**77.93**	**72.82**	
16	30	20	25	84	64	70	72.7	
17	31	20	25.5	84	64	64	70.7	
18	30	19	24.5	76	64	70	70.0	
19	30	18	24	84	64	70	72.7	
20	31	17	24	84	64	84	77.3	
21	30	16	23	84	57	84	75.0	
22	31	16	23.5	84	58	83	75.0	
23	31	15	23	76	57	79	70.7	
24	31	15	23	75	63	69	69.0	
25	31	15	23	81	63	76	73.3	
26	30	15	22.5	81	63	66	70.0	
27	30	15	22.5	81	63	66	70.0	
28	29	15	22	81	63	66	70.0	
29	30	15	22.5	82	63	81	75.3	
30	30	17	23.5	90	63	81	78.0	
2nd fortnight	**30.33**	**16.53**	**23.43**	**81.80**	**62.20**	**73.93**	**72.6**	
Monthly	**30.40**	**17.87**	**24.13**	**78.50**	**63.77**	**75.93**	72.73	
December'2015								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	35	27	31	74	56	73	67.7	
2	35	26	30.5	86	56	73	71.7	
3	35	26	30.5	73	69	73	71.7	
4	35	25	30	79	58	73	70.0	
5	35	25	30	85	74	73	77.3	
6	35	25	30	79	68	78	75.0	
7	33	25	29	85	85	85	85.0	
8	33	25	29	78	85	85	82.7	5
9	33	25	29	77	85	85	82.3	
10	33	25	29	84	85	85	84.7	
11	33	24	28.5	85	85	85	85.0	
12	33	24	28.5	77	67	78	74.0	
13	34	24	29	77	51	78	68.7	
14	32	25	28.5	70	73	70	71.0	
15	34	24	29	77	73	85	78.3	
1st fortnight	**34.00**	**25.00**	**29.43**	**79.06**	**71.33**	**78.60**	76.33	5.00
16	34	24	29	85	73	85	81.0	
17	34	24	29	85	67	78	76.7	

18	34	24	29	85	67	85	79.0	
19	33	23	28	85	61	85	77.0	
20	33	24	28.5	85	61	85	77.0	
21	34	24	29	85	68	78	77.0	
22	33	24	28.5	85	72	85	80.7	
23	33	24	28.5	85	67	85	79.0	
24	32	21	26.5	85	61	85	77.0	
25	32	20	26	84	61	85	76.7	
26	34	19	26.5	84	61	77	74.0	
27	33	19	26	77	55	77	69.7	
28	33	19	26	84	55	77	72.0	
29	33	19	26	84	61	77	74.0	
30	34	20	27	84	61	77	74.0	
31	32	22	27	77	61	84	74.0	
2nd fortnight	**33.19**	**21.88**	**27.53**	**83.69**	**63.25**	**81.56**	**76.2**	**0.00**
Monthly	**33.59**	**23.44**	**28.48**	**81.37**	**67.29**	**80.08**	76.25	5.00
January'2016								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	27	16	21.5	98	62	82	80.7	
2	27	13	20	98	56	82	78.7	
3	26	12	19	81	68	91	80.0	
4	27	12	19.5	90	68	82	80.0	
5	26	12	19	81	68	82	77.0	
6	27	12	19.5	80	68	82	76.7	
7	27	12	19.5	81	68	80	76.3	
8	26	12	19	90	68	90	82.7	
9	23	15	19	90	68	90	82.7	
10	26	11	18.5	90	59	98	82.3	
11	24	12	18	88	59	98	81.7	
12	24	11	17.5	94	59	98	83.7	
13	25	11	18	94	66	90	83.3	
14	25	11	18	89	66	90	81.7	
15	25	10	17.5	89	59	91	79.7	
1st fortnight	**25.67**	**13.00**	**18.90**	**88.87**	**61.00**	**88.40**	80.47	
16	25	10	17.5	89	59	91	79.7	
17	24	11	17.5	89	66	91	82.0	
18	25	11	18	89	59	91	79.7	
19	25	10	17.5	90	73	91	84.7	
20	18	26	22	94	90	90	91.3	15
21	21	13	17	90	81	90	87.0	
22	21	11	16	90	81	98	89.7	
23	20	10	15	88	66	90	81.3	
24	19	10	14.5	88	80	98	88.7	
25	18	8	13	94	94	89	92.3	
26	20	9	14.5	93	80	89	87.3	
27	23	11	17	93	65	81	79.7	
28	25	11	18	89	73	81	81.0	
29	25	11	18	89	73	81	81.0	
30	24	13	18.5	89	73	81	81.0	
31	24	14	19	90	68	81	79.7	
2nd fortnight	**22.31**	**11.81**	**17.06**	**90.25**	**73.81**	**88.31**	**84.1**	**15.00**
Monthly	**23.99**	**12.41**	**17.98**	**89.56**	**67.41**	**88.36**	82.30	15.00
February'2016								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	23	12	17.5	90	50	57	65.7	
2	27	11	19	78	46	52	58.7	
3	29	12	20.5	64	55	60	59.7	

4	29	14	21.5	98	44	44	62.0	
5	27	11	19	81	50	35	55.3	
6	27	11	19	80	35	66	60.3	
7	25	11	18	90	60	62	70.7	
8	26	13	19.5	90	68	62	73.3	
9	26	13	19.5	98	61	53	70.7	
10	26	12	19	81	61	53	65.0	
11	26	11	18.5	81	54	53	62.7	
12	26	11	18.5	57	59	53	56.3	
13	26	12	19	80	59	53	64.0	
14	27	13	20	80	47	53	60.0	
15	26	13	19.5	81	55	60	65.3	
1st fortnight	**26.40**	**12.00**	**19.20**	**81.93**	**53.60**	**54.40**	63.31	0.00
16	29	13	21	65	51	61	59.0	
17	29	14	21.5	81	50	61	64.0	
18	29	13	21	81	63	64	69.3	2.5
19	29	13	21	90	68	68	75.3	23
20	29	18	23.5	90	84	84	86.0	
21	29	18	23.5	82	70	84	78.7	
22	28	17	22.5	82	64	84	76.7	
23	31	19	25	73	64	70	69.0	
24	30	20	25	82	59	64	68.3	
25	30	20	25	83	59	64	68.7	
26	30	20	25	91	64	64	73.0	
27	28	20	24	91	70	76	79.0	35.5
28	27	21	24	91	68	74	77.7	
2nd fortnight	**29.08**	**17.38**	**23.23**	83.23	64.15	70.62	72.7	61.00
Monthly	**27.74**	**14.69**	**21.22**	**82.58**	**58.88**	**62.51**	67.99	61.00
March'2016								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	32	18	25	81	65	76	74.0	
2	32	18	25	75	65	76	72.0	
3	32	18	25	75	65	78	72.7	
4	32	19	25.5	75	65	78	72.7	
5	32	20	26	75	62	78	71.7	
6	33	18	25.5	62	62	76	66.7	
7	33	20	26.5	75	59	70	68.0	
8	33	21	27	75	59	70	68.0	
9	33	21	27	75	62	70	69.0	
10	33	21	27	56	74	59	63.0	
11	33	21	27	64	68	59	63.7	
12	35	21	28	64	68	59	63.7	
13	29	21	25	76	63	53	64.0	
14	27	19	23	76	56	53	61.7	
15	33	19	26	76	64	53	64.3	
1st fortnight	**32.13**	**19.67**	**25.90**	**72.00**	**61.00**	**67.20**	67.67	
16	33	17	25	69	59	70	66.0	
17	34	21	27.5	62	49	64	58.3	
18	34	21	27.5	76	49	64	63.0	
19	33	22	27.5	76	62	65	67.7	
20	33	22	27.5	77	62	73	70.7	
21	35	20	27.5	84	62	73	73.0	
22	34	17	25.5	70	62	61	64.3	
23	34	16	25	62	63	70	65.0	
24	34	16	25	70	63	70	67.7	
25	34	21	27.5	70	63	70	67.7	
26	34	23	28.5	70	63	70	67.7	
27	33	21	27	70	73	70	71.0	

28	25	19	22	82	76	84	80.7	4
29	31	22	26.5	84	71	84	79.7	
30	34	20	27	84	71	84	79.7	5
2nd fortnight	**33.00**	**19.87**	**26.43**	**73.73**	**63.20**	**71.47**	**69.5**	**9.00**
Monthly	**32.57**	**19.77**	**26.17**	**72.87**	**62.10**	**69.33**	68.57	9.00
April'2016								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	31	18	24.5	66	73	91	76.7	
2	31	22	26.5	66	78	82	75.3	
3	29	26	27.5	81	83	81	81.7	
4	32	24	28	81	78	81	80.0	
5	33	23	28	82	79	62	74.3	
6	33	24	28.5	84	79	84	82.3	
7	34	24	29	77	69	77	74.3	
8	34	24	29	77	69	61	69.0	
9	36	26	31	77	69	61	69.0	
10	39	27	33	79	69	75	74.3	
11	37	27	32	78	69	80	75.7	
12	38	27	32.5	73	58	80	70.3	
13	37	27	32	73	58	73	68.0	
14	37	24	30.5	85	58	73	72.0	
15	33	24	28.5	76	61	73	70.0	
1st fortnight	**34.27**	**24.47**	**29.37**	**77.00**	**61.00**	**88.40**	74.20	
16	32	23	27.5	85	73	72	76.7	
17	33	23	28	84	73	70	75.7	
18	34	29	31.5	85	62	85	77.3	
19	36	25	30.5	85	68	85	79.3	
20	36	27	31.5	92	68	85	81.7	
21	38	27	32.5	79	63	85	75.7	
22	38	27	32.5	79	63	85	75.7	
23	39	28	33.5	79	63	85	75.7	
24	39	26	32.5	79	53	68	66.7	
25	37	26	31.5	68	53	78	66.3	
26	36	23	29.5	85	53	78	72.0	
27	36	23	29.5	85	53	78	72.0	
28	36	23	29.5	86	53	78	72.3	7.5
29	36	23	29.5	86	53	78	72.3	
30	36	23	29.5	78	73	78	76.3	
2nd fortnight	**36.13**	**25.07**	**30.60**	**82.33**	**61.60**	**79.20**	**74.4**	**7.50**
Monthly	**35.20**	**24.77**	**29.98**	**79.67**	**61.30**	**83.80**	74.29	7.50

Table A.13. Weather data during wheat growing season 2016-17 in Jamalpur, Bangladesh

Nov.2016								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	34	23	28.5	79	74	85	79.3	
2	34	23	28.5	79	74	74	75.7	
3	34	23	28.5	79	75	79	77.7	
4	30	22	26	84	85	92	87.0	
5	26	24	25	85	92	92	89.7	
6	29	19	24	84	85	84	84.3	
7	29	19	24	91	84	84	86.3	
8	29	19	24	91	61	85	79.0	
9	31	19	25	77	67	78	74.0	
10	31	19	25	77	67	78	74.0	
11	31	19	25	77	67	78	74.0	
12	31	19	25	77	67	78	74.0	
13	31	19	25	84	72	85	80.3	
14	31	18	24.5	84	72	85	80.3	
15	29	17	23	92	85	84	87.0	
1st fortnight	**30.67**	**20.13**	**25.40**	**82.67**	**75.13**	**82.73**	80.18	
16	31	16	23.5	92	71	84	82.3	
17	30	15	22.5	84	71	77	77.3	
18	30	15	22.5	84	71	84	79.7	
19	31	15	23	84	77	84	81.7	
20	29	15	22	83	87	84	84.7	
21	29	15	22	82	76	84	80.7	
22	24	15	19.5	82	76	83	80.3	
23	29	16	22.5	82	76	83	80.3	
24	29	16	22.5	82	76	83	80.3	
25	27	15	21	83	76	76	78.3	
26	29	16	22.5	83	84	83	83.3	
27	29	16	22.5	83	77	76	78.7	
28	29	16	22.5	76	70	76	74.0	
29	29	16	22.5	76	70	76	74.0	
30	30	17	23.5	91	59	76	75.3	
2nd fortnight	**29.00**	**15.60**	**22.30**	**83.13**	**74.47**	**80.60**	**79.4**	
Monthly	**29.83**	**17.87**	**23.85**	**82.90**	**74.80**	**81.67**	79.79	
Dec.'2016								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	0	17	8.5	91	59	77	75.7	
2	30	17	23.5	91	59	77	75.7	
3	30	17	23.5	91	59	77	75.7	
4	29	17	23	91	59	77	75.7	
5	29	17	23	83	76	84	81.0	
6	29	13	21	83	76	84	81.0	
7	30	13	21.5	74	70	84	76.0	
8	29	13	21	91	68	82	80.3	
9	28	13	20.5	82	83	82	82.3	
10	25	13	19	82	82	82	82.0	
11	27	14	20.5	82	84	84	83.3	
12	23	13	18	89	82	83	84.7	
13	25	13	19	89	82	82	84.3	
14	22	14	18	90	69	83	80.7	
15	27	14	20.5	98	63	83	81.3	
1st fortnight	**25.53**	**14.53**	**20.03**	**87.13**	**71.40**	**81.40**	**79.98**	
16	27	13	20	81	84	83	82.7	

17	27	16	21.5	81	84	83	82.7	
18	27	15	21	82	77	83	80.7	
19	26	15	20.5	92	76	83	83.7	
20	26	13	19.5	82	76	83	80.3	
21	27	16	21.5	82	76	83	80.3	
22	26	13	19.5	82	76	83	80.3	
23	27	16	21.5	82	76	83	80.3	
24	29	15	22	82	76	83	80.3	
25	28	16	22	82	77	83	80.7	
26	25	13	19	91	84	83	86.0	
27	25	15	20	82	75	83	80.0	
28	25	14	19.5	90	92	83	88.3	
29	25	13	19	94	91	83	89.3	
30	25	13	19	89	91	83	87.7	
31	25	13	19	89	91	83	87.7	
2nd fortnight	**26.25**	**14.31**	**20.28**	**85.19**	**81.38**	**83.00**	**83.2**	
Monthly	**25.89**	**14.42**	**20.16**	**86.16**	**76.39**	**82.20**	81.58	
Jan'2017								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	27	14	20.5	89	92	91	90.7	
2	28	15	21.5	89	84	84	85.7	
3	25	14	19.5	89	84	83	85.3	
4	25	15	20	89	68	68	75.0	
5	21	13	17	90	68	83	80.3	
6	21	13	17	94	75	82	83.7	
7	26	11	18.5	94	75	83	84.0	
8	26	12	19	90	75	83	82.7	
9	26	13	19.5	90	61	68	73.0	
10	18	11	14.5	95	90	89	91.3	5
11	23	11	17	94	68	75	79.0	
12	24	9	16.5	89	59	82	76.7	
13	20	7	13.5	90	59	66	71.7	
14	24	10	17	90	59	66	71.7	
15	23	9	16	90	59	66	71.7	
1st fortnight	**23.80**	**11.80**	**17.80**	**90.80**	**71.40**	**77.93**	**80.16**	**5.00**
16	24	9	16.5	90	59	66	71.7	
17	24	9	16.5	90	59	66	71.7	
18	25	12	18.5	90	59	91	80.0	
19	25	12	18.5	89	59	91	79.7	
20	25	11	18	89	59	82	76.7	
21	27	12	19.5	89	59	82	76.7	
22	27	13	20	90	69	75	78.0	
23	27	13	20	80	69	75	74.7	
24	28	13	20.5	80	63	68	70.3	
25	30	12	21	82	70	68	73.3	
26	29	13	21	80	64	70	71.3	
27	29	13	21	80	64	76	73.3	
28	23	12	17.5	90	15	69	58.0	2
29	24	13	18.5	95	75	69	79.7	
30	24	14	19	90	68	62	73.3	
31	25	12	18.5	90	68	70	76.0	
2nd fortnight	**26.00**	**12.06**	**19.03**	**87.13**	**61.19**	**73.75**	**74.0**	**7.00**
Monthly	**24.90**	**11.93**	**18.42**	**88.96**	**66.29**	**75.84**	77.09	**7.00**
February'2017								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	22	12	17	89	68	70	75.7	
2	22	12	17	94	69	70	77.7	

3	21	12	16.5	89	62	62	71.0	
4	22	13	17.5	89	62	75	75.3	
5	31	13	22	89	58	75	74.0	
6	31	15	23	62	64	70	65.3	
7	29	13	21	83	64	70	72.3	
8	28	13	20.5	81	70	68	73.0	
9	28	13	20.5	81	70	68	73.0	
10	28	13	20.5	81	70	68	73.0	
11	29	13	21	81	70	68	73.0	
12	29	15	22	81	70	75	75.3	
13	29	15	22	83	64	70	72.3	
14	29	15	22	83	64	70	72.3	
15	29	16	22.5	83	64	70	72.3	
1st fortnight	**27.13**	**13.53**	**20.33**	**83.27**	**65.93**	**69.93**	73.04	
16	29	16	22.5	75	64	70	69.7	
17	29	15	22	73	58	70	67.0	
18	31	15	23	73	58	70	67.0	
19	31	17	24	82	58	70	70.0	
20	31	16	23.5	82	92	70	81.3	
21	31	14	22.5	82	85	70	79.0	
22	31	18	24.5	82	79	85	82.0	6.5
23	31	18	24.5	76	79	85	80.0	
24	29	12	20.5	89	79	85	84.3	
25	28	13	20.5	89	64	69	74.0	
26	29	14	21.5	73	64	76	71.0	
27	29	15	22	73	63	69	68.3	
28	29	15	22	73	64	70	69.0	
2nd fortnight	**29.85**	**15.23**	**22.54**	78.62	69.77	73.77	74.1	6.50
Monthly	**28.49**	**14.38**	**21.44**	**80.94**	**67.85**	**71.85**	73.55	6.50
March'2017								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	31	19	25	81	65	70	72.0	
2	31	19	25	74	65	92	77.0	
3	31	19	25	74	62	66	67.3	
4	25	17	21	74	74	90	79.3	
5	26	18	22	95	95	81	90.3	1.5
6	31	16	23.5	83	70	68	73.7	
7	31	16	23.5	91	59	85	78.3	
8	29	18	23.5	82	70	85	79.0	
9	28	18	23	82	84	85	83.7	
10	31	18	24.5	82	76	85	81.0	
11	23	18	20.5	91	91	91	91.0	
12	29	14	21.5	82	75	75	77.3	
13	31	15	23	74	57	68	66.3	
14	30	12	21	74	56	75	68.3	
15	30	13	21.5	73	64	62	66.3	
1st fortnight	**29.13**	**16.50**	**22.90**	**80.80**	**71.40**	**77.93**	**76.73**	**1.50**
16	30	15	22.5	81	70	62	71.0	
17	32	19	25.5	66	70	75	70.3	
18	27	17	22	82	70	75	75.7	
19	31	18	24.5	84	71	75	76.7	
20	28	17	22.5	91	71	91	84.3	14
21	29	18	23.5	91	70	91	84.0	
22	30	20	25	91	77	83	83.7	
23	29	24	26.5	91	83	91	88.3	
24	32	18	25	70	78	83	77.0	
25	29	20	24.5	91	77	92	86.7	30
26	31	23	27	83	73	78	78.0	

27	33	21	27	85	62	78	75.0	
28	29	23	26	95	92	84	90.3	31
29	29	23	26	91	96	92	93.0	30
30	32	24	28	96	79	70	81.7	18.75
31	32	24	28	92	85	85	87.3	
2nd fortnight	**30.19**	**20.25**	**25.22**	**86.25**	**76.50**	**81.56**	**81.4**	**123.75**
Monthly	**29.66**	**18.38**	**24.06**	**83.53**	**73.95**	**79.75**	79.09	**125.25**
April'2017								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	33	24	28.5	92	85	86	87.7	
2	32	24	28	84	73	86	81.0	
3	32	21	26.5	84	74	79	79.0	35
4	32	20	26	91	84	83	86.0	
5	25	20	22.5	81	95	91	89.0	4
6	31	22	26.5	91	78	78	82.3	
7	32	22	27	91	78	78	82.3	
8	32	22	27	72	74	78	74.7	
9	31	21	26	85	81	85	83.7	
10	32	21	26.5	87	81	80	82.7	
11	33	21	27	85	81	80	82.0	
12	36	24	30	84	75	80	79.7	
13	36	24	30	85	69	80	78.0	
14	36	23	29.5	79	75	80	78.0	
15	35	23	29	92	74	80	82.0	65
1st fortnight	**32.53**	**22.28**	**27.33**	**85.53**	**78.47**	**81.60**	81.87	104.00
16	29	20	24.5	95	85	84	88.0	
17	34	20	27	95	86	84	88.3	
18	35	20	27.5	95	87	74	85.3	
19	32	20	26	95	92	91	92.7	16
20	28	20	24	95	84	92	90.3	65
21	26	20	23	95	95	92	94.0	
22	26	20	23	95	92	92	93.0	95
23	27	20	23.5	95	96	96	95.7	
24	28	20	24	95	92	92	93.0	10
25	31	20	25.5	95	92	84	90.3	
26	35	20	27.5	95	80	86	87.0	
27	36	20	28	95	81	80	85.3	
28	36	20	28	95	69	86	83.3	
29	33	20	26.5	95	69	86	83.3	
30	34	20	27	95	74	86	85.0	
2nd fortnight	**31.33**	**20.00**	**25.67**	**95.00**	**84.93**	**87.00**	**89.0**	**186.00**
Monthly	**31.93**	**21.14**	**26.50**	**90.27**	**81.70**	**84.30**	85.42	290.00

Table A.14. Weather data during wheat growing season 2017-18 in Jamalpur, Bangladesh

November'2017								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	31	20	25.5	99	78	84	87.0	
2	31	19	25	76	78	88	80.7	
3	33	20	26.5	99	76	85	86.7	
4	33	21	27	78	79	85	80.7	
5	33	20	26.5	85	78	85	82.7	
6	32	19	25.5	84	78	85	82.3	
7	33	22	27.5	84	79	85	82.7	
8	33	21	27	85	79	85	83.0	
9	31	19	25	84	78	85	82.3	
10	32	20	26	84	78	85	82.3	
11	33	21	27	84	86	85	85.0	
12	33	22	27.5	84	73	85	80.7	
13	32	22	27	84	85	85	84.7	
14	31	20	25.5	84	73	85	80.7	
15	24	21	22.5	83	84	91	86.0	
1st fortnight	**31.67**	**20.46**	**26.07**	**85.13**	**78.80**	**85.53**	83.16	
16	30	22	26	84	84	92	86.7	95
17	31	20	25.5	84	85	84	84.3	
18	31	21	26	84	85	85	84.7	
19	31	20	25.5	84	85	85	84.7	
20	30	21	25.5	84	85	85	84.7	
21	29	16	22.5	83	84	84	83.7	
22	27	15	21	82	84	84	83.3	
23	28	16	22	81	84	84	83.0	
24	29	16	22.5	82	84	84	83.3	
25	29	15	22	82	84	84	83.3	
26	27	16	21.5	82	84	84	83.3	
27	29	16	22.5	83	84	84	83.7	
28	27	16	21.5	83	84	84	83.7	
29	26	15	20.5	98	91	91	93.3	
30	27	15	21	98	92	84	91.3	
2nd fortnight	**28.73**	**17.33**	**23.03**	**84.93**	**85.27**	**85.20**	**85.1**	95
Monthly	**30.20**	**18.90**	**24.55**	**85.03**	**82.03**	**85.37**	84.14	95
December'2017								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	29	16	22.5	91	84	84	86.3	
2	28	15	21.5	82	84	84	83.3	
3	27	16	21.5	82	84	84	83.3	
4	28	14	21	82	84	84	83.3	
5	28	15	21.5	82	84	84	83.3	
6	27	15	21	91	84	83	86.0	
7	27	16	21.5	82	84	84	83.3	
8	27	17	22	82	84	84	83.3	
9	27	18	22.5	83	92	91	88.7	250
10	24	21	22.5	83	92	91	88.7	
11	27	18	22.5	91	84	84	86.3	
12	29	17	23	91	85	84	86.7	
13	29	19	24	91	84	92	89.0	
14	28	18	23	91	84	84	86.3	
15	27	14	20.5	91	83	83	85.7	
1st fortnight	**27.47**	**16.60**	**22.03**	**86.33**	**85.13**	**85.33**	**85.58**	**250.00**
16	27	12	19.5	82	92	91	88.3	

17	23	16	19.5	98	91	91	93.3	
18	24	17	20.5	98	83	91	90.7	
19	25	16	20.5	91	91	91	91.0	
20	28	15	21.5	98	84	83	88.3	
21	28	15	21.5	98	84	84	88.7	
22	27	14	20.5	91	84	84	86.3	
23	28	15	21.5	91	84	83	86.0	
24	27	14	20.5	91	84	83	86.0	
25	28	14	21	81	84	83	82.7	
26	27	15	21	91	84	84	86.3	
27	28	14	21	98	84	83	88.3	
28	28	13	20.5	90	82	91	87.7	
29	28	14	21	98	84	82	88.0	
30	27	14	20.5	91	84	84	86.3	
31	27	14	20.5	98	84	83	88.3	
2nd fortnight	**26.88**	**14.50**	**20.69**	**92.81**	**85.19**	**85.69**	**87.9**	
Monthly	**27.17**	**15.55**	**21.36**	**89.57**	**85.16**	**85.51**	86.74	**250.00**
Jan'2018								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	28	14	21	80	84	83	82.3	
2	22	12	17	89	81	91	87.0	
3	22	8	15	89	82	80	83.7	
4	23	10	16.5	88	81	80	83.0	
5	22	10	16	78	82	80	80.0	
6	20	9	14.5	89	82	81	84.0	
7	18	7	12.5	87	79	88	84.7	
8	18	6	12	77	80	89	82.0	
9	22	10	16	78	83	80	80.3	
10	22	9	15.5	88	80	78	82.0	
11	20	9	14.5	87	89	89	88.3	
12	20	8	14	87	79	89	85.0	
13	19	9	14	88	80	78	82.0	
14	17	10	13.5	88	80	89	85.7	
15	21	10	15.5	88	82	80	83.3	
1st fortnight	**20.93**	**9.40**	**15.17**	**85.40**	**81.60**	**83.66**	**83.56**	
16	20	10	15	88	41	80	69.7	
17	22	11	16.5	88	82	80	83.3	
18	22	12	17	88	83	81	84.0	
19	21	10	15.5	89	83	81	84.3	
20	23	8	15.5	78	82	80	80.0	
21	23	12	17.5	89	82	81	84.0	
22	19	13	27	89	83	81	27	
23	26	11	18.5	90	83	81	84.7	
24	19	13	16	90	76	82	82.7	
25	22	12	17	80	83	82	81.7	
26	22	10	16	79	84	81	81.3	
27	21	8	14.5	79	82	81	80.7	
28	23	9	16	89	82	81	84.0	
29	22	9	15.5	89	82	98	89.7	
30	23	12	17.5	89	82	98	89.7	
31	24	11	17.5	89	83	82	84.7	
2nd fortnight	**22.00**	**10.69**	**17.03**	**86.44**	**79.56**	**83.13**	**79.5**	
Monthly	**21.47**	**10.04**	**16.10**	**85.92**	**80.58**	**83.39**	81.51	
February'2018								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	24	11	17.5	90	83	82	85.0	
2	25	12	18.5	90	83	82	85.0	

3	25	11	18	90	83	91	88.0	
4	25	12	18.5	98	84	83	88.3	
5	26	13	19.5	91	84	83	86.0	
6	27	13	20	98	84	82	88.0	
7	27	14	20.5	81	84	82	82.3	
8	27	13	20	81	76	83	80.0	
9	27	13	20	91	84	83	86.0	
10	28	12	20	98	84	83	88.3	
11	26	12	19	82	76	83	80.3	
12	28	15	21.5	91	86	83	86.7	
13	28	15	21.5	91	70	84	81.7	
14	26	13	19.5	91	84	83	86.0	
15	27	13	20	91	86	83	86.7	
1st fortnight	**26.40**	**12.80**	**19.60**	**90.27**	**82.07**	**83.33**	85.22	
16	28	14	21	82	77	84	81.0	
17	29	14	21.5	82	84	84	83.3	
18	29	15	22	82	85	84	83.7	
19	30	17	23.5	91	78	84	84.3	
20	30	15	22.5	91	78	76	81.7	
21	30	15	22.5	91	78	84	84.3	
22	31	16	23.5	91	77	84	84.0	
23	31	16	23.5	83	78	84	81.7	
24	31	16	23.5	84	78	84	82.0	
25	32	18	25	84	79	85	82.7	
26	32	18	25	82	77	84	81.0	3.2
27	30	17	23.5	83	78	84	81.7	
28	30	17	23.5	91	72	77	80.0	
2nd fortnight	**30.23**	**16.00**	**23.12**	85.92	78.38	82.92	82.4	3.20
Monthly	**28.32**	**14.40**	**21.36**	**88.09**	**80.23**	**83.13**	83.82	3.20
March'2018								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	30	17	23.5	91	74	77	80.7	
2	31	18	24.5	85	78	84	82.3	
3	32	18	25	84	85	77	82.0	
4	34	21	27.5	84	79	78	80.3	
5	33	18	25.5	85	86	85	85.3	
6	33	16	24.5	84	78	85	82.3	
7	31	16	23.5	84	78	64	75.3	
8	32	16	24	76	73	71	73.3	
9	32	16	24	76	79	79	78.0	
10	33	16	24.5	84	79	78	80.3	
11	34	18	26	84	73	85	80.7	
12	34	19	26.5	84	79	78	80.3	
13	31	18	24.5	84	92	84	86.7	
14	31	20	25.5	84	92	84	86.7	
15	31	20	25.5	96	92	84	90.7	
1st fortnight	**32.13**	**17.80**	**24.97**	**84.33**	**81.60**	**79.53**	**81.67**	
16	32	20	26	84	79	84	82.3	
17	33	20	26.5	84	86	85	85.0	
18	34	19	26.5	84	79	78	80.3	
19	33	17	25	84	62	85	77.0	
20	34	18	26	84	73	85	80.7	
21	34	19	26.5	84	74	79	79.0	
22	35	19	27	84	80	79	27	
23	35	19	27	84	86	86	85.3	
24	36	21	28.5	85	92	85	87.3	9.5
25	35	19	27	92	80	79	83.7	
26	34	21	27.5	92	85	85	87.3	

27	32	21	26.5	92	92	92	92.0	
28	33	23	28	92	92	85	89.7	
29	34	18	26	84	86	79	83.0	17.45
30	33	17	25	91	86	85	87.3	12
31	33	16	24.5	89	85	85	86.3	22
2nd fortnight	**33.75**	**19.19**	**26.47**	**86.81**	**82.31**	**83.50**	**80.8**	**60.95**
Monthly	**32.94**	**18.49**	**25.72**	**85.57**	**81.96**	**81.52**	81.25	**60.95**
April'2018								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	33	20	26.5	84	92	85	87.0	
2	32	22	27	85	92	85	87.3	
3	34	21	27.5	85	78	85	82.7	
4	34	23	28.5	85	80	92	85.7	
5	33	22	27.5	92	81	85	86.0	2.75
6	35	22	28.5	92	85	85	87.3	
7	35	21	28	85	86	85	85.3	
8	31	21	26	85	86	85	85.3	
9	34	21	27.5	85	79	85	83.0	2.75
10	34	22	28	78	73	85	78.7	
11	34	22	28	85	80	86	83.7	
12	34	22	28	77	67	65	69.7	
13	34	21	27.5	77	67	72	72.0	
14	34	21	27.5	71	70	78	73.0	
15	35	23	29	71	73	67	70.3	25
1st fortnight	**33.73**	**21.60**	**27.67**	**82.47**	**78.47**	**81.66**	81.13	30.50
16	35	21	28	72	62	67	67.0	
17	31	20	25.5	91	91	71	84.3	25.95
18	34	22	28	77	67	70	71.3	
19	33	22	27.5	84	67	70	73.7	18.25
20	32	21	26.5	84	67	70	73.7	7.6
21	32	21	26.5	84	79	85	82.7	
22	31	23	27	84	79	78	80.3	
23	33	19	26	77	79	73	76.3	
24	34	21	27.5	78	80	79	79.0	27.25
25	34	21	27.5	71	58	85	71.3	2.5
26	34	21	27.5	77	61	84	74.0	0.25
27	34	20	27	77	85	85	82.3	1.5
28	34	21	27.5	85	73	84	80.7	
29	34	20	27	91	84	85	86.7	30
30	34	21	27.5	91	91	91	91.0	76
2nd fortnight	**33.27**	**20.93**	**27.10**	**81.53**	**74.87**	**78.47**	**78.3**	**189.30**
Monthly	**33.50**	**21.27**	**27.38**	**82.00**	**76.67**	**80.06**	79.71	**219.80**

Table A.15. Weather data during wheat growing season 2018-19 in Jamalpur, Bangladesh

Nov'2018								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	34	22	28	78	73	85	78.7	
2	34	23	28.5	77	62	72	70.3	
3	32	23	27.5	92	73	72	79.0	
4	32	21	26.5	92	73	78	81.0	
5	31	21	26	92	78	85	85.0	
6	31	20	25.5	84	78	85	82.3	
7	30	18	24	91	85	92	89.3	
8	32	22	27	83	70	84	79.0	
9	31	20	25.5	76	78	56	70.0	
10	30	18	24	76	78	70	74.7	
11	31	17	24	83	72	70	75.0	
12	31	17	24	83	72	84	79.7	
13	30	17	23.5	83	70	76	76.3	
14	31	19	25	83	71	84	79.3	
15	30	16	23	84	78	84	82.0	
1st fortnight	**31.33**	**19.60**	**25.47**	**83.80**	**74.07**	**78.47**	78.78	
16	31	18	24.5	75	77	68	73.3	
17	30	16	23	69	77	68	71.3	
18	29	14	21.5	74	70	68	70.7	
19	29	15	22	90	63	68	73.7	
20	28	16	22	91	70	68	76.3	
21	28	16	22	82	68	81	77.0	
22	30	16	23	82	77	75	78.0	
23	31	17	24	82	75	73	76.7	
24	30	17	23.5	75	68	73	72.0	
25	31	15	23	82	70	82	78.0	
26	31	15	23	83	64	82	76.3	
27	31	15	23	82	70	83	78.3	
28	30	14	22	83	58	83	74.7	
29	30	16	23	82	65	84	77.0	
30	31	17	24	81	83	82	82.0	
2nd fortnight	**30.00**	**15.80**	**22.90**	**80.87**	**70.33**	**75.87**	**75.7**	
Monthly	**30.67**	**17.70**	**24.18**	**82.33**	**72.20**	**77.17**	77.23	
Dec'2018								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	30	15	22.5	91	62	81	78.0	
2	28	15	21.5	81	63	82	75.3	
3	29	14	21.5	82	63	82	75.7	
4	29	14	21.5	82	64	83	76.3	
5	26	16	21	82	68	83	77.7	
6	27	14	20.5	82	69	83	78.0	
7	27	15	21	82	70	83	78.3	
8	28	13	20.5	82	69	83	78.0	
9	27	12	19.5	82	69	83	78.0	
10	28	12	20	82	77	83	80.7	
11	29	13	21	82	76	82	80.0	
12	29	14	21.5	82	63	83	76.0	
13	28	13	20.5	82	70	83	78.3	
14	28	14	21	82	77	83	80.7	
15	29	13	21	82	70	83	78.3	
1st fortnight	**28.13**	**13.80**	**20.97**	**82.83**	**68.67**	**82.67**	**77.96**	
16	29	13	21	82	76	83	80.3	

17	24	18	21	82	83	81	82.0	
18	21	15	18	90	90	90	90.0	15.5
19	23	13	18	82	83	82	82.3	
20	24	13	18.5	90	83	81	84.7	
21	25	12	18.5	81	84	82	82.3	
22	26	12	27	81	76	82	27	
23	27	13	20	81	69	82	77.3	
24	22	13	17.5	81	68	82	77.0	
25	25	12	18.5	82	68	81	77.0	
26	24	10	17	89	75	81	81.7	
27	24	10	17	80	66	81	75.7	
28	25	10	17.5	80	66	73	73.0	
29	26	9	17.5	80	62	82	74.7	
30	27	8	17.5	81	76	82	79.7	
31	28	7	17.5	81	76	82	79.7	
2nd fortnight	**25.00**	**11.75**	**18.88**	**82.69**	**75.06**	**81.69**	**76.5**	**0.00**
Monthly	**26.57**	**12.78**	**19.92**	**82.76**	**71.87**	**82.18**	77.24	**15.50**
Jan'2019								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	26	10	18	79	68	74	73.7	
2	27	10	18.5	90	68	74	77.3	
3	25	10	17.5	80	76	82	79.3	
4	24	10	17	79	66	80	75.0	
5	26	10	18	79	75	81	78.3	
6	27	9	18	80	68	81	76.3	
7	28	11	19.5	80	68	73	73.7	
8	25	11	18	80	61	73	71.3	
9	25	10	17.5	80	68	73	73.7	
10	25	10	17.5	80	68	73	73.7	
11	26	11	18.5	78	66	71	71.7	
12	26	11	18.5	84	59	80	74.3	
13	27	12	19.5	84	69	80	77.7	
14	26	9	17.5	89	55	80	74.7	
15	24	10	17	80	62	73	71.7	
1st fortnight	**25.80**	**9.40**	**18.03**	**81.47**	**66.45**	**76.53**	**74.82**	
16	28	10	19	80	61	73	71.3	
17	26	12	19	90	62	74	75.3	
18	27	12	19.5	84	62	80	75.3	
19	26	12	19	80	62	80	74.0	
20	26	12	19	80	68	79	75.7	
21	28	11	19.5	80	63	75	72.7	
22	29	11	27	80	77	75	27	
23	28	12	20	81	70	75	75.3	
24	27	14	20.5	81	69	73	74.3	
25	28	14	21	80	68	81	76.3	
26	29	15	22	81	68	82	77.0	
27	30	15	22.5	82	64	74	73.3	
28	27	15	21	81	69	81	77.0	
29	22	10	16	80	68	81	76.3	
30	24	10	17	80	62	82	74.7	
31	28	13	20.5	80	76	81	79.0	
2nd fortnight	**27.06**	**12.38**	**20.16**	**81.25**	**66.81**	**77.88**	**72.2**	
Monthly	**26.43**	**10.89**	**19.09**	**81.36**	**66.63**	**77.20**	73.49	
February'2019								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	28	12	20	81	82	82	82.0	
2	27	12	19.5	91	68	81	80.0	

3	27	13	20	90	74	80	81.3	
4	26	13	19.5	81	61	91	77.7	
5	26	12	19	90	75	91	85.3	
6	26	12	19	79	61	82	74.0	
7	26	12	19	90	63	82	78.3	
8	26	13	19.5	89	91	80	86.7	1.5
9	27	15	21	89	90	88	89.0	1
10	23	12	17.5	89	74	73	78.7	
11	27	15	21	90	68	82	80.0	
12	27	16	21.5	81	63	74	72.7	
13	29	17	23	82	70	66	72.7	
14	28	18	23	81	77	74	77.3	
15	29	18	23.5	81	76	91	82.7	
1st fortnight	**26.80**	**13.71**	**20.40**	**85.6**	**72.87**	**81.13**	79.9	2.50
16	29	17	23	91	56	66	71.0	
17	26	14	20	81	60	73	71.3	
18	27	13	20	81	69	74	74.7	
19	29	16	22.5	82	63	82	75.7	
20	28	16	22	91	84	82	85.7	
21	29	15	22	81	77	74	77.3	
22	30	15	22.5	82	76	73	77.0	
23	31	15	23	81	68	82	77.0	
24	29	15	22	74	64	83	73.7	
25	29	17	23	75	63	75	71.0	
26	22	19	20.5	82	63	75	73.3	
27	23	17	20	91	91	90	90.7	19.75
28	26	14	20	91	91	81	87.7	
2nd fortnight	**27.54**	**15.62**	**21.58**	**83.31**	**71.15**	**77.69**	77.4	19.75
Monthly	**27.17**	**14.66**	**20.99**	**84.46**	**72.01**	**79.41**	78.6	22.25
March, 2019								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	27	11	19	82	69	82	77.7	
2	27	10	18.5	82	62	82	75.3	
3	27	15	21	82	62	75	73.0	
4	28	18	23	91	63	75	76.3	
5	27	19	23	91	76	82	83.0	9.75
6	28	16	22	82	77	75	78.0	
7	28	17	22.5	82	82	75	79.7	
8	28	19	23.5	66	76	75	72.3	
9	29	19	24	83	70	75	76.0	
10	28	18	23	81	76	83	80.0	
11	32	17	24.5	74	78	76	76.0	
12	34	20	27	87	61	77	75.0	
13	35	18	26.5	77	62	65	68.0	
14	29	19	24	76	51	65	64.0	
15	30	20	25	92	67	70	76.3	
1st fortnight	**29.13**	**9.40**	**23.10**	**81.87**	**66.45**	**76.53**	**75.38**	**9.75**
16	30	20	25	84	72	76	77.3	
17	29	20	24.5	84	78	84	82.0	
18	32	20	26	76	77	70	74.3	
19	33	18	25.5	76	61	64	67.0	
20	35	18	26.5	76	73	71	73.3	
21	32	17	24.5	91	62	71	74.7	
22	34	18	27	82	72	75	27	5
23	35	17	26	83	73	85	80.3	
24	36	17	26.5	77	46	78	67.0	
25	31	19	25	70	51	72	64.3	
26	26	20	23	91	85	84	86.7	

27	31	20	25.5	76	53	57	62.0	
28	32	20	26	76	62	65	67.7	
29	34	19	26.5	76	61	77	71.3	
30	35	20	27.5	84	59	70	71.0	
31	31	17	24	92	78	83	84.3	
2nd fortnight	**32.25**	**18.75**	**25.56**	**80.88**	**66.44**	**73.88**	**70.6**	**5.00**
Monthly	**30.69**	**14.08**	**24.33**	**81.37**	**66.44**	**75.20**	73.01	**14.75**
April, 2019								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	30	22	26	82	71	70	74.3	46.75
2	31	18	24.5	83	74	92	83.0	2
3	31	21	26	75	71	83	76.3	
4	30	18	24	91	78	84	84.3	
5	30	19	24.5	91	77	83	83.7	
6	30	19	24.5	76	83	84	81.0	30
7	31	20	25.5	84	78	77	79.7	
8	30	19	24.5	84	84	77	81.7	11.5
9	29	18	23.5	91	70	81	80.7	13.5
10	32	22	27	84	65	70	73.0	
11	32	22	27	77	73	72	74.0	
12	31	21	26	85	73	85	81.0	33
13	32	21	26.5	92	86	85	87.7	
14	31	22	26.5	85	79	92	85.3	
15	32	24	28	83	73	84	80.0	
1st fortnight	**30.80**	**19.60**	**25.60**	**84.20**	**78.47**	**81.66**	80.38	**136.75**
16	34	21	27.5	85	80	85	83.3	12.5
17	34	22	28	85	73	85	81.0	15.75
18	34	22	28	70	72	85	75.7	
19	35	21	28	77	73	77	75.7	
20	36	21	28.5	76	73	77	75.3	
21	34	22	28	77	74	85	78.7	
22	35	23	29	77	85	84	82.0	15.25
23	37	24	30.5	77	74	79	76.7	
24	38	26	32	78	63	68	69.7	
25	37	24	30.5	79	64	74	72.3	
26	38	24	31	78	68	73	73.0	
27	39	24	31.5	84	73	78	78.3	39.25
28	32	24	28	85	73	78	78.7	1
29	36	26	31	85	69	86	80.0	
30	35	25	30	92	74	79	81.7	2.25
2nd fortnight	**35.60**	**23.27**	**29.43**	**80.33**	**72.53**	**79.53**	**77.5**	**86.00**
Monthly	**33.20**	**21.43**	**27.52**	**82.27**	**75.50**	**80.60**	78.92	**222.75**

Table A.16. Weather data during wheat growing season 2019-20 in Jamalpur, Bangladesh

November, 2019								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	32	22	27	84	78	77	79.7	
2	33	21	27	85	78	85	82.7	
3	31	21	26	85	78	85	82.7	
4	33	21	27	84	72	84	80.0	
5	28	21	24.5	84	78	85	82.3	
6	32	19	25.5	84	78	84	82.0	
7	28	21	24.5	83	72	84	79.7	
8	28	22	25	83	77	84	81.3	
9	27	21	24	83	77	84	81.3	4.25
10	30	20	25	91	77	84	84.0	3
11	29	20	24.5	84	77	84	81.7	
12	30	20	25	84	77	84	81.7	
13	32	20	26	84	78	85	82.3	
14	31	20	25.5	84	78	85	82.3	
15	32	19	25.5	84	78	85	82.3	
1st fortnight	**30.40**	**20.53**	**25.47**	**84.40**	**78.47**	**81.66**	81.73	**7.25**
16	32	19	25.5	84	78	84	82.0	
17	30	18	24	84	71	84	79.7	
18	30	18	24	83	77	84	81.3	
19	29	16	22.5	83	77	84	81.3	
20	29	18	23.5	82	70	84	78.7	
21	29	17	23	91	91	84	88.7	
22	29	16	22.5	91	77	83	83.7	
23	30	17	23.5	83	77	84	81.3	
24	29	17	23	83	70	92	81.7	
25	29	17	23	83	70	84	79.0	
26	30	17	23.5	91	70	92	84.3	
27	30	17	23.5	83	77	92	84.0	
28	30	17	23.5	83	78	76	79.0	
29	30	16	23	83	78	84	81.7	
30	31	16	23.5	82	70	83	78.3	
2nd fortnight	**29.80**	**17.07**	**23.43**	**84.60**	**75.40**	**84.93**	**81.6**	**0.00**
Monthly	**30.10**	**18.80**	**24.45**	**84.50**	**76.94**	**83.30**	81.69	**7.25**
December, 2019								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	28	14	21	91	70	83	81.3	
2	28	14	21	91	69	83	81.0	
3	27	15	21	91	68	82	80.3	
4	28	13	20.5	91	69	82	80.7	
5	28	14	21	82	64	83	76.3	
6	29	13	21	91	70	83	81.3	
7	30	13	21.5	81	70	83	78.0	
8	28	14	21	90	69	82	80.3	
9	28	14	21	81	69	82	77.3	
10	26	13	19.5	81	68	82	77.0	
11	25	13	19	81	68	82	77.0	
12	26	13	19.5	81	68	82	77.0	
13	26	13	19.5	81	68	81	76.7	
14	27	13	20	90	62	81	77.7	
15	27	14	20.5	90	68	82	80.0	
1st fortnight	**27.40**	**13.53**	**20.47**	**86.20**	**66.45**	**76.53**	**78.80**	
16	26	14	20	90	68	82	80.0	

17	25	13	19	90	82	81	84.3	
18	23	13	18	90	81	80	83.7	
19	23	12	17.5	90	81	80	83.7	
20	19	12	15.5	90	81	80	83.7	
21	18	12	15	89	90	90	89.7	
22	18	12	27	89	80	89	27	
23	21	12	16.5	89	81	80	83.3	
24	21	10	15.5	89	90	90	89.7	
25	21	9	15	89	90	89	89.3	
26	22	10	16	89	82	81	84.0	
27	23	10	16.5	89	81	89	86.3	
28	23	11	17	89	81	80	83.3	
29	25	8	16.5	89	73	81	81.0	
30	26	11	18.5	89	60	73	74.0	
31	27	13	20	80	61	82	74.3	
2nd fortnight	**22.56**	**11.38**	**17.72**	**88.75**	**78.88**	**82.94**	**79.8**	
Monthly	**24.98**	**12.45**	**19.09**	**87.48**	**72.66**	**79.73**	79.32	
January, 2020								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	28	16	22	81	62	75	72.7	
2	28	14	21	82	69	83	78.0	
3	28	15	21.5	90	60	82	77.3	8.75
4	29	14	21.5	81	61	62	68.0	
5	23	10	16.5	90	82	90	87.3	
6	20	10	15	89	81	79	83.0	
7	19	11	15	88	80	89	85.7	
8	21	13	17	89	82	90	87.0	
9	21	13	17	90	82	90	87.3	
10	22	12	17	89	82	80	83.7	
11	22	12	17	89	81	90	86.7	
12	21	11	16	89	81	80	83.3	
13	23	12	17.5	90	74	90	84.7	
14	23	13	18	89	74	81	81.3	
15	26	13	19.5	90	75	81	82.0	
1st fortnight	**23.60**	**12.60**	**18.10**	**87.73**	**66.45**	**76.53**	**81.87**	**8.75**
16	25	13	19	90	64	73	75.7	
17	26	12	19	81	68	81	76.7	
18	27	12	19.5	81	75	81	79.0	
19	22	13	17.5	91	90	90	90.3	
20	21	10	15.5	89	73	80	80.7	
21	23	9	16	89	73	80	80.7	
22	21	8	27	88	73	80	27	
23	22	9	15.5	89	66	81	78.7	
24	23	9	16	89	66	81	78.7	
25	24	8	16	89	62	81	77.3	
26	23	11	17	89	66	80	78.3	
27	22	10	16	89	73	81	81.0	
28	23	12	17.5	89	66	73	76.0	
29	23	16	19.5	90	68	81	79.7	
30	23	12	17.5	81	68	82	77.0	
31	24	11	17.5	89	74	81	81.3	
2nd fortnight	**23.25**	**10.94**	**17.88**	**87.69**	**70.31**	**80.38**	**76.1**	**0.00**
Monthly	**23.43**	**11.77**	**17.99**	**87.71**	**68.38**	**78.45**	79.00	**8.75**
February'2020								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	24	9	16.5	89	81	80	83.3	
2	24	9	16.5	79	74	81	78.0	

3	23	8	15.5	79	75	81	78.3	
4	24	10	17	79	62	72	71.0	
5	25	9	17	80	59	72	70.3	
6	24	10	17	80	53	73	68.7	
7	25	10	17.5	80	60	81	73.7	
8	26	9	17.5	80	59	81	73.3	
9	24	11	17.5	80	54	73	69.0	
10	22	10	16	71	53	73	65.7	
11	26	11	18.5	81	61	81	74.3	
12	26	10	18	80	61	81	74.0	
13	25	11	18	80	53	81	71.3	
14	25	10	17.5	90	61	81	77.3	
15	26	11	18.5	90	59	81	76.7	
1st fortnight	**24.60**	**9.87**	**17.23**	**81.20**	**66.45**	**76.53**	**73.67**	0.00
16	28	14	21	81	62	82	75.0	
17	27	14	20.5	91	61	81	77.7	
18	28	14	21	91	62	82	78.3	
19	29	14	21.5	91	56	74	73.7	
20	29	14	21.5	82	64	75	73.7	
21	30	14	22	91	63	82	78.7	
22	31	14	22.5	91	70	75	27	
23	30	15	22.5	91	70	82	81.0	
24	31	14	22.5	91	82	82	85.0	
25	23	16	19.5	91	91	91	91.0	1.75
26	28	15	21.5	82	68	82	77.3	
27	29	14	21.5	81	61	82	74.7	
28	30	14	22	81	68	82	77.0	
29	31	14	22.5	91	69	82	80.7	
2nd fortnight	**31.00**	**14.29**	**21.50**	**87.33**	**67.03**	**80.72**	**75.1**	1.75
Monthly	**27.80**	**12.08**	**19.37**	**88.66**	**64.01**	**80.86**	76.20	1.75
March, 2020								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	32	16	24	91	53	75	73.0	
2	30	17	23.5	83	58	75	72.0	
3	24	18	21	83	59	68	70.0	
4	30	17	23.5	75	52	68	65.0	2.5
5	28	17	22.5	75	52	68	65.0	
6	28	18	23	81	82	81	81.3	14.25
7	30	18	24	81	68	82	77.0	
8	29	15	22	91	70	75	78.7	
9	29	15	22	75	52	75	67.3	
10	30	15	22.5	62	64	76	67.3	
11	31	15	23	75	52	75	67.3	
12	31	16	23.5	83	53	76	70.7	
13	32	17	24.5	83	53	76	70.7	
14	33	18	25.5	75	76	83	78.0	
15	27	17	22	75	75	82	77.3	
1st fortnight	**29.60**	**16.15**	**23.10**	**79.20**	**66.45**	**76.53**	**72.04**	**16.75**
16	29	17	23	76	47	68	63.7	
17	31	17	24	76	53	76	68.3	
18	31	16	23.5	68	65	76	69.7	
19	32	16	24	76	72	76	74.7	
20	34	17	25.5	83	78	84	81.7	
21	34	17	25.5	83	72	84	79.7	
22	29	18	27	82	76	83	27	
23	31	18	24.5	76	78	84	79.3	
24	32	18	25	76	78	84	79.3	
25	32	18	25	76	73	77	75.3	

26	32	17	24.5	85	79	85	83.0	
27	34	17	25.5	76	79	85	80.0	
28	33	18	25.5	84	86	85	85.0	
29	34	18	26	85	74	85	81.3	
30	35	18	26.5	84	68	85	79.0	
31	34	17	25.5	84	68	85	79.0	
2nd fortnight	**32.31**	**17.31**	**25.03**	**79.38**	**71.63**	**81.38**	**74.1**	**0.00**
Monthly	**30.96**	**16.73**	**24.07**	**79.29**	**69.04**	**78.95**	73.08	**16.75**
April, 2020								
	Temp			RH(%)				Rainfall (mm)
Days	Max	Min	Mean	Morning	Noon	Afternon	Mean	
1	34	18	26	77	74	85	78.7	
2	35	17	26	85	74	85	81.3	
3	35	18	26.5	85	74	85	81.3	
4	36	18	27	77	74	78	76.3	
5	35	18	26.5	77	63	78	72.7	
6	36	17	26.5	78	68	78	74.7	
7	36	18	27	78	68	78	74.7	
8	36	18	27	78	68	78	74.7	
9	37	18	27.5	78	63	85	75.3	
10	37	18	27.5	85	63	85	77.7	
11	37	18	27.5	85	63	78	75.3	8.75
12	36	17	26.5	85	74	78	79.0	2
13	35	17	26	85	73	85	81.0	
14	35	23	29	84	73	85	80.7	
15	34	22	28	84	91	83	86.0	35.25
1st fortnight	**35.60**	**19.60**	**26.97**	**81.40**	**78.47**	**81.66**	77.96	**46.00**
16	33	21	27	84	73	85	80.7	
17	33	22	27.5	92	79	85	85.3	
18	31	20	25.5	92	85	84	87.0	
19	31	20	25.5	92	85	85	87.3	2.25
20	32	21	26.5	92	85	92	89.7	3
21	32	21	26.5	92	79	85	85.3	
22	33	20	26.5	85	79	85	83.0	
23	32	21	26.5	85	79	85	83.0	
24	32	20	26	92	78	84	84.7	0.75
25	32	21	26.5	84	85	85	84.7	
26	33	21	27	85	79	85	83.0	
27	34	22	28	85	79	85	83.0	
28	29	21	25	92	85	84	87.0	12.5
29	33	23	28	92	85	85	87.3	
30	32	22	27	85	73	85	81.0	
2nd fortnight	**32.13**	**21.07**	**26.60**	**88.60**	**80.53**	**85.27**	**84.8**	**18.50**
Monthly	**33.87**	**20.33**	**26.78**	**85.00**	**79.50**	**83.46**	81.38	**64.50**

Appendix B - Supplementary Material Chapter 3

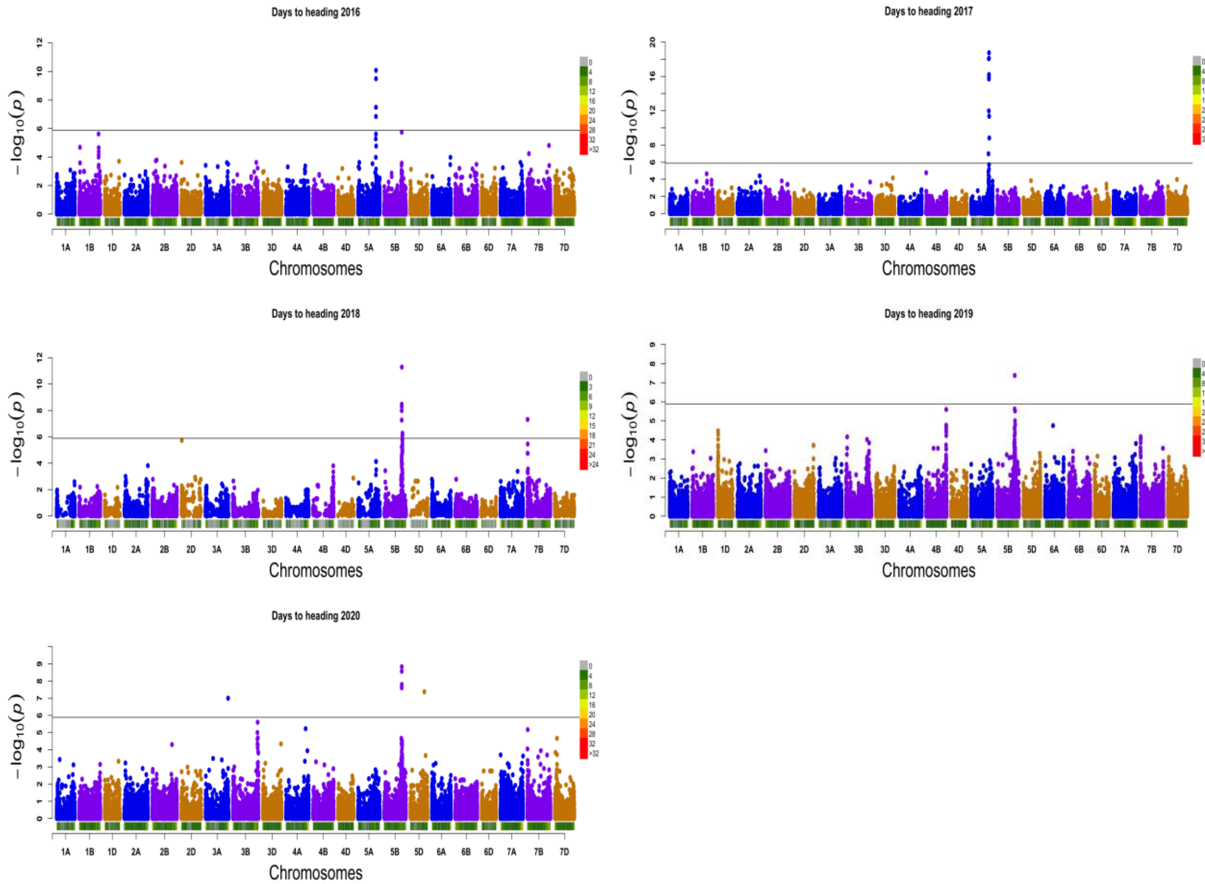

Figure B.1. A panel of Manhattan plots showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.05 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

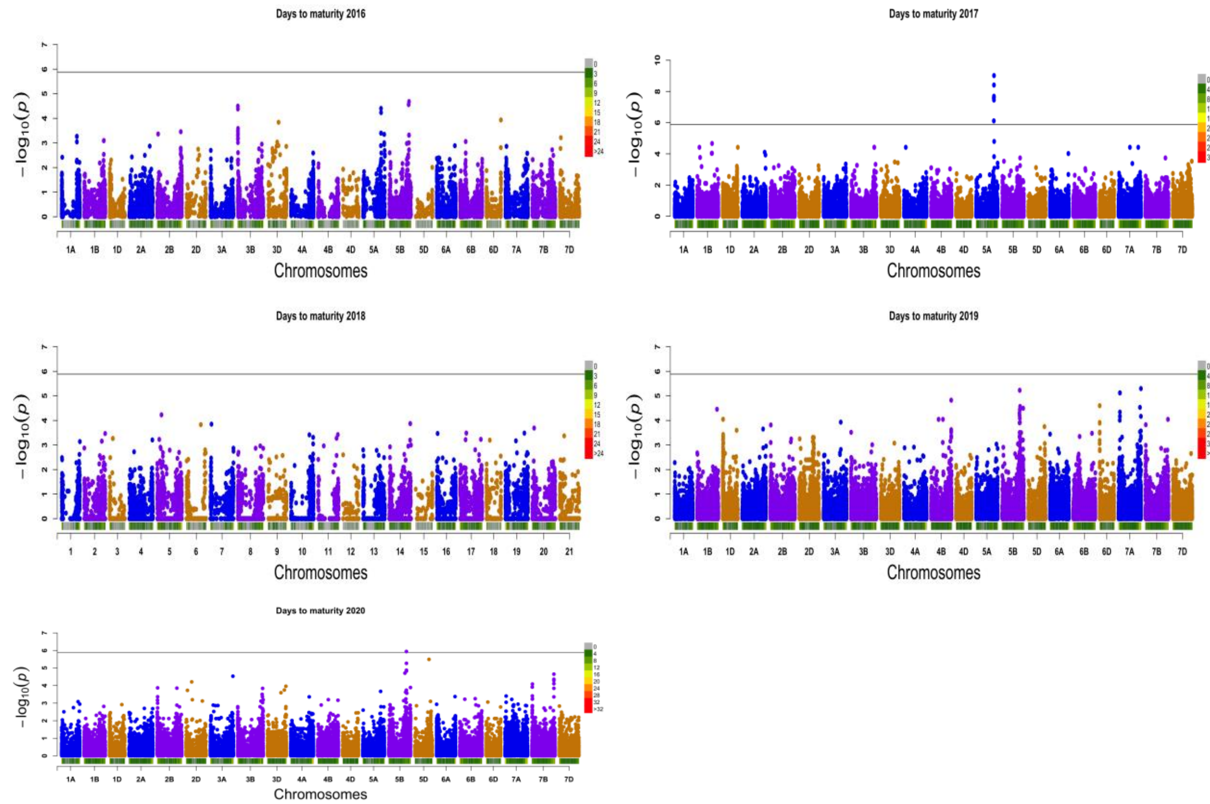

Figure B.2. A panel of Manhattan plots showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.05 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

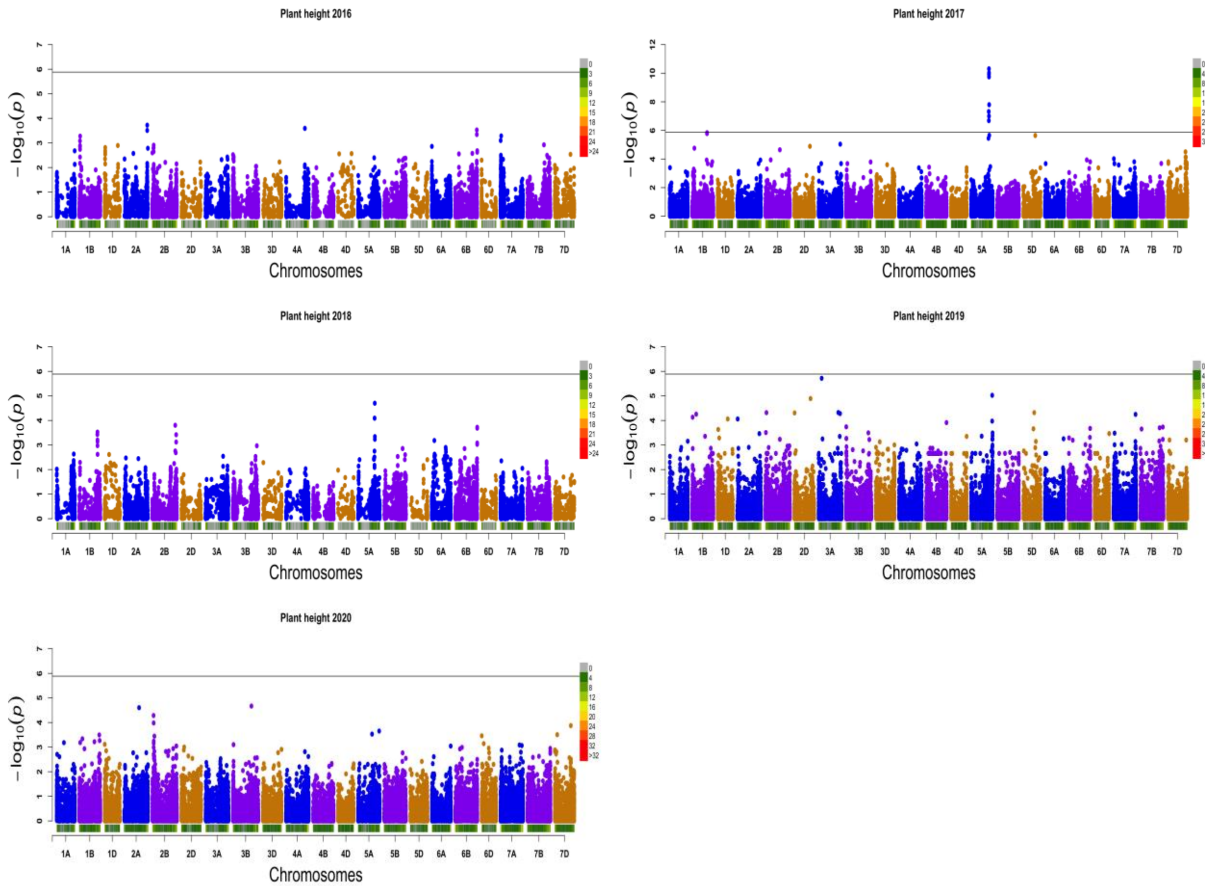

Figure B.3. A panel of Manhattan plot showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.05 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

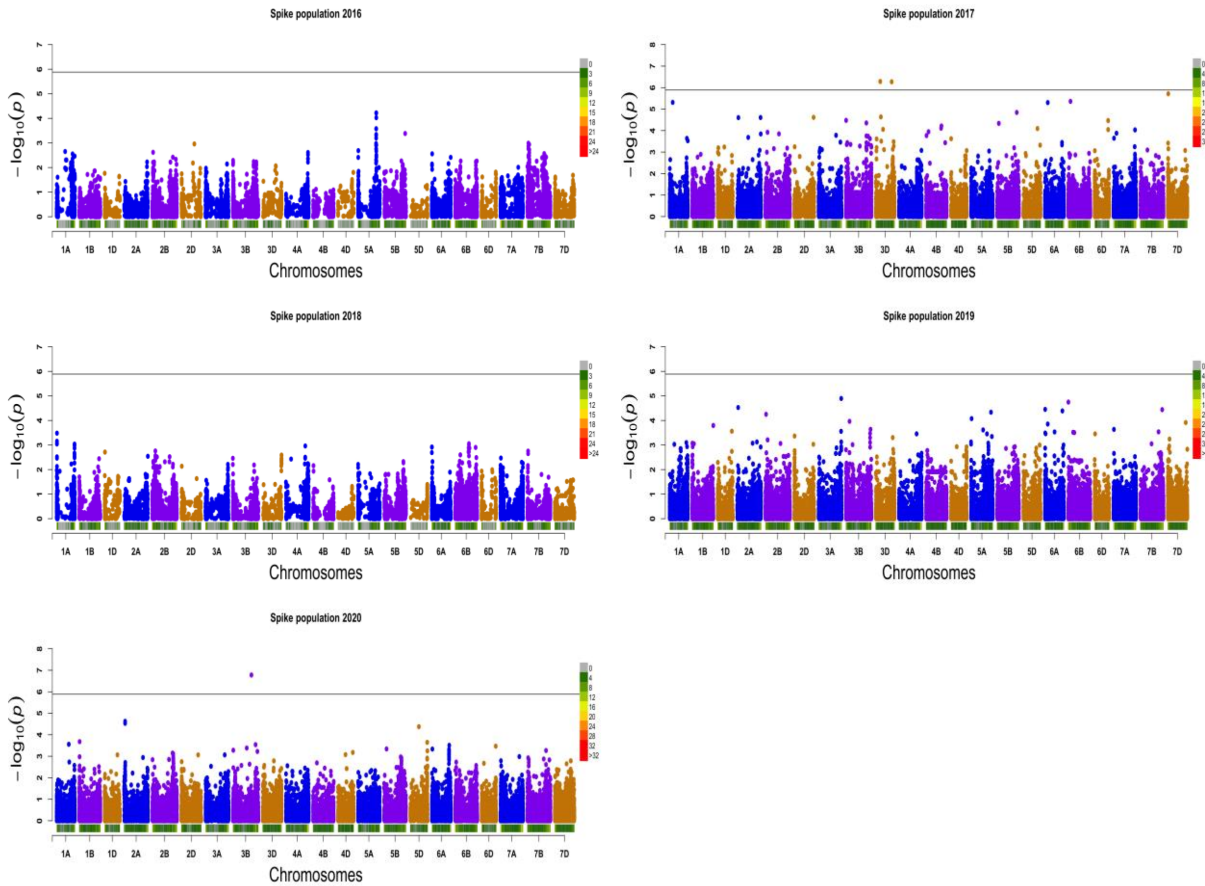

Figure B.4. A panel of Manhattan plot showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.20 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

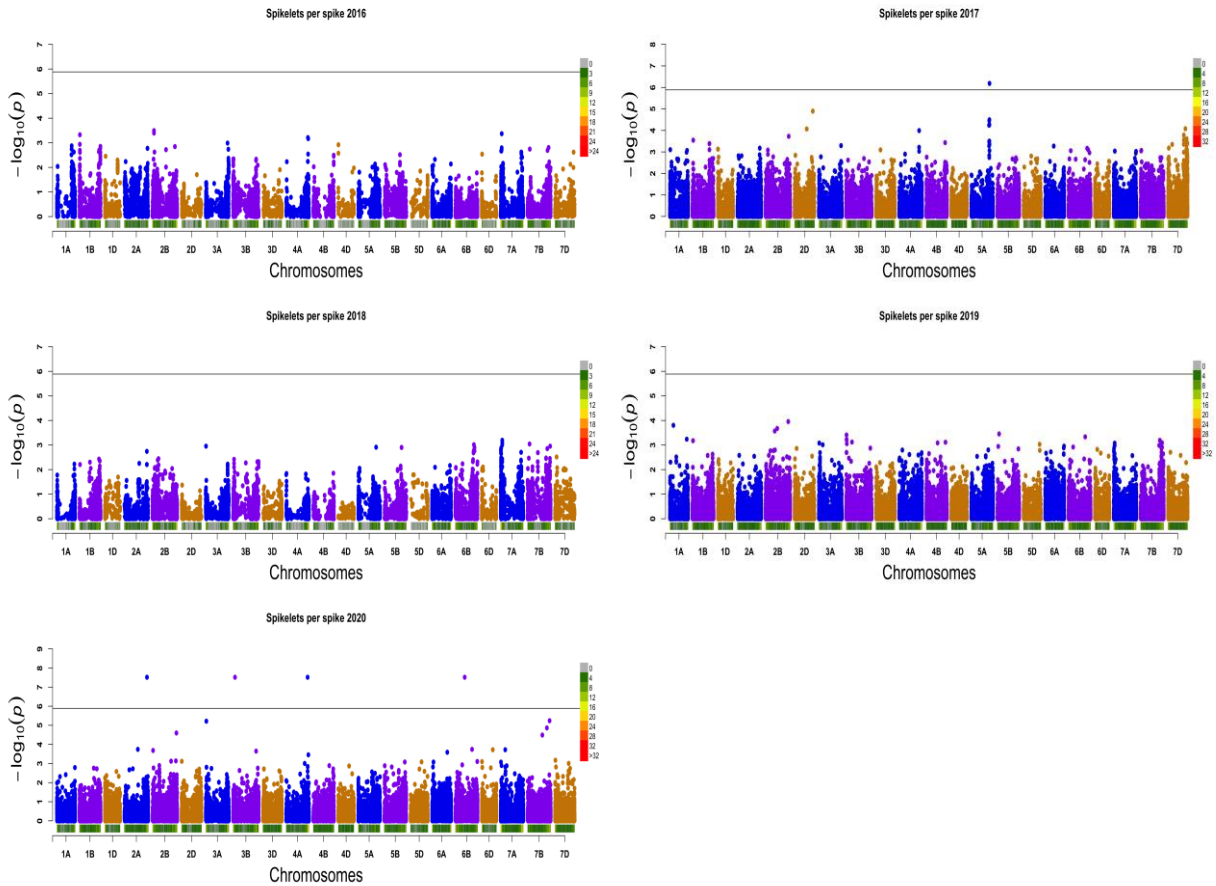

Figure B.5. A panel of Manhattan plot showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.20 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

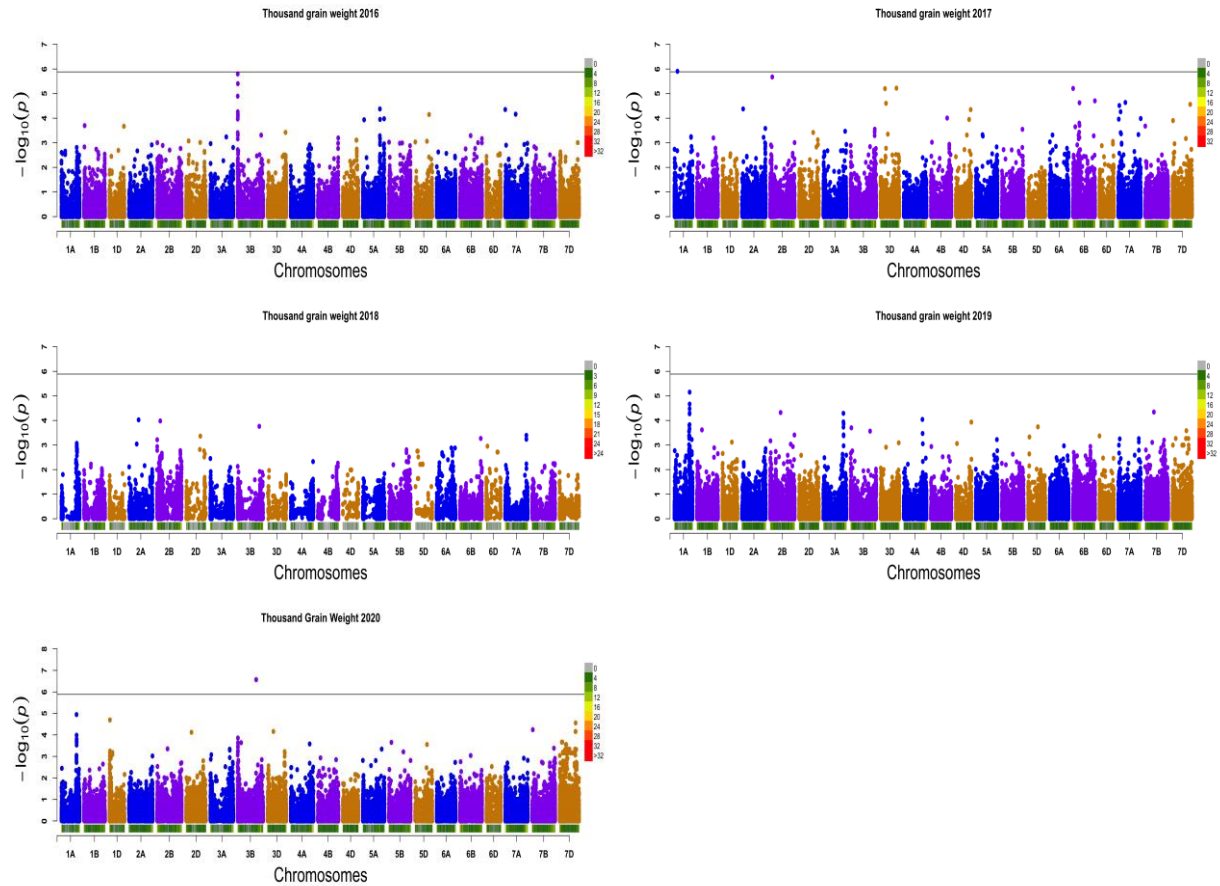

Figure B.6. A panel of Manhattan plot showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.20 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

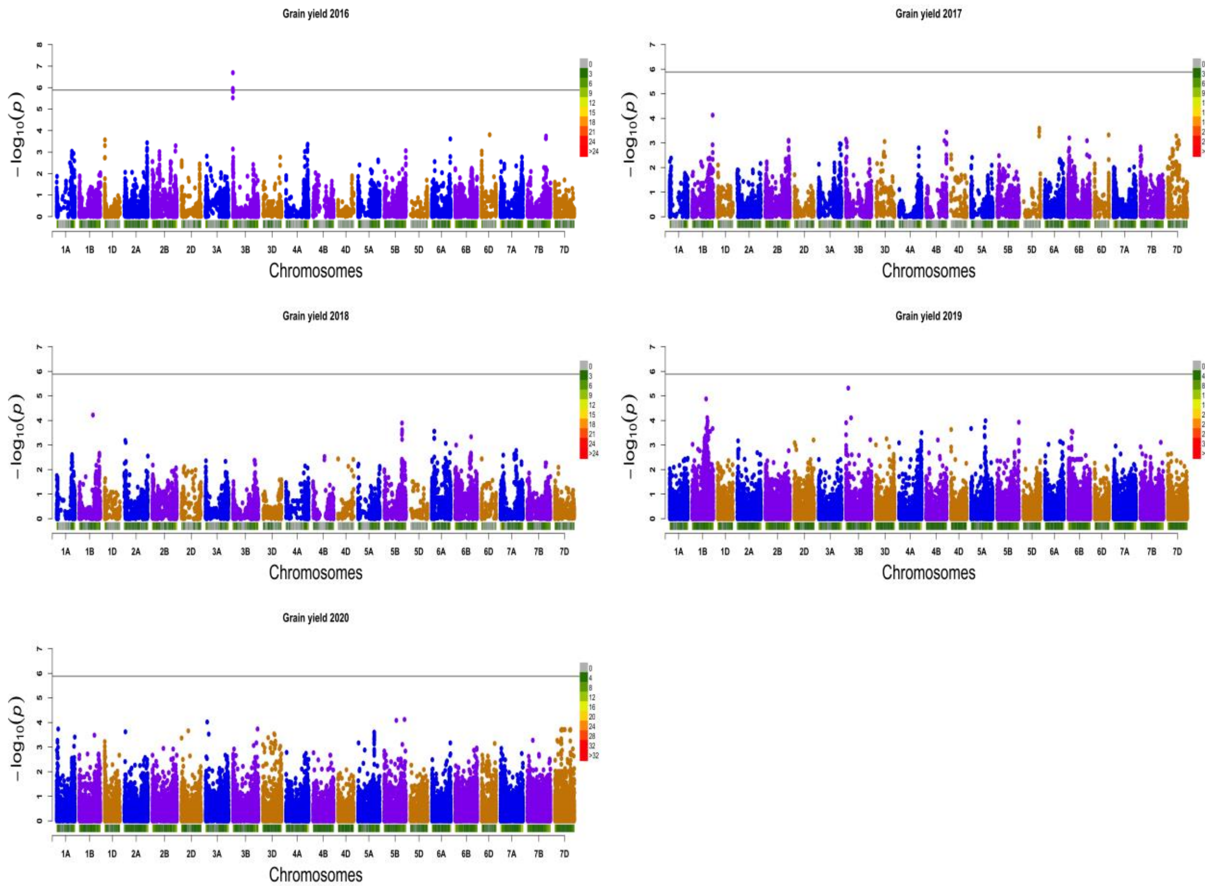

Figure B.7. A panel of Manhattan plot showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.20 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

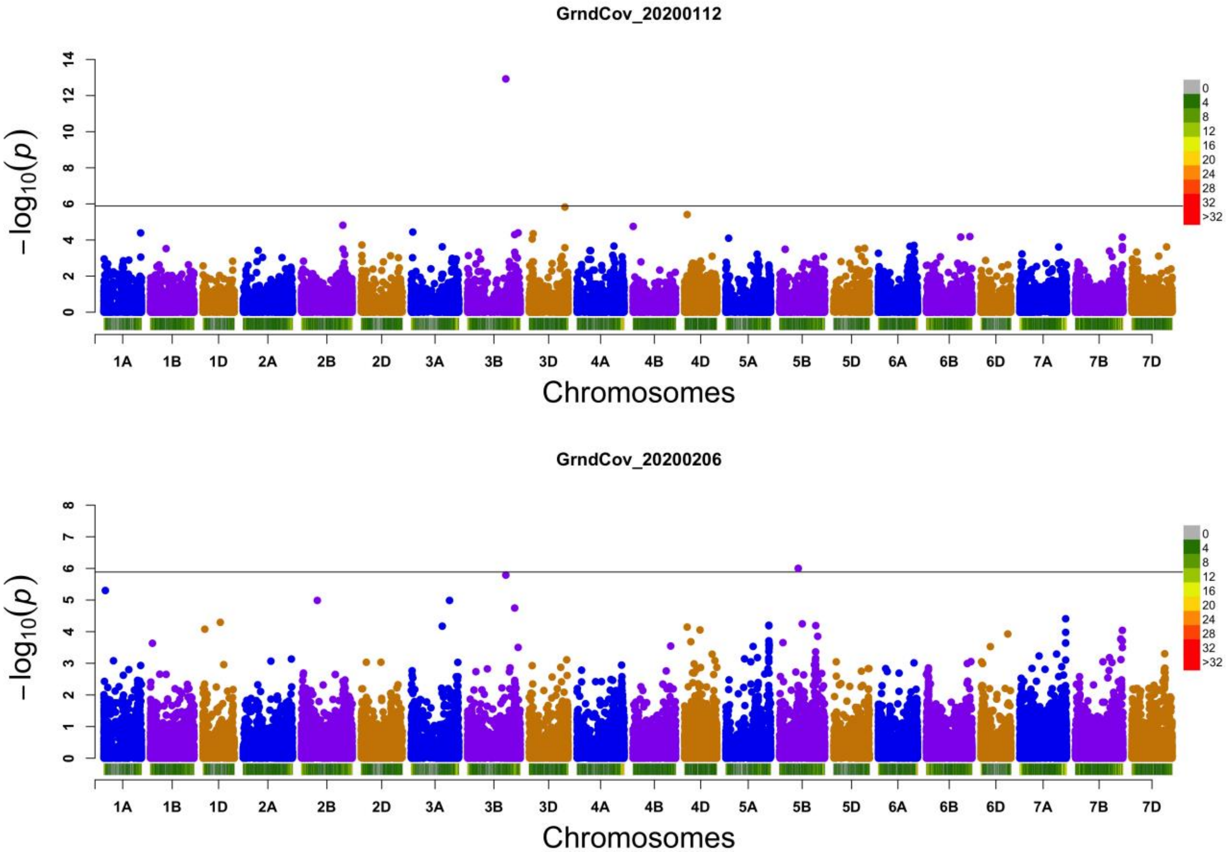

Figure B.8. A panel of Manhattan plot showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.20 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

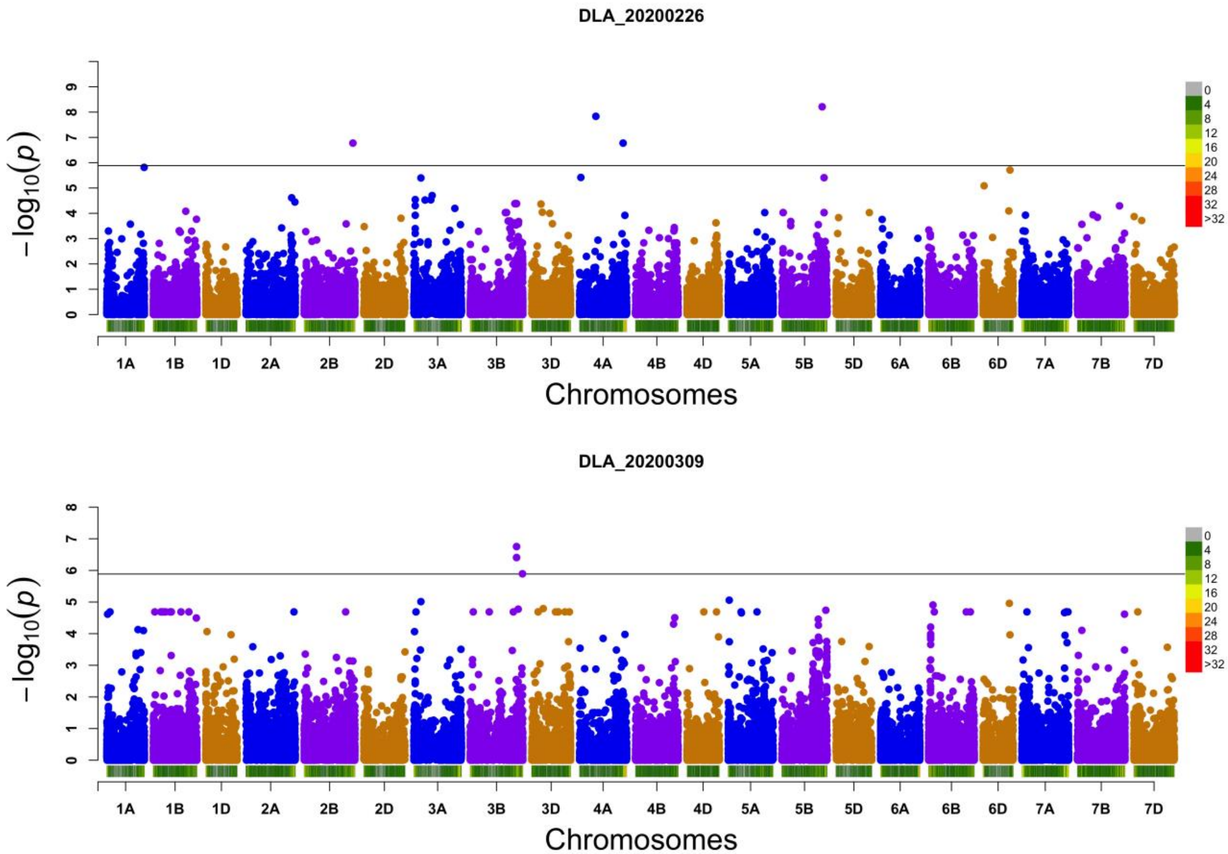

Figure B.9. A panel of Manhattan plot showing marker-trait associations from a GWAS. A Bonferroni ∝ level of 0.20 was used to correct for multiple testing and identify significant markers is shown with horizontal line. The title of the Manhattan plot indicates the trait.

Appendix C - Supplementary Material Chapter 4

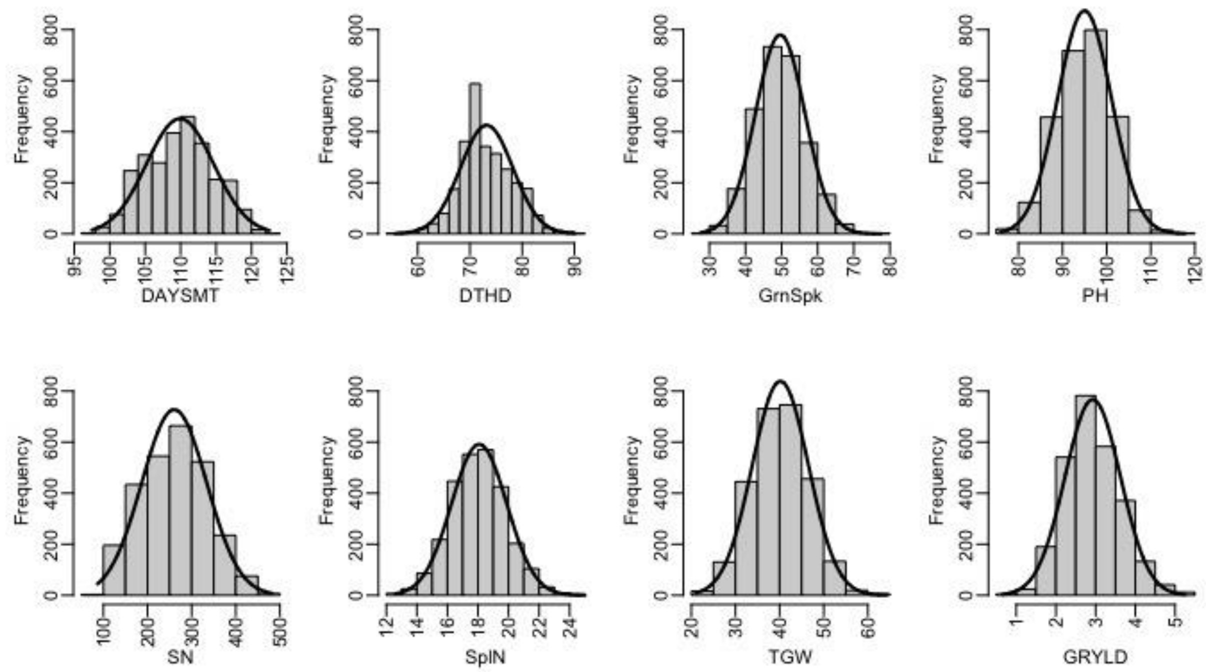

Figure C.1. Distribution of the traits across trials and years

Table C.1. Mean and SE of different traits across trials.

year	trait	mean	SE
2016	DAYSMT	103.8	0.09
2017	DAYSMT	109.2	0.14
2018	DAYSMT	110.5	0.13
2019	DAYSMT	115.2	0.15
2020	DAYSMT	111	0.14
2016	DTHD	70.1	0.11
2017	DTHD	69.8	0.2
2018	DTHD	75.1	0.2
2019	DTHD	77.9	0.16
2020	DTHD	73.2	0.17
2016	GrnSpk	46.7	0.3
2017	GrnSpk	49.9	0.28
2018	GrnSpk	50.6	0.3
2019	GrnSpk	49.7	0.27
2020	GrnSpk	51.3	0.29
2016	GRYLD	2.4	0.02
2017	GRYLD	3.1	0.03
2018	GRYLD	2.6	0.02
2019	GRYLD	3.1	0.03
2020	GRYLD	3.5	0.03
2016	PH	97	0.21
2017	PH	100.8	0.21
2018	PH	89.3	0.2
2019	PH	94.5	0.23
2020	PH	93.4	0.18
2016	SN	258.3	1.77
2017	SN	317.5	2.27
2018	SN	229.9	1.81
2019	SN	166.4	1.4
2020	SN	329.2	1.99
2016	SplN	17	0.07
2017	SplN	18.1	0.07
2018	SplN	18.4	0.08
2019	SplN	18.7	0.07
2020	SplN	18.1	0.08
2016	TGW	34.4	0.22
2017	TGW	36	0.18
2018	TGW	41.6	0.19
2019	TGW	44.3	0.19
2020	TGW	44.6	0.25