STUDY ON THE EFFECTS OF HEAT STRESS ON HSTU-DEVELOPED ADVANCED LINES IN WHEAT (*Triticum aestivum* L.)

A THESIS

BY

AMORISH CHANDRA MOHANTO

STUDENT NO. 1601210

SEMESTER: July -December, 2023

SESSION: 2022-23

MASTER OF SCIENCE (MS)
IN
GENETICS AND PLANT BREEDING

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Certification

This is to certify that the thesis entitled **"STUDY ON THE EFFECTS OF HEAT STRESS ON HSTU-DEVELOPED ADVANCED LINES IN WHEAT (*Triticum aestivum* L.)"** is a study, prepared by the examinee, bearing Registration No. 1601210 of the Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The author has submitted this thesis to the Department as a partial fulfillment of the requirements of the degree "Master of Science in Genetics and Plant Breeding", is a record of original research work carried out by him under my supervision. The work is an original, unique one and to the best of my knowledge, no part of the thesis has been produced elsewhere for any other degree or diploma.

…………………………………..….

(Prof. Dr. Md. Hasanuzzaman)
Supervisor

In the Name of God

&

My Beloved Parents and
Honorable Teachers

ACKNOWLEDGEMENTS

All praises are due to God, the omniscient and omnipotent Authority of this Universe, who enabled me to pursue my education in Agriculture and to complete as well as to submit the thesis for the degree of Master of Science (MS) in Genetics and Plant Breeding.

*It is a pleasure for me to express my heartiest gratitude and profound respect to my honorable teacher, research supervisor, **Professor Dr. Md. Hasanuzzaman,** Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur for his helpful advice, scholastic guidance during planning and execution of the research, valuable suggestions, continuous support and all kind of support and help throughout research work and preparation of manuscript of the dissertation, otherwise it would be too tough to complete the thesis with the stipulated period. Sincere appreciation is also due to his constructive criticism and meticulous corrections of this dissertation.*

*I would like to express my heartiest respect, and immense indebtedness to my respectable teacher and research Co-Supervisor, **Dr. Md. Abdul Hakim**, Principal Scientific Officer, Wheat Breeding Division, Bangladesh Wheat and Maize Research Institute (BWMRI), Dinajpur for his valuable suggestions, sincere help, scholar instructions and careful corrections of the manuscript of the dissertation.*

*I recognize a profound thanks to all the respected teachers, **Professor Dr. Bhabendra Kumar Biswas**, **Professor Dr. Md. Abul Kalam Azad, Professor Dr. Md. Arifuzzaman, Assistant Professor Mst. Tanjina Shahanaj Turin, Assistant Professor Sohana Jui, and Lecturer Mst. Salma Masuda,** Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their kind co-operation, constant encouragement, constructive suggestions, valuable instructions and immense help in successful completion of this thesis.*

*I also recognize a profound thanks to **Md. Monwar Hossain** and **Dr. Md. Farhad,** Senior Scientific Officer, Bangladesh Wheat and Maize Research Institute (BWMRI), Dinajpur for their valuable suggestions and sincere help.*

I extend my gratitude to all the staff (Tajul Islam, Nazir Hosain & Abul Hosain) of Dept. of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their help and assistance during the period of the study.

I would like to say thanks to my seniors, classmates, friends, and junior students for their active encouragement and inspiration.

I express my boundless gratitude to my beloved parents, wife, and other relatives whose sacrifices, inspiration, and continuous blessings paved the way for my higher studies.

Finally, the financial support for this study provided by the Ministry of National Science and Technology, Government of the People`s Republic of Bangladesh is gratefully acknowledged.

December, 2023 *The Author*

STUDY ON THE EFFECTS OF HEAT STRESS ON HSTU-DEVELOPED ADVANCED LINES IN WHEAT (*Triticum aestivum* L.)

ABSTRACT

The experiment was conducted with 11 wheat genotypes (10 HSTU-developed advance lines and 1 check variety) at Hajee Mohammad Danesh Science and Technology University, Dinajpur to identify heat tolerant genotypes during Rabi season, 2022-2023 in Randomized Complete Block Design with 3 replications. The analysis of variance revealed significant differences among the genotypes for most of the characters studied. Mean values of days to heading ranged from 51.67-69.33 days under optimum sowing (OS) and 59.33-66.33 days under late sowing (LS); days to maturity 102.33-121.33 days (OS) and 94.00-106.33 days (LS); plant height 92.35-116.33 cm (OS) and 91.76-115.67 cm (LS); number of grains per spike 30.93-45.20 (OS) and 34.27-47.90 (LS); spike length 8.58-10.73 cm (OS) and 9.25-10.05 cm (LS); number of spikes per square meter 351.67-505.67 (OS) and 332.33-440.00 (LS); grains weight per spike 1.32-1.88 g (OS) and 1.40-1.86 g (LS); 1000-grains weight 31.99-47.23 g (OS) and 30.23-47.04 g (LS); grains yield per plot 2087.2-2812.7 g (OS) and 2155.6-2751.0 g (LS). Judging the mean performance of 11 wheat genotypes it was observed that there may be some genotypes that were adapted to a heat-stressed environment and they could perform better even under heat-stressed conditions. In the study on the grain yield, yield contributing, phenological, and physiological characters, it was observed that the genotypes HSTUW8, HSTUW1, and HSTUW4 ranked better category for the maximum number of characters indicating their high tolerance to heat stress under heat-stressed environment. In the study on the grain yield and stress indices, it was observed that the genotypes HSTUW8, HSTUW1, and HSTUW4 ranked better category for the maximum number of stress indices indicating their high tolerance to heat stress under a heat-stressed environment. 4 SSR markers (TaGwm291, TaXgwm294, TaBarc68, and Xgwm296) were evaluated in 11 wheat genotypes for molecular characterization and to identify the variation among the genotypes. A total of 28 alleles were detected and the number of alleles per locus ranged from 5-8 with an average of 7 alleles per locus. In population genetic structure analysis, population I consisted of 54.55% of the genotypes (6 genotypes: HSTUW4, HSTUW6, HSTUW7, HSTUW8, HSTUW9, and HSTUW10) where all the genotypes were pure. Population II consisted of 45.45% of the genotypes (5 genotypes: HSTUW1, HSTUW2, HSTUW3, HSTUW5, and BARI Gom 32) where all the genotypes were pure. The cluster analysis revealed two major clusters (Group I and II) with a similarity coefficient varying between 0 and 5 indicating significant genetic variation among the wheat accessions studied. In cluster I, the higher genetic similarity was observed among HSTUW4, HSTUW6, HSTUW7, HSTUW8, HSTUW9, and HSTUW10 were less genetic distances and genetically identical to each other. In cluster II, the highest similarity was observed between HSTUW1 and HSTUW2 were less genetic distances and genetically identical to each other, while the lowest similarity was observed between HSTUW5 and BARI Gom 32 were more genetic distances and genetically diverse.

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LIST OF ACRONYMS AND ABBREVIATIONS

%	Percent
@	At the rate of
^{0}C	Degree Celsius
AEZ	Agro Ecological Zone
ANOVA	Analysis of Variance
BARI	Bangladesh Agricultural Research Institute
bp	Base pair
BWMRI	Bangladesh Wheat and Maize Research Institute
cm	Centimeter
CV	Coefficient of Variation
DAP	Diammonium phosphate
DAS	Days after Sowing
DH	Days to Heading
DM	Days to Maturity
FAO	Food and Agriculture Organization
g	Gram
GMP	Geometric Mean Productivity
GPB	Department of Genetics and Plant Breeding
GWPS	Grains weight per spike
GYPP	Grains Yield per Plot
HSTU	Hajee Mohammad Danesh Science and Technology University
iPASTIC	An online toolkit to estimate plant abiotic stress indices
Kg	Kilogram
LS	Late Sowing
m	Meter
m^2	Square meter
mm	Millimeter
MOP	Muriate of Potash
MP	Mean productivity
MS	Mean Sum of Square
NGPS	Number of Grains per Spike
NS	Number of Spikes per square meter
OS	Optimum Sowing
PAGE	Polyacrylamide Gel Electrophoresis

PCR	Polymerase Chain Reaction
PH	Plant height
PIC	Polymorphism Information Content
RCBD	Randomized Complete Block Design
RSI	Relative Stress Index
SE	Standard Error
SL	Spike Length
sq. m	Square Meter
SRDI	Soil Resource Development Institute
SSI	Stress Susceptibility Index
SSR	Simple Sequence Repeat
STI	Stress Tolerance Index
TGW	1000-grains Weight
TM	Temperature
UGPP	Unfilled Grain Per Panicle
UPGMA	Unweight Pair Group Method Using Arithmetic Mean
Viz.	Namely
WP	Wettable powder
Y_O	Grain Yield under Optimum Field Condition
Y_S	Grain Yield under Heat-stressed Condition

CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a self-pollinated crop belonging to the Poaceae family. Species *T. aestivum* is grouped into three ploidy levels: diploid (2n = 14), tetraploid (2n = 28), and hexaploid (2n = 42). The majority of cultivated wheat varieties are from one of three species of the genus *Triticum*. *T. aestivum* (bread wheat) is a hexaploid, and the tetraploids are *T. durum*, *T. dicoccum*, and the diploid is *T. monococcum* (Kimber and Feldman, 1987). Wheat (*T. aestivum*) is one of the most widely cultivated cereal crops all over the world, contributing significantly to global cereal production (28%) and trade (41.5%) (FAO, 2020). Because of its extensive cultivation, great productivity potential, and significant role in the global food grain trade, it has been referred to as the "King of cereals." It has a high nutritional profile, with 12.1% protein, 1.8% lipids, 1.8% ash, 2.0% reducing sugars, 6.7% pentosans, 59.2% starch, 70% total carbohydrates, and 314 Kcal/100 g. It also provides calcium (37 mg/100 g), iron (4.1 mg/100 g), thiamine (0.45 mg/100 g), riboflavin (0.13 mg/100 g), and nicotinic acid (5.4 mg/100 mg) as a source of minerals and vitamins (Lorenz and Kulp, 1991). About 198 million tonnes of additional wheat grain will be required to feed the increasing human population on the planet predicted to be 9.8 billion by 2050 (Akter and Islam, 2017). More than 40 countries around the world view wheat as a staple food, providing 85% of people with basic calories and 82% of people with protein (Sharma *et al*., 2019; Chaves *et al*., 2013). According to FAO, annual cereal production must increase by nearly one billion to feed the projected population of 9.1 billion by 2050. To meet the increased food demand, crop production and productivity must increase in the 21st century (Iqbal *et al.*, 2017). Throughout the world, wheat is grown in tropical and subtropical regions which experience various kinds of abiotic stresses. Crop production is significantly reduced by adverse environmental stress (Rahaie *et al*., 2013). Heat, drought, salinity, cold, chemical, and water excess are the major abiotic stresses. However, heat and drought are the main abiotic stresses affecting wheat production all over the world (Lesk *et al.*, 2016; Liu *et al.*, 2016). Wheat is very sensitive to heat stress. According to Asseng *et al.* (2011), each 1°C increase in temperature reduces global wheat production by 6%. In another study on wheat, a one-degree Celsius increase in minimum or maximum temperatures during the cropping season could reduce global wheat production by 5.6% (Lobell and Field, 2007). Barkley *et al.* (2014) found that a one-degree Celsius increase in projected temperature during reproductive stages reduced grain yield by 21%. So, it is important to breed high temperature tolerant genotypes to sustain wheat production. A temperature increases of 1°C above the mean temperature during the reproductive stage may result in a greater loss of grain yield (Bennett *et al.*, 2012; Yu *et al.*, 2014). Hyperthermal conditions have a significant impact on wheat production and other yield-related characteristics (Gupta *et al.*, 2013). Wheat crops are generally found to be heat tolerant in lower altitude areas with extensive wheat cultivation (Braun *et al.*, 2010). Higher temperatures significantly reduced wheat yields, according to research using various mean temperature models. As a result of higher temperatures, there is a change in seed characteristics, panicle exertion, fertilization, pollination, dry matter production, root emergence, stem development, tillering, and germination (Iqbal *et al.*, 2017).

Compared to vegetative stages, wheat is more susceptible to high temperatures (HT) during the reproductive stages (Farooq *et al.*, 2011). The optimum temperature for wheat production during reproductive stages is between 15 and 20°C (Wardlaw *et al.*, 1989). However, an increased frequency of high daytime temperatures (> 34 °C) is projected in wheat-growing regions around the world (Asseng *et al.*, 2011). If high temperatures occur during sensitive phases of wheat, it will have a considerable negative influence on grain yield. Temperature increases during critical growth phases in field crops can reduce output by 2.5 to 10% (Hatfield *et al.*, 2011). High temperature stress harms plant growth and development by reducing leaf photosynthesis (Wahid *et al.*, 2007). Heat stress (HS) in wheat causes poor seed germination, decreased grain filling duration, decreased grain number, deactivation of the Rubisco enzyme, decreased photosynthetic capacity, decreased rate of assimilate translocation, premature leaf senescence, decreased chlorophyll content, and ultimately decreased yield. (Lukac *et al.*, 2012; Hossain *et al.*, 2013; Bala *et al.*, 2014; Kumar *et al.*, 2016; Raines, 2011; Haque *et al.*, 2014; Pandey *et al.*, 2019). Heat stress also has an impact on grain starch and protein content. Heat stress causes the production of reactive oxygen species, which changes membrane stability as well as lipid peroxidation, protein oxidation, and nucleic acid damage. Mishra *et al.*, 2011; Mittler *et al.*, 2011). Furthermore, heat stress causes a rise in the production of reactive oxygen species, such as superoxide radicals, hydrogen peroxide, and lipid peroxidation, which leads to increased membrane damage (Narayanan *et al.*, 2016; Djanaguiraman *et al.*, 2018). However, to prevent the injury and damage caused by heat stress, wheat has evolved different tolerance mechanisms to ensure its survival and growth.

Heat stress-induced heat shock proteins (HSPs) maintain correct protein folding, refolding, and synthesis while also degrading protein aggregates (Hasanuzzaman *et al.,* 2013; Tripp *et al*., 2009; Sharma *et al*., 2019). The antioxidative defense system detoxifies the accumulated ROS via several enzymatic and non-enzymatic antioxidants (Sharma *et al*., 2012). Stay green (SG), chlorophyll fluorescence and canopy temperature are heat tolerance traits in wheat (Pandey *et al.,* 2019).

High temperature stress causes thylakoid membrane expansion and leakiness (Djanaguiraman *et al*., 2018), which results in the physical separation of chlorophyll light-harvesting complex II from the photosystem II core complex (Pastenes and Horton, 1999). Ristic *et al*. (2007) noticed a strong negative relationship between chlorophyll content and thylakoid membrane damage in winter wheat ($r^2 = 0.78$). The lower photosynthetic rate in wheat under high temperature stress is caused by a combination of thylakoid membrane degradation, membrane lipid composition, and oxidative damage to cell organelles (Djanaguiraman *et al*., 2018).

High temperatures have the potential to significantly damage a variety of physiological processes in wheat, including photosynthetic rate, transpiration activity, and grain development rate. As photosynthesis is one of the important physiological processes affecting grain yield in wheat (Ristic *et al*., 2007), the net photosynthetic rate can be used to predict heat stress tolerance in wheat. In addition, heat stress has been reported to decrease the number of grains per spike, grain size, grain weight, plant biomass, plant height, and shorten the life cycle, resulting in lower grain yields in wheat.

Terminal heat stress is defined as high temperature stress during reproductive development. According to Porter and Gawith (1999), the ideal temperature for grain set and grain filling in wheat is between 19°C and 22°C. The threshold temperature considered as the value of the daily mean temperature at which a detectable reduction in growth begins is 26 °C for wheat at the post-anthesis stage (Suryavanshi and Buttar, 2016). Terminal heat stress with an ambient temperature more than 30˚C during the reproductive development stage has been shown to reduce productivity in wheat (Khajuria *et al.,* 2016). Accompanied by rising mean temperatures as a result of global warming, high temperatures during grain filling affect grain yield in many environments (Hays and Mason, 2007). It is one of the major reasons of yield decrease in temperate environments, affecting more than 36 million hectares (Bala *et al*., 2014).

The first stage in developing or improving wheat genotypes for heat stress tolerance is to evaluate the genetic diversity of cultivated germplasm for heat stress tolerance and choose genotypes with a higher level of heat tolerance. Estimating combing ability effects for this purpose provides useful information for selecting suitable parents to operate an effective breeding program. Estimating combining capacities (both general and specific) provides a framework for evaluating the genetic potential of grain production and quality-related plant characteristics under optimal and heat stress circumstances. It also defines the breeding value of parental lines for the production of wheat hybrids (Romanus *et al.,* 2008).

Grain yield and its quality are the main characters of a cereal crop. They are complicated quantitative traits impacted by a number of yield-contributing characteristics. As a result, selecting favorable genotypes should take into account not just yield but also other yield components. Heat stress is a major environmental yield constraint, and its degree of severity depends on its intensity, duration, and crop stage (Wahid *et al*., 2007). Heat stress is more detrimental during the reproductive stage than during the vegetative stage because it has a direct effect on grain number, grain size, dry weight, and finally, crop yield (Wollenweber *et al*., 2003). It has been reported that the sowing dates in terms of changes in temperature are crucial in determining appropriate crop yields. As a result, the present research was carried out to evaluate the performance of novel wheat advance lines in comparison to check varieties, as well as to estimate the impacts of early and terminal heat stresses on yield and its related traits.

Many stress tolerance indices have been proposed by researchers for the identification of stress-tolerant cultivars; however, few are more useful for the selection of heat-tolerant genotypes in wheat. The tolerance index (TOL) was defined as the difference between grain yield under normal and stressful conditions. The average yield of genotypes under normal (Yp) and stress conditions (Ys) is defined as the mean productivity index (MP) (Rosielle & Hamblin, 1981). Fernandez (1992) developed the stress tolerance index (STI), which detects tolerant genotypes in both normal and heat-stressed situations. Its foundation is the geometric mean production index. Mousavi *et al.* (2008) suggested the stress susceptibility percentage index (SSPI) for examining trait stability and differences in traits under both conditions. Fisher and Wood (1979) developed the relative stress index (RSI) in wheat cultivars under drought stress.

Farshadfar *et al.* (2013) used harmonic mean (HM) in wheat-rye disomic addition lines, as it is the ratio of a doubled product of genotypes yield and their sum under both conditions. The mean relative performance (MRP) is another stress index. The yield stability index (YSI) was calculated as the ratio between the yield under stress and normal conditions (Bouslama and Schapaugh, 1984). Gavuzzi *et al.* (1997) developed the yield index (YI) as the ratio of yield of genotypes under normal and the mean yield of all genotypes under stress conditions. Basavaraj *et al.* (2021) used the index was percent yield reduction (PYR) in their research on low phosphorus stress in rice. High MP, GMP, HM, STI, YSI, and YI values, as well as low values for the TOL, SSI, RSI, and PYR, are better ways to select stable and tolerant genotypes (Pour-Aboughadareh *et al.*, 2019).

Table 1. Optimal temperature requirements of wheat at different growth stages (Adopted from Khan *et al.*, 2021).

Stages	**Optimum temperature (°C)**	**Minimum temperature (°C)**	**Maximum temperature (°C)**
Seed germination	20–25 ± 1.2	3.5–5.5 ± 0.44	35 ± 1.02
Root growth	17.2 ± 0.87	3.50 ± 0.73	24.0 ± 1.21
Shoot growth	18.5 ± 1.90	4.50 ± 0.76	20.1 ± 0.64
Leaf initiation	20.5 ± 1.25	1.50 ± 0.52	23.5 ± 0.95
Terminal spikelet	16.0 ± 2.30	2.50 ± 0.49	20.0 ± 1.60
Anthesis	23.0 ± 1.75	10.0 ± 1.12	26.0 ± 1.01
Grain filling duration	26.0 ± 1.53	13.0 ± 1.45	30.0 ± 2.13

The present study was undertaken with the following objectives:

1. To study the effects of heat stress on HSTU-developed advanced lines in wheat;
2. To study the performance of HSTU-developed advanced lines under heat stress;
3. To select heat tolerant advanced lines for candidate variety suitable for Bangladesh; and
4. To study the molecular genetic variation of HSTU-developed advanced lines in wheat.

CHAPTER II

REVIEW OF LITERATURE

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops and large portions of human populations in many parts of the world depend on as a source of food as well as for animal feed. It is an important staple crop all over the world. It is grown in the tropical and subtropical regions of the world, which are subject to various kinds of abiotic stresses. Extreme weather occurrences, particularly variations in temperature and rainfall, pose a significant threat to the successful production of field crops to feed the world's rapidly rising population. Heat stress is one of the most common forms of abiotic stress among other environmental conditions.

The findings of many researchers on the effects of high temperature stress on wheat were examined in the hope that it would assist Bangladeshi wheat breeders in developing an appropriate breeding strategy for the production of varieties with improved heat stress tolerance. A brief review of literature relevant to the objectives of the current study, "Study on the effects of heat stress on HSTU-developed advanced lines in wheat (*Triticum aestivum* L.)," was presented under the following headings.

2.1 Performance of wheat varieties in Bangladesh

Rashid *et al*. (2004) evaluated the performance of different wheat genotypes under late sown conditions (sowing date: December 15 and December 30) during 1999-2000. Grain yield (1.62 t ha^{-1}) obtained from December 15 sowing were significantly higher than that of December 30 sowing (0.98 t ha^{-1}). Different genotypes showed no significant variation in respect of grain yield. The interaction effect showed that plant height, ear length, no. of grains/ear and straw yield were significantly better in December 15 sowing than December 30 sowing while no. of effective tillers/plant and grain yield were not significantly influenced by date of sowing.

Sikder *et al*. (2001) conducted a field experiment of ten recommended wheat varieties were exposed to two sowing conditions i.e. optimum sowing (November 30) and late sowing (December 30) for evaluating heat tolerance and relative yield performance of wheat varieties under late seeded conditions. Based on membrane thermostability (MT) test, four varieties (Ananda, Pavon, Aghrani, and Barkat) took maximum heat killing time and were classified as relative heat tolerant, three varieties (Akbar, Kanchan and Protiva) as moderately tolerant and the rest three varieties (Balaka, Sawgat and Sonora) took the shortest heat killing time and considered as heat sensitive. The grain number per ear, 1000 grain weight and main shoot grain weight of tolerant and moderately tolerant varieties showed higher relative performance compared to sensitive varieties, But the relative ear number per plant and relative grain yield were found to range low-high in heat tolerant and moderately tolerant varieties. In heat sensitive varieties the relative ear number per plant and relative grain yield were moderate to high. Thus, the results suggest in addition to membrane thermostability test, the high relative grain number per ear, 1000 grain weight and main shoot grain weight can be used to determine the heat tolerance of wheat varieties under late seeded warmer conditions.

Joshi *et al.* (2007) evaluated seven hundred twenty-nine lines of diverse what germplasm lines were evaluated in eight locations of three countries (India, Nepal and Bangladesh) of South Asia for 5 years (1999–2000 to 2003–2004) for agronomic performance and tolerance to spot blotch of wheat. Many lines yielded significantly more than the best check and possessed high levels of spot blotch resistance under warm humid environments of South Asia. The most promising 25 lines have been listed as sources of strong resistance, with 9 lines better yielding than the best resistant check PBW 343 in fewer days to maturity. Most of these superior lines represented elite CIMMYT germplasm and around half were derived from Kauz and Veery. The line EGPYT 67, Kauz// Kauz/ Star/3/Prinia/4/Milan/Kauz, was the best for spot blotch resistance, yield, days to maturity, and 1000 grain weight. The next two lines in the order of merit were EGPYT 84 and EGPYT 69.

Yasmin (2007) conducted a wheat performance trial with 10 promising lines (BAW-1021, BAW-1024, BAW-1028, BAW-1030, BAW-1033, BAW-1035, BAW-1036, BAW-1038, along with two control cultivars, Kanchan (BAW-28) and Shatabdi (BAW-936)) under optimum seeding time at Dinajpur, Jamalpur, Jessore, Ishurdi and Joydebpur. The experiments were laid out in Randomized Complete Block Design (RCBD) during 2002-2003 with four replicates at each location under the supervision of WRC (Wheat Research Center. From the statistical analysis she observed that no significant differences in rank stability were found among the ten genotypes grown in five environments. Genotypes 6 (BAW-1030), 8 (BAW-1035) and 10 (BAW-1038) are the most stable and well adapted to all environments due to non-significant $sd_i^{\ 2}$ value, $b_i \leq 1$ and lower S1(1) values than other genotypes with mean yield ≥ grand mean.

On the other hand, genotypes 4 (BAW-1028), 7 (BAW-1033) and 9 (BAW-1036) have an increasing sensitivity to environmental change and greater specificity of adaptability to high-yielding environments. However, BAW-1021 (G3) was poorly adapted genotypes to all environments and only one genotype BAW-28 (G1) that response greater resistance to environmental fluctuation, and therefore increasing specificity of adaptability to low-yielding environments.

2.2 Constraints of wheat production in Bangladesh

There are several constraints observed on wheat production in Bangladesh. Wheat plants are exposed to many stressors including salinity, drought, heat, cold, flooding, ultraviolet radiation, and metal toxicity, restricting their growth and limiting their productivity. Abiotic stress decreases productivity by 50% in most agriculturally valuable plants, including wheat (Vandenbroucke and Metzlaff, 2013). Heat-stress is the major problem for wheat production in Bangladesh. Wheat agronomists' research into the constraints of wheat production in Bangladesh from 1988 to 1990 found that a variety of natural and managerial factors influence wheat yield. Wheat yield is estimated to be reduced by: (a) 23-42% due to foliar diseases; (b) 8-16% due to soil pathogens; (c) 25-46% due to farmers' fertilizer doses (which are lower than recommended doses); (d) 2.1% and 33.7% due to lack of irrigation in high- and low-fertility situations; and (e) 1.3% per day of delay after November 30th (Ahmed and Meisner, 1998). However, the typical farmer produces 1.9 t/ha, whereas best-practice farmers can produce 2.8 t/ha, indicating a 29% yield differential (Hasan, 2005). The difference in production between farmers who follow optimal practices and average farmers is equivalent to a 25% loss on the gross margin, or Tk. 9,875/ha or US$169/ha. Consequently, there is a great deal of possibilities for improvement in the average farmer's productivity performance. The key objective of this research is to objectively determine technical efficiency in wheat production and identify its causes as a means to determine the scope of this type of research.

2.3 Effect of heat stress on wheat

Heat stress affects the different stages of wheat growth and development, resulting in severe yield losses. However, the effect of heat stress on plants is dependent on the length of heat exposure and growth stage during the high temperature (Ruelland and Zachowski, 2010; Balla *et. al.*, 2012).

Heat stress reduces wheat photosynthesis by causing poor germination, lower leaf area, early leaf senescence, and impaired photosynthetic machinery (Asseng *et al*., 2015). Heat tolerance changes wheat's physiology, biochemistry, and morphology. Wheat undergoes morphological, physiological, and biochemical alterations as a result of heat stress.

2.3.1 Effect on wheat morphology

In various crops, including wheat, heat stress negatively influences the seed germination and plant establishment (Hossain *et al.,* 2013). High temperatures affect the survivability of the productive tiller, which results in a decrease in yield. Heat stress in wheat result in a decrease in grain yield (53.57%) and tiller number (15.38%) (Riaz-ud-Din *et al*., 2010). Heat stress causes a decrease in root growth, which ultimately affects the crop production (Huang *et al*., 2012). The effect of heat stress is highly significant during the reproductive phase (Nawaz *et al*., 2013)**.** Riaz-ud-Din *et al*. (2010) investigated the effect of temperature on grain formation and development in ten spring wheat genotypes. Tillers indicated a 15.38% reduce under late planting conditions, compared to a 53.75% reduction in grain yield**.** Microspore and pollen cell development is negatively affected due to heat stress during of floral initiation stage (Kaur and Behl, 2010). The grain development phenomenon depends on the grain filling rate and duration, which are highly sensitive to heat stress (Gourdji *et al*., 2013; Lobell and Gourdji, 2012). 1°C-2°C As the temperature rises, the time of grain filling decreases, lowering seed weight (Nahar K. *et al*., 2010). The size and number of grains are susceptible to heat stress, and the severity depends on the developmental stage (Ferris *et al.* 1998). Heat stress has an effect on spikelet initiation, male and female pollination, pollination, and fertilization. Short-term heat stress during grain filling can result in a 23 percent reduction in grain production (Mason RE. *et al*., 2010). Wheat grain yields can be greatly reduced when exposed for a short period of time to ambient temperature (>35°C) (Sharma P. *et al*., 2017).

2.3.2 Effect on wheat physiology

Photosynthesis is the most important physiological process in plants which is strongly influenced by high temperatures. Wheat's stroma and thylakoid lamellae are the most sensitive to heat stress (Mathur *et al.,* 2014). The quantity of photosynthetic pigments is decreased by heat shock (Marchand *et al.,* 2005). High temperatures (~ 40°C) result in permanent interchange of RuBisCO, Rubisco Activase, and Photosystem II (Mathur et al., 2011). The RuBisCO enzyme was observed to be deactivated in less than 7 days after being exposed to heat stress conditions in wheat (Kumar *et al.,* 2016). Heat tolerance is directly related to a plant's ability to maintain leaf gas exchange and CO2 assimilation rates under heat stress (Yang *et al*., 2006; Kumar *et al*., 2005). Plant starvation and the loss of glucose stores are also observed under prolonged heat stress (Djanaguiraman *et al*., 2009). Heat stress alters mitochondrial activity by affecting respiration. The rate of respiration increases with increasing temperature, but at a certain level of temperature, it diminishes due to damage to the respiratory apparatus (Prasad *et al*., 2008). Heat stress induces leaf senescence in plants during maturity (Haque *et al.,* 2014). High temperature affects the water relation and content in the plant. Cell dehydration is observed under heat stress as a result of a decrease in osmotic potential (Ahmad *et al*., 2010). Dhyani *et al*. studied heat tolerance and heat-sensitive wheat genotypes grown under normal and late-sown conditions and discovered that chlorophyll content and leaf area index were significantly lower in heat-sensitive genotypes, but Proline content was higher in heat-tolerant genotypes grown late (Dhyani *et al*., 2013). Under heat stress condition, plants highly produce reactive element known as Reactive Oxygen Species (ROS).

Because of their detrimental effects on DNA, lipids, and proteins, ROS disrupt cell activity. A 54% reduction in membrane thermostability is caused by oxidative damage linked to heat stress (Savicka and Škute, 2010). ROS accumulated under heat stress stimulates protein denaturation and formation of unsaturated fatty acids, which finally increases cell membrane permeability (Cossani and Reynolds, 2012).

2.3.3 Effect on wheat biochemistry

Starch is one of the vital constituents of wheat and is composed of amylose and amylopectin. Amylose content is an essential parameter to mark starch quality. Starch properties are affected by variation in amylose content. High temperatures are associated with with an increase in amylose content and the amylose: amylopectin ratio (Cossani and Reynolds, 2012). At high temperatures, there is a decrease in starch content in grain, up to one-third of total endosperm starch, which is caused by the decreased efficiency of enzymes involved in starch biosynthesis (Liu *et al*., 2011).

Rampino *et al*. (2009) investigated the expression of HSPs genes in durum wheat cultivars and the acquisition of thermo-tolerance. Plants are susceptible to heat stress, and they tend to overcome by modifying their many physiological and biochemical mechanisms. Plants depend on HSP production at the cellular and molecular levels to avoid or mitigate the negative effects of high temperatures. The importance of heat stress response and

HSPs expression in cereal yield and quality thermo-tolerance has been investigated. As proposed by Blumenthal *et al*. (1991) wheat high temperature during grain filling stimulates heat shock genes, causing mature grains to have more protein and so generate weaker dough.

According to Mumtaz *et al.* (1995) proline improves protein and membrane stability under high temperature or moisture stress. While Heber and Santarius (1973) reported that sugar alcohols improved membrane and protein stability to high temperature denaturation, Zhu *et al.* (2004) discovered that during the grain filling stage, higher accumulation of proline occurs in stressed leaves, indicating that under moderate stress, the role of proline is related to a protective action.

2.4 Mechanism of Heat Tolerance

Heat tolerance is defined as a plant's ability to survive and maintain normal development under heat stress (Wahid *et al*., 2007). To cope with heat stress, wheat has evolved adaptive mechanisms such as escape, avoidance, and/or staying green (Farooq *et al*., 2011; Wahid *et al*., 2007). Plants can avoid terminal heat stress by reducing grain filling length and increasing grain filling rate with stem reserves to preserve production. Avoidance is the maintenance of an optimal plant water status through the reduction of water loss (via stomatal closure, trichomes, wax on leaves, leaf rolling, change in leaf angle, senescence of older leaves, etc.) or the enhancement of water availability (via improved root architecture and growth). Plants have evolved various mechanisms for survival under higher prevailing temperatures. They include mechanisms for short-term avoidance/acclimation as well as long-term evolutionary adaptations. Ion transporters, late embryogenesis abundant (LEA) proteins, osmo-protectants, antioxidant defense, and components involved in signaling cascades and transcriptional regulation are all important in mitigating the consequences of stress (Rodríguez *et al*., 2005; Wang *et al*., 2004).

Rampinoa *et al*. (2009) investigated the development of thermo-tolerance and HSPs gene expression in durum wheat (*Triticum durum* Desf.) cultivars. The synthesis of heat-shock proteins (HSPs) is crucial for mitigating or avoiding the harmful effects of high temperatures on cells and molecules. Protein synthesis and folding are critical to protein function. Protein misfolding has a profound impact on the cell's working mechanism. Protein folding and synthesis are disrupted under heat stress conditions (Sharma *et al*., 2019).

HSPs are classified into families based on their molecular mass, amino acid sequence homologies, and functions (Gupta *et al*., 2013). The HSP100 family, HSP90 family, family, HSP70 family, HSP60 family, and small HSPs family are among the families. Under heat stress conditions, several HSPs are related with various functions. Heat stress transcription factors (Hsfs) are present in the cytoplasm in an inactive condition, which acts as a regulatory protein in the transcription of the genes that encode for HSPs. These Hsfs function as transcriptional activators in response to heat stress (Hu *et al.*, 2009). HSPs act as molecular chaperons to inhibit protein denaturation and aggregation under heat stress (Hasanuzzaman *et al*., 2013; Al-Whaibi, 2011).

Unfavorable reactive oxygen species (ROS), such as hydroxyl radical (•OH), superoxide (O_2^-), and singlet oxygen ($^1O_2$), are produced when a plant is under heat stress (Marutani *et al*., 2012; Suzuki *et al*., 2012). Redox homeostasis, defined as the balance between ROS generation and scavenging, occurs in a normal cell (Caverzan *et al*., 2016). Oxidative stress is the state in which a cell experiences stress due to the generation of more ROS than it can scavenge (Mullineaux and Baker, 2010). Heat stress causes changes in membrane potential (depolarization), lipid peroxidation, protein oxidation, nucleic acid damage, obstructs enzyme function, and activates programmed cell death (Mishra *et al*., 2011; Mittler *et al*., 2011; Srivastava and Dubey, 2011). To protect the plant from oxidative damage, ROS must be detoxified by an antioxidative defense mechanism (Xin *et al*., 2019). The antioxidative defense mechanism in plants is extremely powerful, because to the involvement of many enzymatic and non-enzymatic antioxidants (Puthur, 2016). Superoxide dismutase (SOD), ascorbate peroxidase, catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), and peroxidase (POX) are enzymatic antioxidants, whereas non-enzymatic antioxidants include ascorbic acid, glutathione, tocopherols, carotenoids, and phenolic compounds (Caverzan *et al*., 2016; Suzuki *et al*., 2011). Balla *et al*. (2009) reported that the activity of S-transferase (GST), APX, and CAT is increased in heat-tolerant cultivars. Although ROS production is related with oxidative stress, it may also function as a signaling molecule in response to various abiotic stresses, triggering tolerance to such stress. As a result, ROS should not be fully eliminated and should be maintained at a level sufficient to prevent oxidative injury.

Stay green (SG) genotype provides photosynthesis and grain filling in heat stress conditions via late expression of senescence-related genes (Vijayalakshmi *et al*., 2010). Stay green is an important heat stress tolerance strategy in wheat because it conserves photosynthetic area and increases nitrogen remobilization to maturing grains (Poiroux-Gonord *et al*., 2013).

A study was done to investigate the relationship between remain green qualities and canopy temperature depression (CTD) (Dolferus *et al*., 2011). They discovered that stay green genotypes had higher CTD (air temperature-canopy temperature) values under heat stress conditions and concluded that stay green is strongly associated with CTD. As a result, the stay green trait can be adopted as a selection criterion in wheat genotypes under heat stress (Huang *et al*., 2012).

2.5 Mean performances of different morpho-physiological traits in wheat genotypes

Abro *et al*. (2019) conducted a research on twenty bread wheat genotypes viz. AS-11, AS-12, AS-13, AS-14, AS-15, AS-16, AS-17, AS-18, AS-19, AS-20, AS-21, AS-22, AS-23, AS-24, AS-25, AS-26, AS-27, AS-28, AS-29 and Chakwal-86 (check), at the Experimental Field, Nuclear Institute of Agriculture (NIA), Tandojam. The experiment was conducted in Randomized Complete Block Design having three replications during Rabi season, for evaluating the response of terminal heat stress in wheat genotypes. Reduction in various traits was observed due to late planting, which indicated visible effects of high temperature on physio-yield traits. On the basis of average performance, tillers per plant, spikelet per spike, grain yield per plant, grains per spike, grain weight of mean per spike and biological weight per plant showed reduction of 24.83, 7.1, 39.78, 14.87, 25.49 and 37.41, respectively under heat stress conditions. However, minimum reduction under heat stress was manifested in the wheat genotypes as AS-15, AS-26 and AS-13 for various traits, suggesting their presence of heat tolerance.

Zcgeyc *et al.* (2020) found the result in an experiment, average wheat production level per hectare (2.7 tons/ha) has been on the increase with inter annual variability, but the yield level is very low as compared to that of the research station (6-7 tons/ha) and the estimated average potential (5 tons/ha) in highland areas of the country. The yield gap analysis shows that 61 %, 55% and 46% of wheat yield gap existed when the national average yield was compared with that of the actual yield at research station, farmers' plot and potential yield at highland part of the country, respectively. The empirical analysis found there is statistically significant level of yield variability among wheat growers.

Rana (2019) included ten genotypes in his experiment namely BARI Gom-25, BARI Gom-26, BARI Gom-27, BARI Gom-28, BARI Gom-29, BARI Gom-30, BARI Gom-31, BARJ

Gom 32 and two advanced line BA W-1203 and BA W-1194. The results of the experiment revealed that there were a genetic variability and significant variations between genotypes were observed for all the characters. In case of genotypes BARI Gom-26 requires comparatively fewer days and BARI Gom-31 requires more days for 50% heading than other cultivars. Among the cultivars BARI Gom-27 is longer whereas, BARI Gom 32 is comparatively shorter. The maximum thousand grain weight was recorded in BA W-1194 whereas the minimum thousand grain weight was recorded from BARI Gom-26. Random forest method was highly capable of predicting crop yields. The distribution of the number of nodes for the tree and it is 82.5. The highest yield/plant was found in BAW-1194. The lowest yield/plant found in BARI Gom 32. The highest yield/plot was found in BARI Gom-30 and the lowest yield found in BARI Gom-25. The number of grains per spike, has the highest value this mean it bad a maximum importance in terms of contributing accuracy. Compared to them, it can be seen that at the effective tillers per plant contribution was negative. So, this variable was not that important for prediction.

2.6 Stress tolerance indices under heat stress

Lamba *et al*. (2023) investigated 50 wheat genotypes to find heat-stress-tolerant genotypes for stress tolerance improvement. The field experiment was carried out in the Research Area of Wheat and Barley Section, Department of Genetics & Plant Breeding, CCS Haryana Agricultural University, Hisar. The experiment was laid out in two replications in Randomized Block Design (RBD). All genotypes were grown for two years (2018-2020) under normal and late-sown conditions. The study's findings found that the combined analysis of variance revealed significant variations among genotypes for all of the stress indices observed. Heat stress significantly decreases grain yield in all genotypes as compared to non-stress conditions, indicating that heat stress has a significant effect on grain yield. The correlation analysis demonstrated a negative correlation of the tolerance index and the stress susceptibility percentage index with the grain yield of genotypes under heat stress (Ys) and a highly positive correlation of the stress tolerance index, mean productivity, geometric mean, harmonic mean, and mean relative performance with grain yield (Yp and Ys) under both conditions, which assisted in accurately identifying the desirable genotypes. According to the results of principal component, biplot, and cluster analysis, HD 2967, WH 1249, HI 1617, WH 1202, WH 1021, and WH 1142 are suitable and high-yielding genotypes under both conditions. As a result, the mentioned genotypes can be used for high-temperature cultivation or as genetic resources for introducing genetic variations in wheat genotypes to improve stress tolerance.

Poudel *et al*. (2021) carried out a field experiment for twenty wheat genotypes were evaluated during wheat growing season (2019–2020) under normal and late sown (heat stress) conditions. The experiment was conducted at Institute of Agriculture and Animal Science, Bhairahawa, Nepal using alpha lattice design with two replications. The results of the study reveal that the mean grain yield of all genotypes was decreased by 47.58% under stress condition as compared to normal sowing condition, suggesting significant effect of heat on grain yield. Tolerance index (TOL) and stress susceptibility index (SSI) exhibited a significant negative correlation with grain yield (Ys) of genotypes under stress conditions (0.804 to 0.945). Similarly, grain yield of genotypes was shown to be significantly and positively correlated (0.899 to 0.965) with mean productivity (MP), geometric mean productivity (GMP), and stress tolerance index (STI) under both conditions. According to the findings, these indices could be used to identify high-yielding genotypes under both conditions. Bhrikuti, NL1420, BL4669, NL1350, and NL1368 were discovered to be superior genotypes with high yielding capability under both conditions using principal component and biplot analysis.

Kamrani *et al*. (2018) conducted a field experiment at the research farm of Mohaghegh Ardabili University located at Moghan, Iran during 2012–2013 and 2013–2014 growing seasons. Forty-five durum wheat (*Triticum turgidum* var. *durum*) genotypes were grown during two growing seasons (2012–2013 and 2013–2014) under non-stress (normal sowing) and heat-stress (late sowing) conditions by using randomized complete block design (RCBD) with three replications. Based on the grain yield under normal sowing (Yp) and late sowing (Ys) conditions, the heat tolerance indices were estimated. The combined analysis of variance results demonstrated the significant effects of heat stress on grain yield in addition to significant differences among genotypes for grain yield and the indices. Results of correlation coefficients and multivariate analyses shown that the stress tolerance index (STI), geometric mean productivity (GMP), and mean productivity (MP) indices were the most profitable criteria for the selection of heat-tolerant and high-yielding genotypes. By using STI, GMP, and MP, the genotypes G29, G41, and G10 were found to be the best genotypes with a relatively high yield and suitable for both normal and heat-stressed conditions. It was possible to identify superior genotypes across the conditions based on biplot analysis with Yp, Ys, and the indices.

2.7 Importance of genetic diversity analysis of wheat

Wani *et al*. (2022) found that every successful crop improvement program is dependent on genetic diversity. In the face of climate change, higher food production must be performed with fewer agricultural inputs in order to feed more than 9 billion people by 2050. The wild cousins of wheat (*Triticum aestivum* L.) had a variety of features that could increase wheat productivity and quality, as well as its resistance to biotic and abiotic stresses. The use of high-throughput (i.e., genomic and phenomic) technologies and the creation of creative breeding strategies were required to quickly introgress advantageous alleles from the reservoir of genetic diversity prevalent in wild wheat relatives into modern wheat in order to increase genetic gains and make agricultural systems climate resilient.

Mourad *et al*. (2020) studied an important crop such as wheat (*Triticum aestivium* L.), which was grown all over the world and had a complex genome. Understanding the genetic diversity among worldwide wheat genotypes was crucial for finding parents with relevant agronomic traits that might be exploited in the various breeding programs. Understanding genetic variation was also helpful in breeding research like genome-wide association studies (GWAS), marker-assisted selection (MAS), and genomic selection.

According to Mujeeb-Kazi *et al.* (2013), genetic diversity is present in three gene pools of *Triticeae* and is necessary for the improvement of all crops. In order to provide the necessary defense against diverse abiotic and biotic stresses, expanding the genetic base of cultivated wheat will ensure that increasing wheat production is more sustainable. Based on the genetic separation between the relatives of wild species and the wheat genomes, accessed to this diversity in wheat and its exploitation were determined. Global wheat development tactics focus on the diversity that was there and can be used to create improved high-yielding cultivars. The focus was either on improving yield or developing resistances or tolerances for important biotic or abiotic stressors. The breeding situation for wheat had been dominated by conventional germplasm, and the genetic diversity that was currently accessible had been able to meet the demands of wheat breeders for novel types.

2.8 Importance and uses of SSR markers in wheat

Kumar *et al*. (2021) observed that for breeding programs, simple sequence repeat (SSR) markers were taken into consideration due to their merits, which include repeatability, multi-allelic nature, co-dominant inheritance, relative abundance, and good genome coverage.

Swetha *et al*. (2022) observed that microsatellites were DNA repeating sequences with a 2–5 base set, sometimes referred to as simple sequence repeats (SSRs) or short tandem repeats (STRs). Compared to other types of markers, SSR markers had a number of benefits. SSR markers had two advantages: first, they had excellent repeatability, and second, they included genetic information that was polymorphic. SSR markers were repeating sequences that make it simple to find and identified the gene of interest for specific chromosomal features. In comparison to other genetic markers, SSR markers showed a higher level of polymorphism. Compared to other forms of cell markers, they also had automatic power and dominant inheritance benefits.

Shafi *et al*. (2021) found that the identification of quantitative trait loci, marker-assisted selection (MAS), genetic diversity, labeling of stress-tolerant genes in wheat or its wild relatives, and genetic variability research in wheat seed-borne illnesses were all possible uses for SSR markers.

Kumar *et al*. (2016) conducted a study to determine the degree of genetic similarity in diversity research. In this case, Microsatellites or Simple Sequence Repeats (SSRs), were a useful tool. SSR markers were suitable for detecting allele frequency within the population and for determining population structure because of their high rate of polymorphism, or high Polymorphic Information Content (PIC), co-dominant character, selective neutrality, distribution across the genome, environment-independent characteristics, and cost and labor efficiency.

Masoumi *et al*. (2012) studied that, by using SSR markers, numerous studies have looked at the genetic diversity among various plant species. SSRs were repeats of one to six nucleotides that can be found in both coding and non-coding regions of the genome. SSRs were a preferred genotype marker because of their high frequency, significant allelic diversity, co-dominant inheritance, and simplicity of analysis.

2.9 Molecular characterization of wheat genotypes using SSR markers

Meena *et al*. (2022) reported that 82 SSR markers were used to assess the molecular diversity in a collection of 32 wheat genotypes; among them, only 45 SSRs were polymorphic. Estimates for marker index ranged from 0.22 (WMC213) to 1.33 (WMC232), resolving power ranged from 1.6 (GWM194 and CFD127) to 4.69, and estimates for polymorphism information content ranged from 0.09 (GWM297) to 0.50 (GWM111 and CFD46) (WMC505). A total of 102 alleles were found, including 20 uncommon and three unique ones. The average number of alleles per marker was 2.2, while the number of alleles per marker ranged from 2 to 4.

Kara *et al.* (2020) studied a total of 16 SSR primers tested; only 11 showed polymorphic bands (WMC 14, WMC 15, WMC 17, WMC 20, WMC 21, WMC 24, WMC 25, WMC 27, WMC 48, WMC 50, and WMC 283). No amplified products were obtained with WMC 16, WMC 18, WMC 19, WMC 22, and WMC 23 primers. The polymorphism information content (PIC) varied from 0.14 (WMC 21) to 0.70 (WMC 50 and WMC 17), with an average of 0.48 and 0.49. This implies that the markers were highly informative.

Farshadar and Farshadar (2022) used 20 SSR markers, among which 16 primers with appropriate polymorphisms were chosen. XCFD168-2D, XGWM350-7D, and XGWM136-1A primers showed 100% polymorphism, the greatest number of alleles, high amounts of polymorphic information content indices, marker indices, effective multiplex ratio indices, and resolving indices. These were introduced as the best primers for wheat in subsequent studies due to the high proliferation of bands and the production of high polymorphic bands.

Odindo (2020) observed that polymorphic information content (PIC) correlates positively with the number of alleles per locus and useful for assessing the discriminating power of markers. The PIC values for the SSR loci ranged from 0.28–0.77, with a mean of 0.58, which is higher than the mean PIC (0.33) and lower than the PIC value of 0.60 in wheat. Simple sequence repeats primers with high PIC also exhibited a high number of effective alleles per locus (i.e., Wmc596 [PIC = 0.73, Ne = 3.66], Xwmc182a [PIC = 0.76, Ne = 4.24], and Xwmc707-4a [PIC = 0.77, Ne = 4.40]), showing high marker ability for genetic analysis among the studied heat-tolerant and drought-tolerant wheat genotypes.

Hasanuzzaman *et al.* (2022) examined 44 genotypes of wheat and were subjected to marker-assisted selection using 15 simple sequence repeats (SSRs). The TSSRI and principal component analysis (PCA) revealed that Akbar was the most salt-tolerant wheat genotype, followed by Barigom-20, Barigom-22, BW-1284, BW-1262, BW-1237, and Barigom-24. The highest value (0.7127) of polymorphism information content (PIC) was shown by the marker Xwmc17. Considering the morphological characterization and molecular marker response, Akbar, Barigom-20, Barigom-22, BW-1284, and BW-1262 are identified as tolerant genotypes.

Khodadadi *et al.* (2022) worked under control and water-deficit stress conditions, and a group of Iranian accessions of the *Aegilops tauschii* Coss. and *Triticum aestivum* L. species were assessed. 50 fragments were produced by 25 SSR markers, 49 of which (98%) were polymorphic. The markers Xgwm-111, Xgwm-44, Xgwm-455, Xgwm-272, and Xgwm-292 may be valuable genetic tools for gene tagging, population characterization, and other molecular breeding investigations.

Ateş and Terzi (2018) observed that 10 bread wheat cultivars (*Triticum aestivum* L.) and 9 breeding lines were examined by using SSR microsatellite markers. The genotypes were screened with molecular markers for the presence of QTLs mapping to different chromosomes. The molecular study results identified and detected 15 polymorphic SSR markers, which gave the clearest PCR bands among the control genotypes. At the end of the research, bread wheat genotypes, which were classified as tolerant or sensitive to drought, and the genetic similarity within control varieties were determined by molecular markers.

Kumar *et al.* (2016) studies showed that advancements in the field of molecular markers have made the genetic characterization of genotypes rapid, reliable, and reproducible. In this study, 10 wheat genotypes were characterized at the molecular level using 12 simple sequence repeat (SSR) markers. Individual distinctness of the genotypes had become evident from the dendrogram prepared on the basis of allelic diversity revealed by the molecular markers. Among the 12 SSR markers used, 2 had been observed to be monomorphic, whereas the rest of the markers revealed polymorphic information content values ranging from 0.17 to 0.50.

2.10 Population structure of wheat genotypes

Pour-Aboughadareh *et al*. (2022) population structure of 100 individuals from four *Triticum* and *Aegilops* species (including *T. aestivum*, *Ae. tauschii*, *Ae. cylindrica*, and *Ae. crassa*) were investigated using simple-sequence repeats (SSRs). The population structure analysis using SSR data showed that K = 4, and the samples were grouped into four distinct subpopulations. A cluster analysis using each marker system and combined data showed that the SSR marker had greater efficiency in a grouping of tested accessions, such that the results of principal coordinate analysis (PCoA) and population structure confirmed the obtained clustering patterns.

Belete *et al.* (2021) conducted a research in which fifty-two selected bread wheat genotypes were evaluated at five test sites. The test genotypes were assessed using eight phenotypic traits and 20 SSR markers. SSR analysis identified a total of 181 alleles, with a mean of 10.1 alleles per locus. Population structure analysis grouped the test genotypes into three main populations. Cluster analysis based on phenotypic and SSR data grouped the test genotypes into three major groups. Three genotypes [10 (ETW17-295), 37 (ETW17-385) and 38 (ETW17-386)] were identified as the most divergent and were recommended for drought-tolerance breeding. Overall, the SSR markers were useful and provided complementary data for selecting agronomically suitable parental lines for drought-tolerance breeding.

Ouaja *et al.* (2021) found that 10 simple sequence repeat (SSR) markers with a polymorphism information content (PIC) of 0.69 were used to evaluate the 304 local accessions. 11 genetic groupings were discovered by population structure analysis, and they were significantly associated with the morphological characteristics.

Mdluli *et al.* (2020) assessed the genetic diversity and population structure of 47 bread wheat genotypes using 10 polymorphic simple sequence repeat (SSR) markers. Results showed that, at the locus level, marker Xgwm 132 had the highest Na (21), Ne (14.5), Ho (1.0), and He (0.94), while at the population level, Population III had the highest Na (21), Ne (5.59), He (0.83), and I (1.78). Four populations were indicated based on pedigrees, with GD ranging from 0.01 (Populations III and IV) to 0.31 (Populations II and III), while GI ranged from 0.74 (Populations II and III) to 0.99 (Populations III and IV). The selected markers successfully grouped test genotypes by using the most informative marker, Xgwm 132. Populations II and III were most distinct, therefore suitable for parental selection and further drought tolerance breeding.

Khan *et al.* (2021) studied Bayesian-based population structure, with a total of 158 wheat genotypes classified into seven groups based on SSR data. The groups are based on genetic similarity, indicating their ancestral origin and geographical ecotype.

Khan *et al.* (2020) performed a study in which the genetic association of 95 tetraploid and hexaploid wheat genotypes originating from India and Turkey was evaluated for the first time. Turkish hexaploid varieties were basically dispersed into two clusters; one group revealed a close association with Indian hexaploid varieties and the other with Indian tetraploid varieties. Analysis of molecular variance revealed high (77%) genetic variation within Indian and Turkish populations. Population structure analysis elucidated distinct clusterings of wheat genotypes on the basis of both geographical origin and ploidy. The results will support worldwide wheat breeding programs and aid in achieving the target of sustainable wheat production.

Mourad *et al*. (2020) based on examinations of population structure, primary coordinates, and kinship relationships, the 103 wheat genotypes that were evaluated had three distinct subpopulations. Based on the AMOVA, considerable variation was discovered both within and between the subpopulations. Based on the various allelic patterns, Subpopulation 1 was shown to be the more varied subpopulation (Na, Ne, I, h, and uh).

Zhang *et al.* (2010) carried out a study in which the genetic diversity and population structure of 205 elite wheat breeding lines were analyzed using 245 markers across the wheat genomes. This result showed a high level of genetic diversity as reflected by allele number per locus (7.2) and polymorphism information content (0.54). In addition, the diversity of U.S. modern wheat marked to be lower than previously reported diversity levels in worldwide germplasm collections. This collection was highly structured according to geographic origin and market class, with soft and hard wheat clearly separated from each other. Hard wheat accessions were further classified into three subpopulations.

Bhatta *et al.* (2018) suggested a diverse source of 143 synthetic and bread wheat accessions for identifying potentially rich genetic resources in wheat. The population structure analysis implied three distinct clusters of wheat genotypes on the basis of the type and geographical origin of wheat accessions. Population differentiation using the analysis of molecular variance indicated 21% of the total genetic variance among subgroups and the remainder within subgroups.

Ya *et al*. (2017) examined 200 wheat accessions using 15 SSR markers dispersed throughout the wheat genome to ascertain genetic diversity and population structure. Based on structural and phylogenetic investigations, the 200 Mongolian wheat accessions were primarily divided into two subgroups, and certain traits were different between the subgroups.

2.11 Genetic diversity assessment based on SSR markers in wheat

Ahmed *et al*. (2020) carried out an experiment in which a total of 105 bread wheat genotypes were studied to determine the genetic diversity and genome-wide allelic variation. A natural population of 105 bread wheat genotypes was analyzed. The 302 polymorphic SSR markers, distributed among homoeologous genomes A, B, and D, were manipulated. A total of 2308 alleles of 302 markers, with an average density of 7.6 alleles per marker, were observed. Among the observed polymorphic alleles, 685, 869, and 754 for 102, 100, and 100 polymorphic SSR loci were found in the A, B, and D genomes, respectively.

Tahir *et al*. (2022) observed that the phenotypic and genetic diversity of 20 wheat lines were assessed using eight SSR markers. With an average of 2 alleles per location, the genetic diversity at eight SSR markers indicated a total of 16 alleles. Six of the eight SSR markers were dimorphic, indicating two alleles at each locus, whereas one marker (WMC105) was monomorphic. For marker WMC78, the highest number of alleles (3) was seen, and genotypes AC and AA were mostly observed in the high-yielding lines Borlaug-2016 and Zincol-2016, which were not closely connected to other kinds.

Thungo *et al.* (2020) examined twenty-four agronomically selected wheat genotypes from the International Maize and Wheat Improvement Centre's (CIMMYT) drought tolerance nursery, and four local check varieties were genotyped using 12 selected polymorphic SSR markers. Using cluster analysis, the studied wheat genotypes were plotted into six genetic groups. Significant genotypic differences were observed for agronomic traits and GPC under NS and DS conditions. Genetically unrelated breeding parents, viz., LM02, LM13, LM23, LM41, LM44, LM71, LM73, and LM75, were selected for population development to enhance grain yield and protein content under heat and drought-stressed environments.

Koli *et al.* (2022) studied nine wheat cultivars' genetic diversity by using 23 polymorphic microsatellite (SSR) markers. Moderate levels of polymorphism were found in the collection of genotypes under study, according to an analysis of molecular diversity. There were found to be 56 alleles, with an average of two alleles per locus. PIC values for allelic polymorphisms ranged from 0.16 to 0.67, with 0.33 serving as the average. The primers gwm192, gwm111, gwm374, STSS5765, XPSP3000, wmc104, and gwm192 might be deemed very informative. The cultivars "WH1184" and "WH1124" had the lowest dissimilarity index and were the most comparable, while "WH711" and "HD3059" had the biggest genetic distance. Indicators of dissimilarity ranged from 0.62 to 0.85. There were three main clusters made up of one, one, and seven genotypes.

Sagwal *et al.* (2022) developed and evaluated a total of 98 simple sequence repeat (SSR) markers, including 66 microRNAs and 32 gene-specific SSRs, on a panel of 10 (N and P efficient/deficient) wheat genotypes. Out of these, 35 SSRs were found to be polymorphic and have been used for the study of genetic diversity and population differentiation. A set of two SSRs, namely miR171a and miR167a, were found to be candidate markers able to discriminate between contrasting genotypes for N and PUE, respectively. Target genes of these miRNAs were found to be highly associated with biological processes (24 GO terms) as compared to molecular function and cellular components and show differential expression under various starving conditions and abiotic stresses.

Wang *et al.* (2017) observed the morphological and genetic diversity and population structure of 238 *T. urartu* accessions. This collection had 19.37 alleles per SSR locus, and its polymorphic information content (PIC) value was 0.76. The PIC and Nei's gene diversity (GD) of high-molecular-weight gluten in subunits (HMW-GSs) were 0.86 and 0.88, respectively.

Islam *et al.* (2012) performed research to examine the genetic diversity of 12 wheat genotypes using 4 simple sequence repeats (SSRs). The polymorphic information content (PIC) values ranged from 0.2755 to 0.5411, with an average of 0.3839. The average gene diversity over all SSR loci for the 12 wheat genotypes was 0.4688, ranging from 0.3299 to 0.6042. Genetic diversity was the highest between varieties Gourab and Akbar as well as Gourab and BAW-1064, showing a genetic distance value of 0.4697. In addition, genetic distance was lowest between Balaka and Aghrani, as well as Triticale and BAW-1036. Between gene diversity, the number of alleles, the allele size range, and the types of repeat motif of microsatellite markers, positive correlations were estimated. From this study, it was found that microsatellite markers could characterize and discriminate all of the genotypes.

Al-Naggar *et al.* (2020) utilized microsatellite markers to assess the genetic diversity of 20 Egyptian wheat landraces and two cultivars (SSRs). In the group of 22 wheat accessions, ten SSR markers amplified a total of 27 alleles, of which 23 (85.2%) were polymorphic. The majority of the markers had high polymorphism information content (PIC) values (0.67–0.94). Utilizing a dendrogram, the genotyping information from the SSR markers was utilized to evaluate genetic variance in the wheat accessions.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted at the Genetics and Plant Breeding research field of Hajee Mohammad Danesh Science and Technology University, Dinajpur.

The molecular work was conducted at both BWMRI Molecular Breeding Laboratory and Biotechnology Laboratory of Genetics and Plant Breeding Department, HSTU, Dinajpur.

The materials and methods of the study were presented in that chapter under the following sub-headings.

The experiment was carried out with two different sowing times, viz,

1. Optimum sowing (15th November 2022) that ensured optimum field condition; and
2. Late sowing (20th December 2022) that ensured heat-stressed condition.

3.1 Experimental site and period

The experimental field was situated under the Dinajpur Sadar upazila and located at $25^0 13''$ North latitude and $88^0 23''$ East longitude at an altitude of 37.5 m above the mean sea level. The land belongs to the agroecological region of the Old Himalayan Piedmont Plain (AEZ-1). The experiment was carried out from November to April 2022-2023.

3.2 Climate

Both the experimental fields belonged to the subtropical climate where the rainfall was heavy in the Kharif season (March–August) and scantiness in the Rabi season (October-February). During the growth period of this crop, the atmospheric temperature decreased as the Rabi season proceeded with occasional gusty winds.

3.3 Soil

Both the experimental fields were of medium-high land belonging to the non-calcareous dark gray floodplain soil under the agroecological zone (AEZ-1) of the Old Himalayan Piedmont plain. The soil was sandy loam under the order Inceptisol. The experimental field had well-organized irrigation and drainage systems.

3.4 Soil sample test

The total land of the experimental site was tested on 18th October 2022. The total number of collected soil samples was nine. During the soil samples collection period, the auger method was followed. Soil sampling test was carried out at the Soil Research and Development Institute (SRDI), and after that, they recommended a fertilizer dose for that experimental site.

3.5 Experimental design and layout

Both experiments were laid out in Randomized Complete Block Design (RCBD) with 3 replications. The total land area was 35 m ×18 m (630 m^2) i.e. individually 17.5 m×18 m (315 m^2) respectively for each experiment.

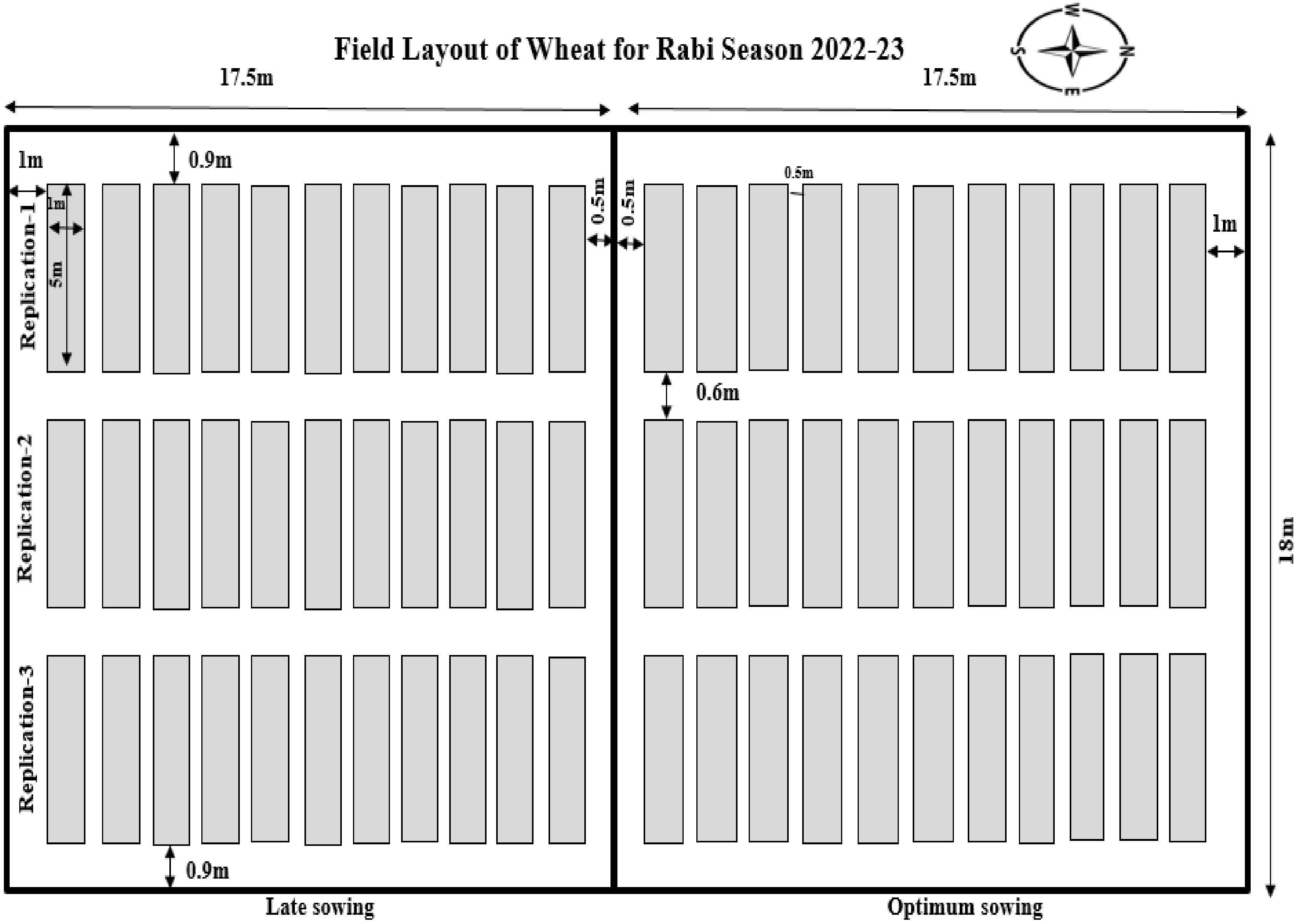


Figure 1: Field experimental layout of wheat for Rabi season 2022-2023

3.6 Experimental materials

In the experiment, both the optimum and late sowing had the same experimental materials. HSTU-developed ten advance lines of wheat, including one check variety; a total of eleven wheat genotypes were used for the preliminary yield trial conducted at the research field of Hajee Mohammad Danesh Science & Technology University, Dinajpur (Table 2).

Table 2. Plant genetic materials with their name used in the experiment

Sl. No.	Entry Name	Source
01	HSTUW1	HSTU-Developed heat tolerance advance lines, Department of Genetics and Plant Breeding, HSTU, Dinajpur.
02	HSTUW2	
03	HSTUW3	
04	HSTUW4	
05	HSTUW5	
06	HSTUW6	
07	HSTUW7	
08	HSTUW8	
09	HSTUW9	
10	HSTUW10	
11	BARI Gom 32	Bangladesh Wheat and Maize Research Institute (BWMRI), Nashipur, Dinajpur.

3.7 Seed rate

The seed rate was followed at 120 kg/ha. In this experiment, 12 g seeds were required per square meter. Since the experimental plot was 5 m^2 and 5 lines for each plot. So, the seeds were required per plot 5×12= 60 g per plot. A total of 180 g seeds were required for 3 replications of each line, respectively.

3.8 Optimum sowing (15th November, 2022)

3.8.1 Layout of the experiment

The experiment was laid out in an RCBD design with 3 replications. The layout of the experiment was made for distributing the genotypes into every line of each block. There were 33 plots, and 11 wheat genotypes were randomly assigned into 5 rows of each plot measuring 5 m × 1 m. The distance maintained between the two blocks was 0.5 m. The line-to-line distance was maintained at 0.20 m. The area of the individual experimental plots was 5 square meter.

3.8.2 Experimental duration

The experiment was performed during the Rabi season (2022- 23) from 15th November, 2022 to May, 2023.

3.8.3 Preparation of the main field

The plot selected for the experiment was opened on the 1st of October, 2022 with a power tiller, and was exposed to the sun for a week. After one week, the land was harrowed, plowed, and cross-plowed several times, followed by laddering to obtain a good tilth. Weeds and stubbles were removed, and we finally obtained the desired soil tilth for planting wheat seed. The experimental plot was partitioned into unit plots by the design of the experiment mentioned earlier. The recommended doses of well-rotten cow dung, dolomite, and dhaincha were incorporated as green manure, and chemical fertilizers, as indicated in the next, were mixed with the soil of each unit plot.

3.8.4 Seeds sowing in the field

The seeds were sown on the field at a depth of 2–2.5 cm on November 15, 2022. The seeds were sown singly for each line by using the line sowing method, where the line-to-line distance was 0.20 m.

3.8.5 Application of manure and fertilizers

Green manure and decomposed organic matter were used at the rate of 5.0 tons/ hectare before final land preparation. Fertilizers were applied @ 7.90, 3.11, 5.48, 3.19, 0.20, 1.56, and 0.31 kg/315 m^2 of N, P, K, S, Zn, Mg, and B, respectively. The doses and methods of application of manure and fertilizers were shown in Table 3.

Table 3. Doses and methods of application of fertilizers in the wheat field

Manure & Fertilizers	**Doses (kg/315 m^2)**	**Methods**
Cow dung	156	Basal application
Dolomite	31	Basal application
Urea	7.90	3 split doses (1/3rd during FLP and rests were top dressed, 1/3rd at 21 DAS, and 1/3rd not applied)
DAP	3.11	½ During final land preparation, rest ½ at 21 DAS
MOP	5.48	½ During final land preparation, rest ½ at 21 DAS
Gypsum	3.19	½ During final land preparation, rest ½ at 21 DAS
Zinc sulphate	0.20	½ During final land preparation, rest ½ at 21 DAS
Magnesium sulphate	1.56	½ During final land preparation, rest ½ at 21 DAS
Boric acid	0.31	½ During final land preparation, rest ½ at 21 DAS

Source: Soil Resources and Development Institute (SRDI)

3.8.6 Seed treatment

Seeds were treated with a fungicide, Provax 200 WP (Carboxin, 17.5% and Thiram, 17.5%) @ 3 g/kg, to protect emerging seedlings from soil-borne fungal diseases.

3.8.7 Intercultural operations

After the emergence of the seedling, various inter-cultural operations were accomplished for better growth and development of the wheat seedlings, described below:

3.8.7.1 Weeding

Weeding was done during the first two top dressings of urea. Weeding was done to break the soil crust, to keep the plots free from weeds, easy aeration of soil and to incorporate urea fertilizer into the soil for reducing the loss of fertilizer through de-nitrification and leaching which ultimately ensured better growth and development of plants.

3.8.7.2 Irrigation

Irrigation was provided at the crown root initiation (CRI), pre-flowering, early booting, and early milking stages for proper growth and development of plants.

3.8.7.3 Rouging

The seedlings were first rouged from all the lines before the flowering stage and 2nd rouging was carried out before harvesting to maintain purity of the genotypes.

3.8.8 Harvesting, threshing, and cleaning

The crops were harvested after maturity was obtained. At that stage, the spike turned into straw color, and the photosynthates were never went up to the spikes as far and the kernels became very hard. Ten plants were collected randomly from each plot for data of yield contributing characters. Plants of one square meter and other areas of each plant were harvested separately, bundled, tagged properly, and carried out to the threshing floor for data collection. The yield of grain was recorded after thoroughly drying in the sun.

3.9 Late sowing (20th December, 2022)

3.9.1 Layout of the experiment

The layout of the experiment was performed as described in the earlier experiment (optimum sowing).

3.9.2 Experimental duration

The experiment was performed during the Rabi season (2022- 23) from 20th December, 2022 to April, 2023.

3.9.3 Preparation of the main field

The plot selected for the experiment was opened on the 1st of November, 2022 with a power tiller, and was exposed to the sun for a week. Other activities were performed as described in the earlier experiment (optimum sowing).

3.9.4 Seeds sowing in the field

The seeds were sown on the field at a depth of 2–2.5 cm on December 20, 2022. Other activities were performed as described in the earlier experiment (optimum sowing).

3.9.5 Application of manure and fertilizers

The application of manure and fertilizers was performed as described in the earlier experiment (optimum sowing).

3.9.6 Seed treatment

Seed treatment was performed as described in the earlier experiment (optimum sowing).

3.9.7 Intercultural operations

The intercultural operations were performed as described in the earlier experiment (optimum sowing).

3.9.8 Harvesting, threshing, and cleaning

Harvesting, threshing, and cleaning were performed as described in the earlier experiment (optimum sowing).

3.10 Measurement of morpho-physiological traits

Ten plants from each plot were randomly selected and the data was recorded on selected plants per genotypes in three replications on nine morpho-physiological characters viz., days to 50% heading, days to 80% maturity, plant height (cm), number of grains per spike, spike length (cm), number of spikes per square meter, Grains weight per spike (g), 1000-grains weight (g), and Grains yield per plot in 5 sq. m (g).

A brief outline of nine morpho-physiological data recording procedures was given in below:

3.10.1 Days to 50% heading

Days to 50% heading were calculated by counting the number of days from the date of sowing till the emergence of a 50% spike (eye estimation) in each plot.

3.10.2 Days to 80% maturity

Days to 80% maturity were calculated by spike color turning into a straw color. The photosynthates were not able to go up to the grain. The grain became harder and produced a stony sound when it was crushed.

3.10.3 Plant height (cm)

Plant height was measured from the base of the plant to the tip of the tallest leaf with the help of a measuring scale and was expressed as centimeter (cm).

3.10.4 Number of grains per spike

The number of grains counted of the individual spike from selected plants in randomly selected 10 spikes from each plot. The average number of grains were counted and recorded.

3.10.5 Spike length (cm)

Length of spikes were measured by scale and data were recorded.

3.10.6 Number of spikes per square meter

Average number of spikes per square meter were counted carefully and data were recorded.

3.10.7 Grains weight per spike (g)

Ten number of dried and matured spikes (g) were collected from each plot. Then average Grains weight per spike was weighed by using an electrical balance.

3.10.8 1000-grains weight (g)

One thousand (1000) clean dried grains were counted from the seed of each plot and weighed by using an electrical balance.

3.10.9 Grains yield per plot in 5 square meter (g)

The grains obtained from each plot were sun-dried and weighed carefully. The dry weight of the grains was recorded to obtain the Grains yield per plot at maturity was expressed as grams.

Table 4. Mathematical formulas of tolerance and susceptibility indices calculated by iPASTIC software

Index	Formula	Pattern of selection	Reference
Mean productivity (MP)	$MP = (Y_P + Y_S) / 2$	Maximum value	Rosielle and Hamblin (1981)
Geometric mean productivity (GMP)	$GMP = \sqrt{}(Y_S \times Y_P)$	Maximum value	Fernandez (1992)
Stress susceptibility index (SSI)	$SSI = \frac{1 - (Y_S / Y_P)}{1 - (\overline{Y_S} / \overline{Y_P})}$	Minimum value	Fischer and Maurer (1978)
Stress tolerance index (STI)	$STI = \frac{Y_S \times Y_P}{(\overline{Y_P})^2}$	Maximum value	Fernandez (1992)
Relative stress index (RSI)	$RSI = \frac{(Y_S / Y_P)}{(\overline{Y_S} / \overline{Y_P})}$	Maximum value	Fischer and Wood (1979)

Whereas, Y_S and Y_P denotes grain yield of each genotype individually under heat stress and optimum field condition; $\overline{Y_S}$ and $\overline{Y_P}$ represents mean grain yield for all genotypes under heat stress and optimum field condition, respectively.

3.11 Statistical Analysis

The data obtained for different characters were recorded first on MS excel sheet. Afterwards, the data were analyzed using the software Statistix10 and iPASTIC.

3.12 Study of molecular genetic variation utilizing Microsatellite/SSR markers

11 Lines including 10 advanced lines and 1 check variety were used for PCR-DNA based assay by using SSR (Simple Sequences Repeat**)** markers was conducted at both BWMRI Molecular Breeding Laboratory and Biotechnology Laboratory of Genetics and Plant Breeding Department, HSTU, Dinajpur.

3.12.1 Microsatellite/SSR markers

A total of 4 microsatellite (SSR) markers primer pairs were selected for the genetic diversity analysis of 11 wheat genotypes as shown in the Table 5. These SSR primers with a distinct chromosome number were used for final polymerase chain reaction (PCR) amplification. The sources, repeat motifs, primer sequences, expected length and chromosomal position, and other relevant information to these markers were published on the Grain Genes website (http://www.wheat.pw.usda.gov). The annealing temperature and primer sequences to SSR markers are shown in the Table 5.

Table 5. List of four SSR markers used for diversity analysis of eleven wheat genotypes

Sl. No.	Primer name	Sequence	Phenotype	Annealing temp. (^{0}C)
1	TaGwm291F	AATGGTATCTATTCCGACCCG	Leaf Curl	57.5
	TaGwm291R	CATCCCTAGGCCACTCTGC		
2	TaXgwm294F	GCAGAGTGATCAATGCCAGA	HSI-single kernel weight of main spike	56.5
	TaXgwm294R	GGATTGGAGTTAAGAGAGAACCG		
3	TaBarc68F	CGATGCCAACACACTGAGGT	Chl. Content	58
	TaBarc68R	GCCGCATGAAGAGATAGGTAGAGAT		
4	Xgwm296F	AATTCAACCTACCAATCTCTG	Leaf rust resistance	52
	Xgwm296R	GCCTAATAAACTGAAAACGAG		

Source: Imported from China and Supplied by Dept. of GPB, HSTU, Dinajpur.

3.12.2 Chemicals name

The chemicals which were used in the research (Table 6, 7, 8, and 9)

Table 6. Chemicals for DNA extraction

Sl. No.	Component	Amount
1.	CTAB	1 ml
2.	Chloroform	0.4 ml
3.	Isopropyl alcohol	0.4ml
4.	Ethyl alcohol	70%

Table 7. Chemicals for CTAB buffer preparation

Sl. No.	Components	Amount
1.	D-sorbitol (1M)	36.44 g
2.	Tris-HCl (1M)	24.23 g
3.	EDTA	148.9 g
4.	NaCl (1M)	58.44 g
5.	CTAB (Hexadecyl trimethyl-ammonium bromide)	20 ml
6.	Sarcosin	20 ml
7.	ddH$_2$O	--

Table 8. Chemical reagents used for PCR, based on SSR markers of wheat genotypes

Sl. No.	**Name**	**Amount**	**Source**	**Washing time**
1.	Acrylamide	500 g	Bio Basic Canada INC	
2.	Ammonium per sulfate (APS)	100 g	SISCO research laboratories pvt Ltd. (SRL)	
3.	N, N Methylene-bis acrylamide (BIS)	100 g	Lobal chemic	
4.	TEMED	100 ml	ROTH	
5.	Boric acid	500 g	ROTH	
6.	TBE Buffer	1L	SIGMA	
7.	EDTA Buffer		SIGMA	
8.	Ethanol+ Acetic acid+ Water	200 ml+10 ml+2000 ml		Washing for 15 minutes
9.	$AgNO_3$ + Water	2 gm+1000 ml		Washing in dark mood for 15 minutes
10.	NAOH+ Formaldehyde +Water	30 gm+8 ml+2000 ml	Sisco research laboratories	Washing for 5 minutes depends on color
11.	Tris (hydroxymethyl aminomethane	500 g	Merck	
12.	Deionized water			
13.	DNA ladder	2 µl		
14.	Loading dye	1 µl		
15.	Master mix	8 µl		
16.	Mineral oil	3 µl		

Table 9. Chemicals used for preparation Polyacrylamide gel (8%)

Sl. No.	Component	Amount (1000 ml)
1.	Acrylamide (40%)	200 ml
2.	BIS (2%)	107 ml
3.	10× TBE	100 ml
4.	dd H_2O	593 ml

3.12.3 Data collection for molecular characterizations

Data collected from the young wheat leaves and the collection procedures were given below:

3.12.4 Sampling and lyophilization of leaves

2-4 pieces of 2 cm leaves were collected in the Eppendorf tube from 15-day-old wheat seedlings and dried for 7 days in silica gel.

3.12.5 Genomic DNA extraction

3.12.5.1 Grinding of leaves

About 200 mg of fresh wheat leaves were cut into small pieces (~2 mm) and put into porcelain mortal, added 0.5 ml (0.8%) of warmed (65^0C) CTAB buffer and crushed the leaves with the pestle.

Figure 2: Showing grinding of lyophilized leaf samples

3.12.5.2 DNA extraction using modified CTAB method

The leaf sap was collected into a 2 ml centrifuge tube and added 800 µl of warmed (65°C) CTAB buffer to each tube and vortex thoroughly and incubated samples at 65°C for 45 minutes and every 10 minutes mixed them gently by inversion (400 µl of 2% β-Mercaptoethanol was added to 200 ml of extraction buffer prior to warming). The tubes were taken out of the water bath and left at room temperature for 5 minutes. 600 µl chloroform Isoamyl alcohol was added (24:1). The sample was mixed by inversion for about 2 minutes (100 times) gently until two layers were mixed. Centrifuged them for 4000 rpm at room temperature for 20 mins. The aqua phase was removed with a wide-bore pipette. The aqua phase was transferred to a clean tube 1.5 ml then added 2/3 or 1 volume of Ethanol and mixed gently to precipitate the nucleic acid. At this stage, the samples were stored at -4°C for overnight. Then the samples were centrifuged at 10000 rpm for 20 minutes discarded the supernatant and dried the DNA so that there was no ethanol. Added 500 µl of washing buffer in each tube and washed it by inversion gently. After centrifuged at 10000 rpm for 20 mins, the supernatant was removed and left on the bench to dry enough.100 µl of TE (PH=8) was added with RNAase (1 µl /100 µl of TE), and the samples were left to dissolve and put at 37°C in the oven for 1-2 hrs. The samples were stored at -4°C for the short term.

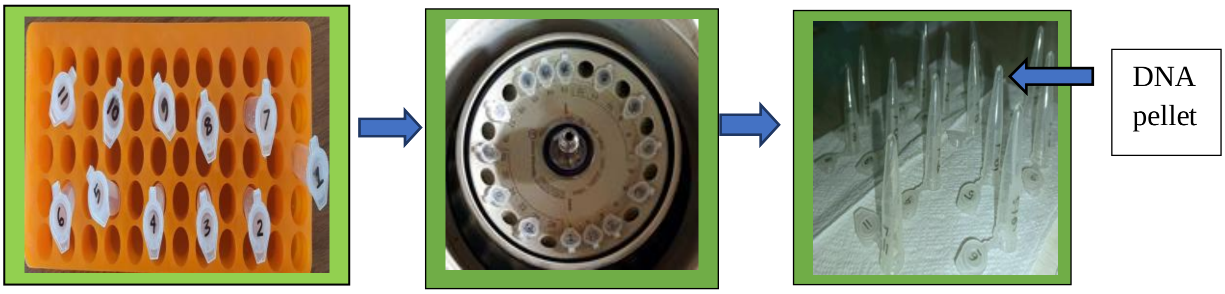
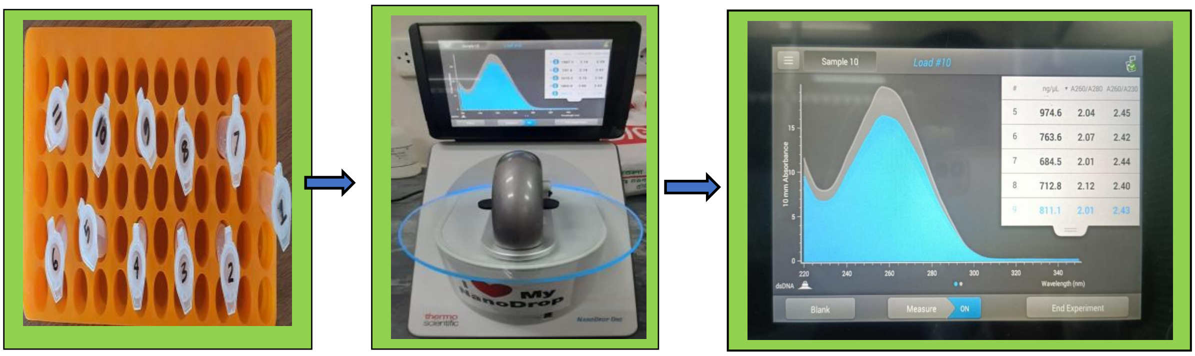


Figure 3: Isolation of genomic DNA

3.12.6 DNA quantification

The quality of the extracted DNA samples was also checked before PCR amplification through quantification using a Thermo Scientific NanoDrop^TM1000 Spectrophotometer (Thermo Fisher Scientific, USA).



#	ng/µL	A260/A280	A260/A230
5	974.6	2.04	2.45
6	763.6	2.07	2.42
7	684.5	2.01	2.44
8	712.8	2.12	2.40
9	811.1	2.01	2.43

Figure 4: Quantification of genomic DNA by Nano Drop spectrophotometer

3.12.7 DNA dilution

DNA samples were diluted four times by using nuclease-free water in 1: 4 proportions.

3.12.8 PCR amplification and electrophoresis separation

PCR amplifications were performed in 10 µL volume using a Verity Thermal Cycler (Applied Biosystems, USA). The reaction mixture contained 10 mM/L Tris-HCl, 50 mM/L KCl, 2 mM/L $MgCl_2$, 200 µmol/L of each dNTP, 250 nM/L of each primer, 20 to 40 ng genomic DNA, and 0.25 U Taq DNA polymerase. The PCR amplification was as follows: one cycle of 94°C for 5 min; 35 cycles of 95°C for 0.5 min, 53 to 58°C (depending on the specific primers) for 0.5 min and extension for 0.5 min; and a final extension at 72°C for 5 min. Reaction products were mixed with one fifth volume of loading buffer (100 mM/L EDTA pH 8.0,10 mM/L Tris-HCl pH 7.5, 5% Ficoll 400; 0.05% bromophenol, 0.05% xylenecyanol) and 8 µL were loaded vertically, for electrophoresis 8% denaturing polyacrylamide gels in 1 × TBE (90 mM/LTris borate pH 8.3, 2 mM/L EDTA) at 50 mA for 2 to 3 h (Wang *et al.*, 2007). Gels were then silver stained and photographed.

Figure 5: Amplification of polymerase chain reaction

3.12.9 Electrophoresis and silver nitrate staining

The reaction products were then run into polyacrylamide gels in 1×TBE buffer (90 mM Tris-borate pH 8.3, 2 mM EDTA) at 50mA for 2 to 3 h (Wang *et al.*, 2007). The polyacrylamide gel was contained 40 ml of 8% non-denaturing polyacrylamide gel (37.5:1 acrylamide-bis), 400 µl of 10% ammonium peroxydisulfate (APS), and 40 µl of TEMED.

Specific banding patterns were detected with silver nitrate staining. In brief, the gel was carefully removed from the glass plate and pretreated with fix/stop [10% alcohol and 0.5% acetic acid (v/v)] solution for 10 min, and stained by $AgNO_3$ (0.2%) solution for 15 min. After a brief rinse (10 sec) by distilled water, the gel was transferred into sodium thiosulfate [(0.002% (w/v)] solution for 1 min followed by incubation in the well chilled developer solution [15% NaOH and 0.4% HCHO] for 5-10 min. The gel was transferred into distilled water on a shaker for 5 minutes until stop the reaction.

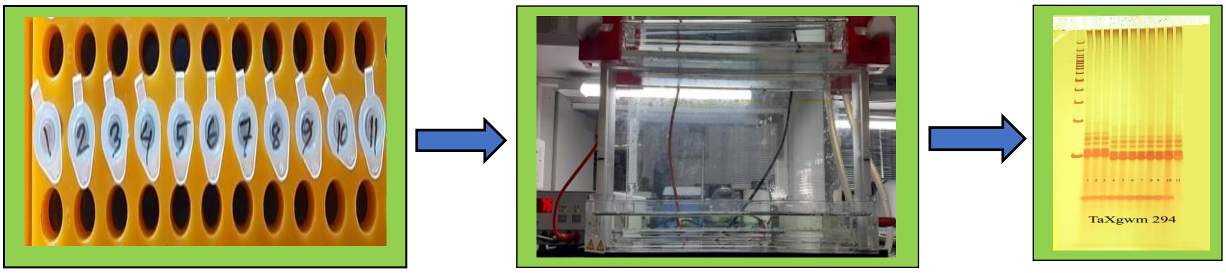


Figure 6: Polyacrylamide gel electrophoresis

3.12.10 Molecular statistical analysis

Polymorphism information content (PIC) will be calculated using the following formula:

$PIC= 1-\sum (Pi)^2$

Note: Pi depicts the proportion of samples carrying the ith allele.

The population structure analysis was done using the Bayesian clustering method in structure software version 2.3.4 (Pritchard *et al.*, 2000). Final delta K value was determined using the Evannos ΔK method and Ln probability data was used to detect presence of genetically distinct populations using graphical approach (Evanno *et al.*, 2005) by running the Structure Harvester software (Earl and VonHoldt, 2012).

Every band was considered as a single locus. All the scorable Loci were considered for generation of bivariate 1-0 data matrix and genetic distances (GD) among the genotypes were estimated using Unwaited Pair Group of Arithmetic Means (UPGMA) as described by Nei and Jin (1989) and for estimation of genetic diversity dendrogram was constructed using the software MetaboAnalyst (Online Version) by Chong and Xia (2018).

CHAPTER IV

RESULTS AND DISCUSSION

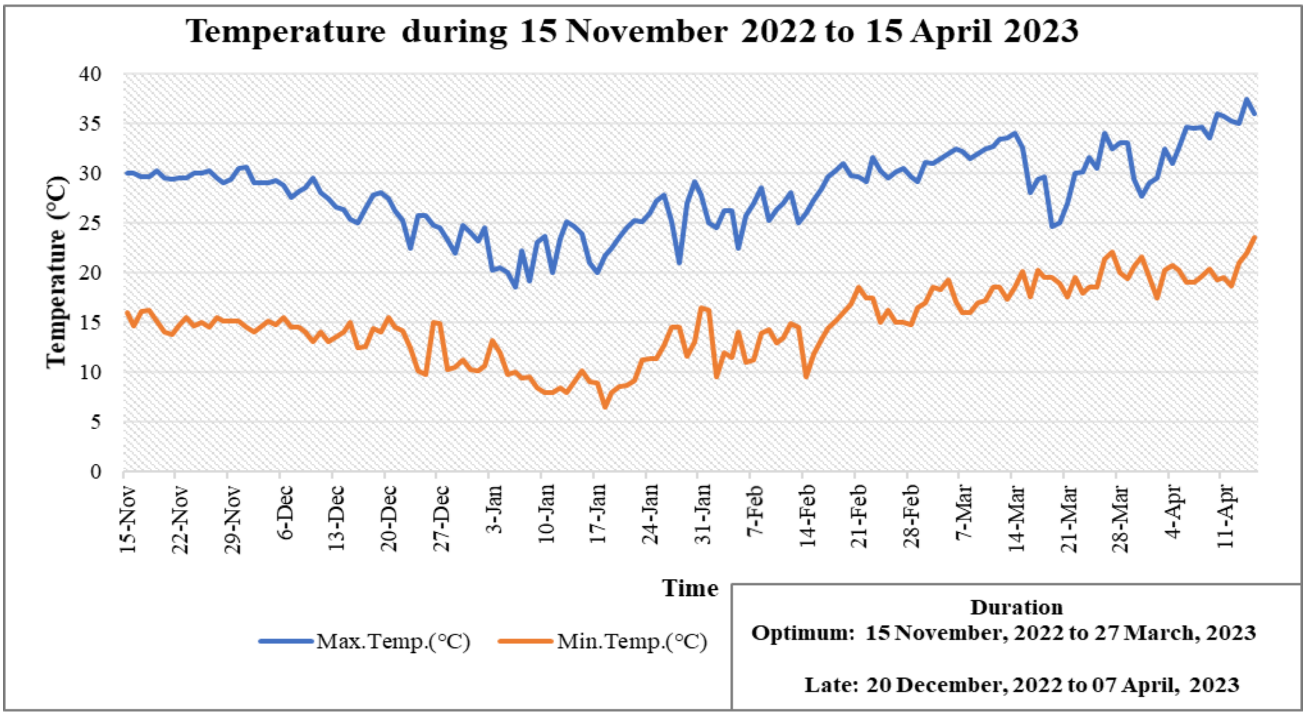


Figure 7: Graphical representation of temperature during the wheat growing season at the experimental site

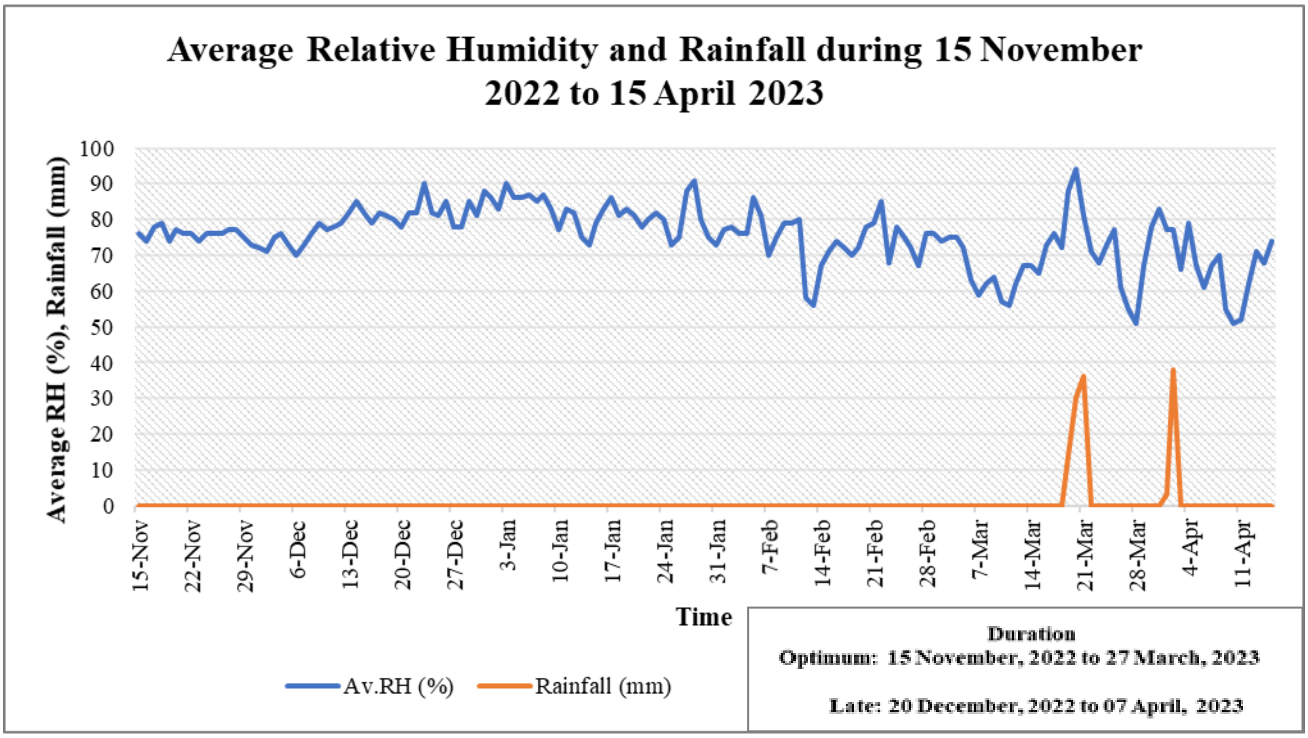


Figure 8: Graphical representation of the average related humidity and rainfall during the wheat growing season at the experimental site

Data source: Bangladesh Meteorological Observatory, Rajbati, Dinajpur

Data collected from the experiment was analyzed to study the effects of heat stress and stress indices under the optimum sowing and the late sowing time. A total number of 11 wheat genotypes (HSTU-developed 10 advance lines and 1 check variety) were used for this experiment. Mean performance and other analyses were done on 9 yield and yield contributing characters viz., days to heading, days to maturity, plant height, number of grains per spike, spike length, number of spikes, grains weight per spike, 1000-grains weight, grains yield per plot. Molecular markers (SSRs) were also applied to characterize these wheat germplasms at the DNA level. The results had been presented and discussed under the following headings.

4.1 Analysis of variance of nine morpho-physiological traits in wheat

The development of heat-tolerant wheat varieties is the goal and major challenge of wheat breeders. Analysis of variance of 9 quantitative characters was accomplished to assess the heat stress pertained to a particular character among the 11 wheat genotypes. The source of variation included replication, sowing time, genotype, genotype × sowing time, and error (Table 10). The analysis of variance revealed highly significant differences among genotypes for the most traits. This indicated that the genotypes possessed inherent genetic variances among themselves concerning the characters studied. This information is needed before initiating any successful heat-stressed tolerance breeding program. Again, the effects of sowing times were no significant difference among most traits except days to maturity, number of spikes, and 1000-grains weight. Rasal *et al*. (2006) reported the duration of thermal requirements for physiological maturity decreased due to heat stress under late sowing conditions. The interaction between genotype and sowing time revealed no significant difference among most traits except days to heading, days to maturity, number of grains per spike, and spike length**.** The coefficient of variation in all the characters was less than 11%. Many researchers also observed that high temperatures during grain filling stages reduce grain filling duration, grain yield, and grain sizes (Wiegand and Cuellar, 1981; Wardlaw *et al*., 1989).

Table 10. Mean squares derived from 9 morpho-physiological traits in wheat

Character	**ANOVA FOR 11 WHEAT GERMPLASM**					
	Replication (2 df)	**Genotype (10 df)**	**Sowing time (1 df)**	**Genotype × Sowing time (10 df)**	**Error (42 df)**	**Coefficient of Variation (%)**
Days to heading	2.02	89.44**	0.74NS	23.31**	1.75	2.08
Days to maturity	1.47	130.78**	1318.56**	7.79**	0.61	0.74
Plant height	350.64	397.89**	4.51NS	0.07NS	4.93	2.14
No. of grains per spike	11.40	81.95**	0.22NS	31.25**	273.54	6.38
Spike length	0.41	1.13**	0.06NS	0.52*	0.19	4.60
No. of spikes	1401.0	10789.5**	15825.5**	1653.3NS	1305.0	9.10
Grains weight per spike	0.05	0.20**	0.04NS	0.04NS	0.03	10.26
1000-grains weight	31.82	169.33**	23.26*	4.07NS	10.41	7.81
Grains yield per plot	1435005	207084**	10008NS	38847NS	25439	6.51

Here, * and ** indicates significant at 5% and 1% level of probability respectively and df indicates degree of freedom and NS = non-significant

4.2 Mean performance of various morpho-physiological traits of wheat genotypes

The mean performances of various morpho-physiological traits of wheat genotypes were presented (Table 11.1, 11.2, and 11.3). The results of mean performances for the optimum sowing (non-stress) and the late sowing (heat stress) were discussed under the following headings.

4.2.1 Days to heading

In the experiment, the average ranges of days to heading were 51.67 to 69.33 days (optimum sowing) and 59.33 to 66.33 days (late sowing), with a mean value of 63.73±1.47 for optimum sowing and 63.52±0.50 for late sowing (Table 11.1). The highest days to heading were observed in HSTUW6 (66.33 days), followed by HSTUW7 (65.67 days), HSTUW3 (65.33 days), HSTUW4 (65.00 days) and HSTUW5 (65.00 days), respectively under the late sowing time, while the lowest one was observed in HSTUW1 (59.33 days). Despite this, HSTUW1 yielded high in both the optimum and late sowing times (Table 11.1). Due to heat stress, the days to heading of most of the wheat genotypes were decreased compared to the optimum sowing time. Narayanan (2018), Cleland *et al.* (2007), and Mondal *et al*. (2013) also found similar results. HSTUW1 was yielded high, even though the genotype had the lowest days to heading and lowest days to maturity (Table 11.1). Therefore, HSTUW1 may be considered as a high-yielding as well as short-duration wheat genotype.

4.2.2 Days to maturity

In the experiment, the average ranges of days to maturity were 102.33 to 121.33 days (optimum sowing) and 94.00 to 106.33 days (late sowing), with a mean value of 110.82±0.85 for optimum sowing and 101.88±0.20 for late sowing (Table 11.1). Due to heat stress, days to maturity of all the wheat genotypes were decreased compared to the optimum sowing time. Rasal *et al.* (2006), Neog *et al.* (2001), Sial *et al.* (2005) and Inamullah *et al*. (2007) were found similar results. The highest days to maturity was observed in HSTUW9 (106.33 days), followed by HSTUW6 (105.33 days), HSTUW7 (105.33 days), and HSTUW8 (105.33 days), respectively under the late sowing time (Table 11.1). Among these genotypes, only HSTU8 was yielded high in both the optimum and late sowing times. The lowest one days to was observed in HSTUW1 under the late sowing time (Table 11.1). Despite the lowest days to heading and days to maturity, HSTUW1 was yielded high in both the optimum and late sowing (Table 11.1). Therefore, the genotype HSTUW1 may be considered as a high-yielding as well as short-duration wheat genotype.

4.2.3 Plant height

In the experiment, the average ranges of plant height were 92.35 to 116.33 cm (optimum sowing) and 91.76 to 115.67 cm (late sowing), with a mean value of 104.22±1.79 for optimum sowing and 103.70±1.91 for late sowing (Table 11.1). The highest plant height was observed in HSTUW4 (115.67 cm), followed by HSTUW5 (113.68 cm), HSTUW6 (109.37 cm), and HSTUW10 (107.03 cm), respectively under the late sowing time, while the lowest one was observed in HSTUW2 (91.76 cm) (Table 11.1). Due to heat stress, the plant height of all the wheat genotypes was decreased compared to the optimum sowing time. The present results were depicted with Shazad *et al*. (2002) and Irfaq *et al*. (2005).

4.2.4 Number of grains per spike

In the experiment, the average ranges of number of grains per spike were 30.93 to 45.20 (optimum sowing) and 34.27 to 47.90 (late sowing), with a mean value of 40.08±2.10 for optimum sowing and 39.97±2.01 for late sowing (Table 11.2). The highest number of grains per spike was observed in HSTUW3 (47.90), followed by HSTUW2 (45.87), HSTUW7 (41.00), and HSTUW9 (40.40), respectively under the late sowing time, while the lowest one was observed in HSTUW4 (34.27) (Table 11.2).

4.2.5 Spike length

In the experiment, the average ranges of spike length were 8.58 to 10.73 cm (optimum sowing) and 9.25 to 10.05 cm (late sowing), with a mean value of 9.61±0.43 for optimum sowing and 9.55±0.26 for late sowing (Table 11.2). The highest spike length was observed in HSTUW5 (10.05 cm), followed by HSTUW10 (9.88 cm), HSTUW6 (9.87 cm), and HSTUW4 (9.63 cm), respectively under the late sowing time, while the lowest one was observed in HSTUW1 (9.25 cm) (Table 11.2).

4.2.6 Number of spikes

In the experiment, the average ranges of number of spikes were 351.67 to 505.67 (optimum sowing) and 332.33 to 440.00 (late sowing), with a mean value of 412.24±26.01 for optimum sowing and 381.27±31.00 for late sowing (Table 11.2). The highest number of spikes was observed in HSTUW1 (440.00), followed by HSTUW2 (435.00), HSTUW3 (414.33), and check variety BARI Gom 32 (384.67), respectively under the late sowing time while the lowest one was observed in HSTUW4 (332.33) (Table 11.2). Due to heat stress, number of spikes of most the wheat genotypes were decreased compared to optimum sowing time. Ansary *et al.* (1989) also reported similar findings.

4.2.7 Grains weight per spike

In the experiment, the average ranges of grains weight per spike were 1.32-1.88 g (optimum sowing) and 1.40-1.86 g (late sowing), with a mean value of 1.65±0.12 for optimum sowing and 1.60±0.15 for late sowing (Table 11.3). The highest Grains weight per spike was observed in HSTUW8 (1.86 g), followed by HSTUW10 (1.84 g), HSTUW7 (1.72 g), and HSTUW9 (1.67 g), respectively under the late sowing time, while the lowest one was observed in HSTUW1 (1.40 g) (Table 11.3). A similar result was reported by Ojha *et al*. (2018).

4.2.8 1000-grains weight

In the experiment, the average ranges of 1000-grains weight were 31.99 to 47.23 g (optimum sowing) and 30.23 to 47.04 g (late sowing), with a mean value of 41.89±2.45 for optimum sowing and 40.70±2.89 for late sowing (Table 11.3). The highest 1000-grains weight was observed in HSTUW10 (47.04 g), followed by HSTUW8 (46.55 g), BARI Gom 32 (45.00 g), and HSTUW6 (44.40 g), respectively under the late sowing time, while the lowest one was observed in HSTUW3 (30.23 g) (Table 11.3). Due to heat stress, 1000-grains weight of most of the wheat genotypes decreased compared to the optimal condition. Tahir *et al.* (2009) also observed decrease in weight of grains under heat-stressed condition. Delayed planting of wheat faced high temperature at anthesis and decreased the weight of grains (Rahman *et al*., 2009).

4.2.9 Grains yield per plot

In the experiment, the average ranges of Grains yield per plot were 2087.2 to 2812.7 g (optimum sowing) and 2155.6 to 2751.0 g (late sowing), with a mean value of 2462.3±129.47 for optimum sowing and 2437.7±103.39 for late sowing (Table 11.3). The highest Grains yield per plot was observed in HSTUW8 (2751.0 g), followed by HSTUW1 (2584.0 g), HSTUW4 (2550.1 g), and HSTUW10 (2500.0 g), respectively under the late sowing time, while the lowest one was observed in check variety BARI Gom 32 (2155.6 g) (Table 11.3). Nahar *et al.* (2010) observed yield reduction of five genotypes (‘Sourav’, ‘Shatabdi’, ‘Sufi’, ‘Bijoy’ and ‘Prodip’) under late heat stress condition. Carvalho *et al.* (1983) stated that the ideal wheat genotype should be high yielding under any environmental conditions. In our experiment HSTUW8, HSTUW1, and HSTUW4 were yielded high in both the optimum and late sowing times.

Table 11.1 Mean performance of wheat genotypes of morpho-physiological traits

Genotypes	**DH**		**DM**		**PH**	
	Optimum sowing	**Late sowing**	**Optimum sowing**	**Late sowing**	**Optimum sowing**	**Late sowing**
HSTUW1	51.67 e	59.33 e	102.33 d	94.00 f	94.57 ef	94.07 ef
HSTUW2	55.67 de	62.67 cd	105.67 c	96.33 e	92.35 f	91.76 f
HSTUW3	65.33 ab	65.33 ab	107.33 c	98.33 d	94.08 ef	93.57 ef
HSTUW4	69.33 a	65.00 ab	113.00 b	104.33 c	116.33 a	115.67 a
HSTUW5	69.00 a	65.00 ab	112.00 b	104.33 c	114.73 ab	113.68 ab
HSTUW6	68.33 a	66.33 a	111.67 b	105.33 b	109.73 bc	109.37 a-c
HSTUW7	68.00 a	65.67 ab	113.00 b	105.33 b	104.98 cd	104.40 cd
HSTUW8	62.00 bc	61.67 d	112.67 b	105.33 b	105.50 cd	105.07 cd
HSTUW9	64.33 a-c	64.33 bc	121.33 a	106.33 a	107.17 c	106.85 bc
HSTUW10	65.00 a-c	64.00 bc	113.00 b	104.33 c	107.55 c	107.03 bc
BARI Gom 32	60.00 cd	61.67 d	107.00 c	96.67 e	99.40 de	99.19 d
Range	51.67-69.33	59.33-66.33	102.33-121.33	94.00-106.33	92.35-116.33	91.76-115.67
Mean	63.73	63.52	110.82	101.88	104.22	103.70
SE (±)	±1.47	±0.50	±0.85	±0.20	±1.79	±1.91

The mean values having same letter (s) did not significantly differ at 5% level of probability.

DH = Days to heading, DM = Days to maturity, PH = Plant height (cm)

Table 11.2 Mean performance of wheat genotypes of morpho-physiological traits

Genotypes	**NGPS**		**SL**		**NS**	
	Optimum sowing	**Late sowing**	**Optimum sowing**	**Late sowing**	**Optimum sowing**	**Late sowing**
HSTUW1	34.13 bc	40.03 bc	8.58 c	9.25 a	505.67 a	440.00 a
HSTUW2	39.43 ab	45.87 ab	9.10 bc	9.46 a	477.00 a-c	435.00 a
HSTUW3	44.50 a	47.90 a	8.80 c	9.38 a	435.33 a-d	414.33 a
HSTUW4	39.57 ab	34.27 c	9.91 a-c	9.63 a	377.00 d	332.33 a
HSTUW5	45.03 a	39.13 bc	10.73 a	10.05 a	359.00 d	355.00 a
HSTUW6	41.63 ab	37.97 c	9.84 a-c	9.87 a	409.33 b-d	361.00 a
HSTUW7	43.57 a	41.00 a-c	9.84 a-c	9.30 a	351.67 d	368.00 a
HSTUW8	38.33 a-c	39.73 bc	9.57 a-c	9.61 a	373.33 d	377.33 a
HSTUW9	45.20 a	40.40 bc	10.58 ab	9.31 a	385.00 cd	363.67 a
HSTUW10	38.57 ab	38.83 bc	9.70 a-c	9.88 a	378.00 d	362.67 a
BARI Gom 32	30.93 c	34.50 c	9.04 bc	9.30 a	483.33 ab	384.67 a
Range	30.93-45.20	34.27-47.90	8.58-10.73	9.25-10.05	351.67-505.67	332.33-440.00
Mean	40.08	39.97	9.61	9.55	412.24	381.27
SE (±)	±2.10	±2.01	±0.43	±0.26	±26.01	±31.00

The mean values having same letter (s) did not significantly differ at 5% level of probability.

NGPS= Number of grains per spike, SL = Spike length (cm), NS = Number of spikes

Table 11.3 Mean performance of wheat genotypes of morpho-physiological traits

Genotypes	**GWPS**		**TGW**		**GYPP**	
	Optimum sowing	**Late sowing**	**Optimum sowing**	**Late sowing**	**Optimum sowing**	**Late sowing**
HSTUW1	1.34 c	1.40 a	39.45 a-c	35.07 b-d	2812.7 a	2584.0 ab
HSTUW2	1.32 c	1.45 a	33.09 bc	33.21 cd	2087.2 d	2214.5 bc
HSTUW3	1.41 b-c	1.40 a	31.99 c	30.23 d	2125.1 c-d	2334.7 bc
HSTUW4	1.76 a-c	1.48 a	45.44 a	42.15 a-c	2665.9 ab	2550.1 ab
HSTUW5	1.88 a	1.60 a	41.98 a	40.23 a-d	2490.8 a-d	2431.0 a-c
HSTUW6	1.85 ab	1.66 a	45.55 a	44.40 ab	2576.1 a-c	2492.9 a-c
HSTUW7	1.84 ab	1.72 a	43.38 a	42.34 a-c	2468.8 a-d	2478.5 a-c
HSTUW8	1.80 ab	1.86 a	47.23 a	46.55 a	2672.0 ab	2751.0 a
HSTUW9	1.84 ab	1.67 a	41.22 ab	41.52 a-c	2243.1 b-d	2322.0 bc
HSTUW10	1.74 a-c	1.84 a	45.46 a	47.04 a	2450.1 a-d	2500.0 a-c
BARI Gom 32	1.42 bc	1.56 a	46.01 a	45.00 ab	2493.5 a-d	2155.6 c
Range	1.32-1.88	1.40-1.86	31.99-47.23	30.23-47.04	2087.2-2812.7	2155.6-2751.0
Mean	1.65	1.60	41.89	40.70	2462.3	2437.7
SE (±)	±0.12	±0.15	±2.45	±2.89	±129.47	±103.39

The mean values having same letter (s) did not significantly differ at 5% level of probability.

GWPS = Grains weight per spike (g), TGW= Thousand grains weight (g), GYPP = Grains yield per plot (g)

Judging the mean performance of 11 wheat genotypes it was observed that there may some genotypes which were adapted to heat-stressed environment and they had the ability to performed better even under late sowing (heat stress) condition. Overall study on the grain yield, yield contributing, phenological and physiological characters, it was observed that the genotypes HSTUW8, HSTUW1, and HSTUW4 ranked better category for maximum number of characters indicating their high tolerance to heat-stress under late sowing time.

4.3 Combined analysis of variance of various important stress tolerance indices

Combined analysis of variance of various important stress tolerant indices were accomplished to assess the heat stress pertained for grain yield and others yield related traits among the 11 wheat genotypes. The source of variation included replication, genotype, and error (Table 12.1 and Table 12.2). The results of the combined analysis of variance were studied a significant influence of heat stress of various important stress tolerant indices. The combined analysis of variance revealed highly significant differences among 11 wheat genotypes in most stress tolerant indices for grain yield and others yield related traits.

Table 12.1 Mean squares derived from various important stress indices on 11 wheat genotypes

Characters	**Source of Variation**	**df**	**ANOVA of various important stress indices**				
			MP	**GMP**	**SSI**	**STI**	**RSI**
Days to heading	Replication	2	1.01	1.03	8.91	0.00	0.00
	Genotype	10	44.72 **	45.54 **	271.75 **	0.04 **	0.01 **
	Error	20	0.77	0.79	23.76	0.00	0.00
Days to maturity	Replication	2	0.73	0.81	0.00	0.00	4.297E-06
	Genotype	10	65.39 **	65.01 **	0.15 **	0.02 **	1.170E-03 **
	Error	20	0.24	0.21	0.01	0.00	9.458E-05
Plant height	Replication	2	175.31	175.39	0.10	0.00	4.395E-06
	Genotype	10	198.95 **	198.94 **	0.36 NS	0.07 **	1.214E-05 NS
	Error	20	5.10	5.10	0.38	0.00	1.399E-05
Number of grains per spike	Replication	2	5.70	5.41	3.67	0.06	0.01
	Genotype	10	40.97 **	40.93 **	13.43 NS	0.10 **	0.04 **
	Error	20	3.38	3.38	19.70	0.01	0.01
Spike length	Replication	2	0.21	0.20	1.72	0.01	0.00
	Genotype	10	0.56 **	0.56 **	11.94 NS	0.02 **	0.01 *
	Error	20	0.11	0.11	12.34	0.00	0.00

Here, * and ** indicates significant at 5% and 1% level of probability, respectively and df indicates degree of freedom and NS = non-significant

MP = Mean productivity, GMP = Geometric mean productivity, SSI= Stress susceptibility index, STI = Stress tolerance index, RSI = Relative stress index

Table 12.2 Mean squares derived from various important stress indices on 11 wheat genotypes

Characters	Source of Variation	df	ANOVA of various important stress indices				
			MP	GMP	SSI	STI	RSI
Number of spikes	Replication	2	700.51	673.69	3.00	0.04	0.00
	Genotype	10	5394.77 **	5315.22 **	6.09 NS	0.13 **	0.02 NS
	Error	20	410.69	430.33	6.62	0.01	0.02
Grains weight per spike	Replication	2	0.03	0.02	0.73	0.03	0.00
	Genotype	10	0.10 **	0.10 **	17.34 NS	0.13 **	0.03 NS
	Error	20	0.02	0.02	31.38	0.02	0.02
1000-grains weight	Replication	2	15.91	16.37	2459.76	0.01	0.01
	Genotype	10	84.67 **	85.29 **	1490.26 NS	0.17 **	0.00 NS
	Error	20	5.69	5.78	705.67	0.01	0.01
Grains yield per plot	Replication	2	717503	727140	1.62	0.03	0.00
	Genotype	10	103542 **	105498 **	2.35 NS	0.07 **	0.02 NS
	Error	20	10068	10293	5.23	0.01	0.01

Here, * and ** indicates significant at 5% and 1% level of probability, respectively and df indicates degree of freedom and NS = non-significant

MP = Mean productivity, GMP = Geometric mean productivity, SSI= Stress susceptibility index, STI = Stress tolerance index, RSI = Relative stress index

4.4 Various important heat tolerant indices on selected morpho-physiological traits

4.4.1 Days to heading

In the experiment, the highest MP, GMP, and STI values were observed in HSTUW6, followed by HSTUW4, HSTUW5, and HSTUW7 (Table 13, 14, and 15). Among these genotypes, only HSTUW4 was yielded high in both the optimum sowing and the late sowing times; HSTUW6 was yielded high under the optimum sowing time and was yielded intermediate under the late sowing time; HSTUW5 and HSTUW7 were yielded intermediate in both the optimum sowing and the late sowing times.

The lowest SSI value was observed in HSTUW4, followed by HSTUW5, HSTUW7, and HSTUW6 (Table 16). Among these genotypes, only HSTUW4 was yielded high in both the optimum sowing and the late sowing times; HSTUW6 was yielded high under the optimum sowing time and was yielded intermediate under the late sowing time; HSTUW5 and HSTUW7 were yielded intermediate in both the optimum sowing and the late sowing times. According to RSI genotypes HSTUW1, HSTUW2, BARI Gom 32, and HSTUW9 were heat tolerant (Table 17).

4.4.2 Days to maturity

In the experiment, the highest MP, GMP, and STI values were observed in HSTUW9, followed by HSTUW7, HSTUW8, and HSTUW4 (Table 13, 14, and 15). Among these genotypes, only HSTUW8 and HSTUW4 were yielded high in both the optimum sowing and the late sowing times; HSTUW7 was yielded intermediate in both the optimum sowing and the late sowing times; HSTUW9 was yielded low in both the optimum sowing and the late sowing times.

The lowest SSI and highest RSI values were observed in HSTUW6, followed by HSTUW8, HSTUW7, and HSTUW5 (Table 16 and 17). Among these genotypes, only HSTUW8 was yielded high in both the optimum sowing and the late sowing times; HSTUW6 was yielded high under the optimum sowing time and was yielded intermediate under the late sowing time; HSTUW5 and HSTUW7 were yielded intermediate in both the optimum sowing and the late sowing time.

4.4.3 Plant height

In the experiment, the highest MP, GMP, and STI values were observed in HSTUW4, followed by HSTUW5, HSTUW6, and HSTUW10 (Table 13, 14, and 15). Among these genotypes, only HSTUW4 was yielded high in both the optimum sowing and the late sowing times; HSTUW6 was yielded high under the optimum sowing time and was yielded intermediate under the late sowing time; HSTUW10 was yielded intermediate under the optimum sowing time and was yielded high under the late sowing time; HSTUW5 was yielded intermediate in both the optimum sowing and the late sowing times.

The lowest SSI and highest RSI values were observed in BARI Gom 32, followed by HSTUW9, HSTUW6, and HSTUW8 (Table 16 and 17). Among these genotypes, only HSTUW8 was yielded high in both the optimum sowing and the late sowing times; HSTUW6 was yielded high under the optimum sowing time and was yielded intermediate under the late sowing time; BARI Gom 32 was yielded intermediate under the optimum sowing time and was yielded low under the late sowing time; HSTUW9 was yielded low in both the optimum sowing and the late sowing times.

4.4.4 Number of grains per spike

In the experiment, the highest MP, GMP, and STI values were observed in HSTUW3, followed by HSTUW9, HSTUW2, and HSTUW7 (Table 13, 14, and 15). Among these genotypes, HSTU7 was yielded intermediate in both the optimum sowing and the late sowing times; HSTUW3 was yielded low under the optimum sowing time and was yielded intermediate under the late sowing time; HSTUW9 and HSTUW2 was yielded low in both the optimum sowing and the late sowing times.

The lowest SSI and highest RSI values were observed in HSTUW1, followed by HSTUW2, BARI Gom 32, and HSTUW3 (Table 16 and 17). Among these genotypes, only HSTUW1 was yielded high in both the optimum sowing and the late sowing times; BARI Gom 32 was yielded intermediate under the optimum sowing time and was yielded low under the late sowing time; HSTUW3 was yielded low under the optimum sowing time and was yielded intermediate under the late sowing time; HSTUW2 was yielded low in both the optimum sowing and the late sowing times.

4.4.5 Spike length

In the experiment, the highest MP, GMP, and STI values were observed in HSTUW5, followed by HSTUW9, HSTUW6, and HSTUW10 (Table 13, 14, and 15). Among these genotypes, HSTUW6 was yielded high under the optimum sowing time and was yielded intermediate under the late sowing time; HSTUW10 was yielded intermediate under the optimum sowing time and was yielded high under the late sowing time; HSTUW5 was yielded intermediate in both the optimum sowing and the late sowing times; HSTUW9 was yielded low in both the optimum sowing and the late sowing times.

The lowest SSI and highest RSI values were observed in HSTUW1, followed by HSTUW3, HSTUW2, and BARI Gom 32 (Table 16 and 17). Among these genotypes, only HSTUW1 was yielded high in both the optimum sowing and the late sowing times; BARI Gom 32 was yielded intermediate under the optimum sowing time and was yielded low under the late sowing time; HSTUW3 was yielded low under the optimum sowing time and was yielded intermediate under the late sowing time; HSTUW2 was yielded low in both the optimum sowing and the late sowing times.

4.4.6 Number of spikes

In the experiment, the highest MP, GMP, and STI values were observed in HSTUW1, followed by HSTUW2, BARI Gom 32, and HSTUW3 (Table 13, 14, and 15). Among these genotypes, only HSTUW1 was yielded high in both the optimum sowing and the late sowing times; BARI Gom 32 was yielded intermediate under the optimum sowing time and was yielded low under the late sowing time; HSTUW3 was yielded low under the optimum sowing time and was yielded intermediate under the late sowing time; HSTUW2 was yielded low in both the optimum sowing and the late sowing times.

The lowest SSI and highest RSI values were observed in HSTUW7, followed by HSTUW8, HSTUW5, and HSTUW10 (Table 16 and 17). Among these genotypes, only HSTUW8 was yielded high in both the optimum sowing and the late sowing times; HSTUW10 was yielded intermediate under the optimum sowing time and was yielded high under the late sowing time; HSTUW5 and HSTUW7 were yielded intermediate in both the optimum sowing and the late sowing times.

4.4.7 Grains weight per spike

In the experiment, the highest MP, GMP, and STI values were observed in HSTUW8, followed by HSTUW10, HSTUW7, and HSTUW9 (Table 13, 14, and 15). Among these genotypes, only HSTUW8 was yielded high in both the optimum sowing and the late sowing times; HSTUW10 was yielded intermediate under the optimum sowing time and was yielded high under the late sowing time; HSTUW7 was yielded intermediate in both the optimum sowing and the late sowing times; HSTUW9 was yielded low in both the optimum sowing and the late sowing times.

The lowest SSI and highest RSI values were observed in HSTUW2, followed by BARI Gom 32, HSTUW10, and HSTUW1 (Table 16 and 17). Among these genotypes, only HSTUW1 was yielded high in both the optimum sowing and the late sowing times; HSTUW10 was yielded intermediate under the optimum sowing time and was yielded high under the late sowing time; BARI Gom 32 was yielded intermediate under the optimum sowing time and was yielded low under the late sowing time; HSTUW2 was yielded low in both the optimum sowing and the late sowing times**.**

4.4.8 1000-grains weight

In the experiment, the highest MP, GMP, and STI values were observed in HSTUW8, followed by HSTUW10, BARI Gom 32, and HSTUW6 (Table 13, 14, and 15). Among these genotypes, only HSTU8 was yielded high in both the optimum sowing and the late sowing times; HSTUW6 was yielded high under the optimum sowing time and was yielded intermediate under the late sowing time; HSTUW10 was yielded intermediate under the optimum sowing time and was yielded high under the late sowing time; BARI Gom 32 was yielded intermediate under the optimum sowing time and was yielded low under the late sowing time.

The lowest SSI and highest RSI values were observed in HSTUW10, followed by HSTUW9, HSTUW2, and HSTUW8 (Table 16 and 17). Among these genotypes, only HSTUW8 was yielded high in both the optimum sowing and the late sowing times; HSTUW10 was yielded intermediate under the optimum sowing time and was yielded high under the late sowing time; HSTUW2 and HSTUW9 were yielded low in both the optimum sowing and the late sowing times.

4.4.9 Grains yield per plot

Several stress tolerance indices are efficiently exploited for the identification of tolerant genotypes under the late sowing (heat-stress) time. Stress tolerance index in wheat was used in various research to select genotypes that could withstand high temperatures (Poudel *et al.*, 2021; Puri *et al.,* 2015; Aberkane *et al.*, 2021). Heat tolerance indices were calculated on the basis of grain yield of 11 wheat genotypes under the optimum sowing and the late sowing times (Table 13, 14, 15, 16, and 17). Many researchers (Puri *et al.,* 2015; Singh *et al.*, 2011) had used stress tolerance indices of grain yield to identify stress tolerance genotypes. When genotypes are selected based on SSI, those with less yield performance under normal conditions and higher yield under stressful conditions are favored. The highest value of SSI belonged to genotypes BARI Gom 32. That genotype had an intermediate grain yield under optimum field condition and low grain yield under heat-stress condition and therefore was identified as heat susceptible genotype. Similar results were reported by Khodarahmpour *et al.* (2011) in maize and Dorostkar *et al.* (2015) in wheat. According to SSI and RSI genotypes HSTUW3, HSTUW2, HSTUW9, and HSTUW8 were revealed tolerant to heat stress. According to Kamrani *et al.* (2018), selection based on SSI helps to determine high yielding genotypes under both conditions. As mentioned above, SSI introduced high yielding genotypes under both conditions as heat tolerant genotypes. Similar results were obtained by Dorostkar *et al.* (2015) in wheat.

A genotype of superior performance in both normal and stressed environments is shown by higher values for STI, MP, and GMP. Furthermore, the genotypes chosen using STI will have higher grain yield and stress tolerance. The selections based on MP typically boost the average performance of genotypes in both stress and non-stress conditions and fail to make a distinction between stress-tolerant and high-yielding genotypes. MP favors higher yield potential and lower stress tolerance (Kumar *et al.*, 2023).

In the experiment studies based on MP, GMP, and STI genotypes, the highest values of STI, GMP, and MP were recorded for HSTUW8, HSTUW1, and HSTUW4 genotypes. HSTUW8, HSTUW1, and HSTUW4 genotypes were more productive under stress conditions than the remaining genotypes. Hence, they were introduced as the most stable and productive genotypes among the cultivated genotypes under both conditions. Basavaraj *et al.* (2021) and Kamrani *et al.* (2018) also presented similar results and suggested higher yielding and heat tolerant genotypes could be selected on the basis of high values of MP, GMP and STI. Fernandez (1992) reported that selection based on STI, GMP, and MP would identify genotypes with higher levels of yield potential and stress tolerance.

Table 13. Mean productivity (MP) of 11 wheat genotypes of morpho-physiological traits

Genotype	DH	Rank	DM	Rank	PH	Rank	NGPS	Rank	SL	Rank	NS	Rank	GWPS	Rank	TWG	Rank	GYPP	Rank
HSTUW1	55.50	11	98.17	11	94.32	9	37.08	9	8.92	11	472.84	1	1.37	11	37.26	9	2698.36	2
HSTUW2	59.17	10	101.00	10	92.06	11	42.65	3	9.28	8	456.00	2	1.39	10	33.15	10	2150.88	11
HSTUW3	65.33	5	102.83	8	93.83	10	46.20	1	9.09	10	424.83	4	1.41	9	31.11	11	2229.91	10
HSTUW4	67.17	2	108.67	4	116.00	1	36.92	10	9.77	5	354.67	11	1.62	7	43.79	5	2608.00	3
HSTUW5	67.00	3	108.17	7	114.21	2	42.08	5	10.39	1	357.00	10	1.74	6	41.11	8	2460.89	7
HSTUW6	67.33	1	108.50	6	109.55	3	39.80	6	9.86	3	385.17	5	1.76	5	44.98	4	2534.54	4
HSTUW7	66.84	4	109.17	2	104.69	7	42.29	4	9.58	7	359.84	9	1.78	3	42.86	6	2473.65	6
HSTUW8	61.84	8	109.00	3	105.29	6	39.03	7	9.59	6	375.33	6	1.83	1	46.89	1	2711.46	1
HSTUW9	64.33	7	113.83	1	107.01	5	42.80	2	9.95	2	374.34	7	1.76	4	41.37	7	2282.55	9
HSTUW10	64.50	6	108.67	4	107.29	4	38.70	8	9.80	4	370.34	8	1.79	2	46.25	2	2475.05	5
BARI Gom 32	60.84	9	101.84	9	99.30	8	32.72	11	9.18	9	434.00	3	1.49	8	45.51	3	2324.56	8
Mean	62.81		106.35		103.96		40.02		9.58		396.76		1.63		41.30		2449.99	

Here, DH = Days to heading, DM = Days to maturity, PH = Plant height, NGPS= Number of grains per spike, SL = Spike length, NS = Number of spikes, GWPS = Grains weight per spike, TGW= Thousand grains weight, GYPP = Grains yield per plot

Table 14. Geometric mean productivity (GMP) of 11 wheat genotypes of morpho-physiological traits

Genotype	DH	Rank	DM	Rank	PH	Rank	NGPS	Rank	SL	Rank	NS	Rank	GWPS	Rank	TWG	Rank	GYPP	Rank
HSTUW1	55.37	11	98.08	11	94.32	9	36.96	9	8.91	11	471.69	1	1.37	11	37.20	9	2695.93	2
HSTUW2	59.07	10	100.89	10	92.05	11	42.53	3	9.28	8	455.52	2	1.39	10	33.15	10	2149.94	11
HSTUW3	65.33	5	102.73	8	93.82	10	46.17	1	9.09	10	424.70	4	1.41	9	31.10	11	2227.45	10
HSTUW4	67.13	2	108.58	4	116.00	1	36.82	10	9.76	5	353.96	11	1.61	7	43.76	5	2607.35	3
HSTUW5	66.97	3	108.10	7	114.20	2	41.98	5	10.38	1	356.99	10	1.73	6	41.10	8	2460.71	7
HSTUW6	67.32	1	108.45	6	109.55	3	39.76	6	9.85	3	384.41	5	1.75	5	44.97	4	2534.20	4
HSTUW7	66.82	4	109.10	2	104.69	7	42.27	4	9.57	7	359.74	9	1.78	3	42.86	6	2473.65	6
HSTUW8	61.83	8	108.94	3	105.28	6	39.02	7	9.59	6	375.32	6	1.83	1	46.89	1	2711.17	1
HSTUW9	64.33	7	113.58	1	107.01	5	42.73	2	9.92	2	374.18	7	1.75	4	41.37	7	2282.21	9
HSTUW10	64.50	6	108.58	4	107.29	4	38.70	8	9.79	4	370.26	8	1.79	2	46.24	2	2474.92	5
BARI Gom 32	60.83	9	101.70	9	99.29	8	32.67	11	9.17	9	431.19	3	1.49	8	45.50	3	2318.41	8
Mean	63.59		106.25		103.95		39.96		9.57		396.18		1.63		41.29		2448.72	

Here, DH = Days to heading, DM = Days to maturity, PH = Plant height, NGPS= Number of grains per spike, SL = Spike length, NS = Number of spikes, GWPS = Grains weight per spike, TGW= Thousand grains weight, GYPP = Grains yield per plot

Table 15. Stress tolerance index (STI) of 11 wheat genotypes of morpho-physiological traits

Genotype	**DH**	**Rank**	**DM**	**Rank**	**PH**	**Rank**	**NGPS**	**Rank**	**SL**	**Rank**	**NS**	**Rank**	**GWPS**	**Rank**	**TWG**	**Rank**	**GYPP**	**Rank**
HSTUW1	0.76	11	0.78	11	0.82	9	0.85	9	0.86	11	1.31	1	0.68	11	0.79	9	1.20	2
HSTUW2	0.86	10	0.83	10	0.78	11	1.13	3	0.93	8	1.22	2	0.70	10	0.63	10	0.76	11
HSTUW3	1.06	5	0.86	8	0.81	10	1.33	1	0.89	10	1.06	4	0.72	9	0.55	11	0.82	10
HSTUW4	1.12	2	0.96	4	1.24	1	0.84	10	1.03	5	0.74	11	0.95	7	1.09	5	1.12	3
HSTUW5	1.11	3	0.95	7	1.20	2	1.10	5	1.17	1	0.75	10	1.10	6	0.96	8	1.00	7
HSTUW6	1.12	1	0.96	6	1.10	3	0.98	6	1.05	3	0.87	5	1.12	5	1.15	4	1.06	4
HSTUW7	1.11	4	0.97	2	1.01	7	1.11	4	0.99	7	0.76	9	1.16	3	1.05	6	1.01	6
HSTUW8	0.95	8	0.97	3	1.02	6	0.95	7	1.00	6	0.83	6	1.22	1	1.25	1	1.21	1
HSTUW9	1.03	7	1.05	1	1.05	5	1.14	2	1.07	2	0.82	7	1.12	4	0.98	7	0.86	9
HSTUW10	1.03	6	0.96	4	1.06	4	0.93	8	1.04	4	0.81	8	1.17	2	1.22	2	1.01	5
BARI Gom 32	0.92	9	0.84	9	0.91	8	0.66	11	0.91	9	1.09	3	0.81	8	1.18	3	0.89	8
Mean	1.01		0.92		1.00		1.00		0.99		0.93		0.98		0.99		0.99	

Here, DH = Days to heading, DM = Days to maturity, PH = Plant height, NGPS= Number of grains per spike, SL = Spike length, NS = Number of spikes, GWPS = Grains weight per spike, TGW= Thousand grains weight, GYPP = Grains yield per plot

Table 16. Stress susceptibility index (SSI) of 11 wheat genotypes of morpho-physiological traits

Genotype	**DH**	**Rank**	**DM**	**Rank**	**PH**	**Rank**	**NGPS**	**Rank**	**SL**	**Rank**	**NS**	**Rank**	**GWPS**	**Rank**	**TWG**	**Rank**	**GYPP**	**Rank**
HSTUW1	44.26	11	1.01	7	1.06	6	-60.49	1	-12.14	1	1.73	10	-1.37	4	3.92	11	8.13	10
HSTUW2	37.54	10	1.10	9	1.28	10	-57.15	2	-6.15	3	1.17	7	-3.25	1	-0.13	3	-6.10	2
HSTUW3	0.00	7	1.04	8	1.08	7	-26.73	4	-10.25	2	0.64	5	0.21	6	1.94	9	-9.86	1
HSTUW4	-18.65	1	0.95	5	1.14	9	46.87	11	4.24	8	1.58	9	5.27	11	2.55	10	4.34	9
HSTUW5	-17.31	2	0.85	4	1.83	11	45.85	10	10.00	10	0.15	3	4.86	10	1.47	8	2.40	7
HSTUW6	-8.74	4	0.70	1	0.66	3	30.76	8	-0.47	7	1.57	8	3.32	9	0.89	7	3.23	8
HSTUW7	-10.23	3	0.84	3	1.11	8	20.64	7	8.68	9	-0.62	1	2.23	7	0.85	6	-0.40	6
HSTUW8	-1.59	6	0.81	2	0.82	4	-12.78	5	-0.65	6	-0.14	2	-1.26	5	0.51	4	-2.96	4
HSTUW9	0.00	7	1.53	11	0.60	2	37.16	9	18.66	11	0.74	6	3.00	8	-0.26	2	-3.52	3
HSTUW10	-4.59	5	0.95	5	0.97	5	-2.36	6	-2.72	5	0.54	4	-1.87	3	-1.23	1	-2.04	5
BARI Gom 32	8.31	9	1.20	10	0.42	1	-40.39	3	-4.29	4	2.72	11	-3.24	2	0.78	5	13.55	11
Mean	2.64		1.00		1.00		-1.69		0.45		0.92		0.72		1.03		0.62	

Here, DH = Days to heading, DM = Days to maturity, PH = Plant height, NGPS= Number of grains per spike, SL = Spike length, NS = Number of spikes, GWPS = Grains weight per spike, TGW= Thousand grains weight, GYPP = Grains yield per plot

Table 17. Relative stress index (RSI) of 11 wheat genotypes of morpho-physiological traits

Genotype	**DH**	**Rank**	**DM**	**Rank**	**PH**	**Rank**	**NGPS**	**Rank**	**SL**	**Rank**	**NS**	**Rank**	**GWPS**	**Rank**	**TWG**	**Rank**	**GYPP**	**Rank**
HSTUW1	1.14	1	1.00	7	1.00	6	1.18	1	1.09	1	0.94	10	1.07	4	0.91	11	0.93	10
HSTUW2	1.12	2	0.99	9	1.00	10	1.17	2	1.05	3	0.99	7	1.13	1	1.03	3	1.07	2
HSTUW3	1.00	4	1.00	8	1.00	7	1.08	4	1.07	2	1.03	5	1.02	6	0.97	9	1.11	1
HSTUW4	0.93	11	1.00	5	1.00	9	0.87	11	0.98	8	0.95	9	0.87	11	0.95	10	0.97	9
HSTUW5	0.94	10	1.01	4	1.00	11	0.87	10	0.94	10	1.07	3	0.88	10	0.99	8	0.99	7
HSTUW6	0.97	8	1.03	1	1.00	3	0.91	8	1.01	7	0.95	8	0.93	9	1.00	7	0.98	8
HSTUW7	0.96	9	1.01	3	1.00	8	0.94	7	0.95	9	1.13	1	0.96	7	1.00	6	1.01	6
HSTUW8	0.99	6	1.02	2	1.00	4	1.04	5	1.01	6	1.09	2	1.07	5	1.01	4	1.04	4
HSTUW9	1.00	4	0.95	11	1.00	2	0.90	9	0.89	11	1.02	6	0.94	8	1.04	2	1.05	3
HSTUW10	0.98	7	1.00	5	1.00	5	1.01	6	1.02	5	1.04	4	1.09	3	1.06	1	1.03	5
BARI Gom 32	1.02	3	0.98	10	1.00	1	1.12	3	1.03	4	0.86	11	1.13	2	1.01	5	0.87	11
Mean	1.00		1.00		1.00		1.01		1.00		1.01		1.01		1.00		1.00	

Here, DH = Days to heading, DM = Days to maturity, PH = Plant height, NGPS= Number of grains per spike, SL = Spike length, NS = Number of spikes, GWPS = Grains weight per spike, TGW= Thousand grains weight, GYPP = Grains yield per plot

4.5 Analysis of DNA fingerprinting based on SSR markers

Initially, DNA was extracted from 2 cm young leaves of 3 weeks old seedlings of 11 wheat genotypes. The DNA was extracted using the modified CTAB method. The quality of the extracted DNA samples was checked before PCR amplification through quantification using a Thermo Scientific NanoDrop^TM1000 Spectrophotometer. The DNA concentrations of 11 wheat genotypes ranged from 747.6 to 1869.8 ng per µl. After that, the DNA concentrations were diluted and finally, the DNA concentrations of 11 wheat genotypes ranged from 170.6 to 235.1 ng per µl. After polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis (PAGE), 3 (out of 4) SSRs/microsatellite markers i.e., TaXgwm294, TaBarc68, and Xgwm296 showed the polymorphic bands. Here, 100 bp DNA ladders were used to count the distance of the DNA bands of SSRs/microsatellite markers. Gel pictures of PCR amplified fragments using those SSR markers were shown (Figure 9, 10, 11, and 12).

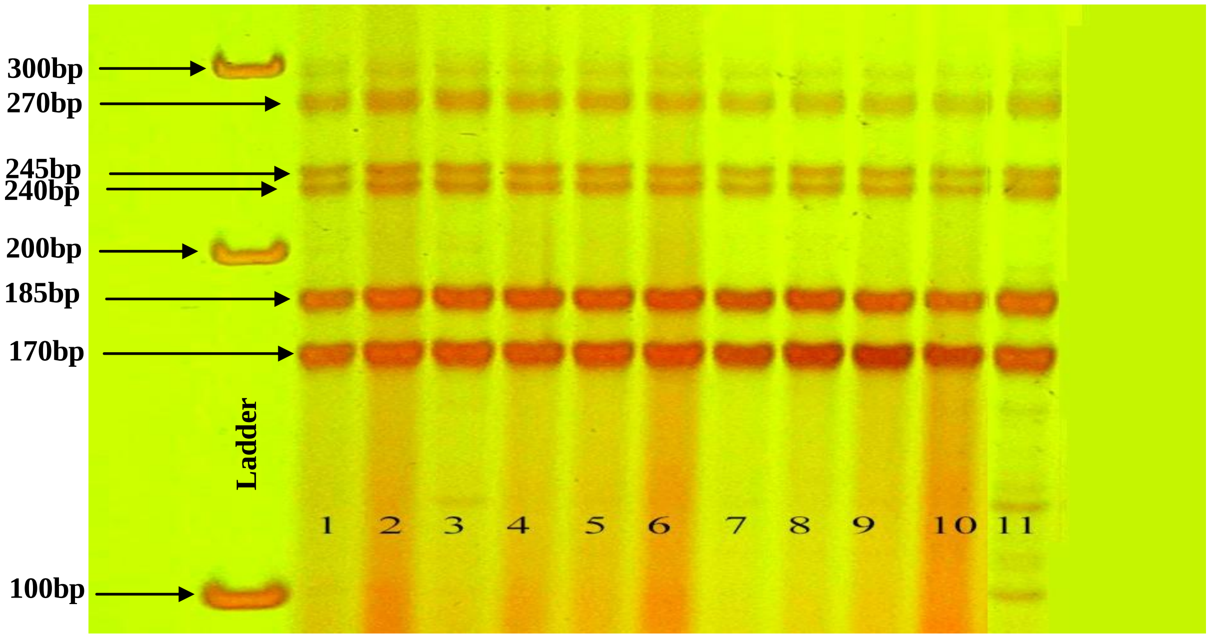


Figure 9: DNA profile of 11 wheat genotypes using TaGwm291

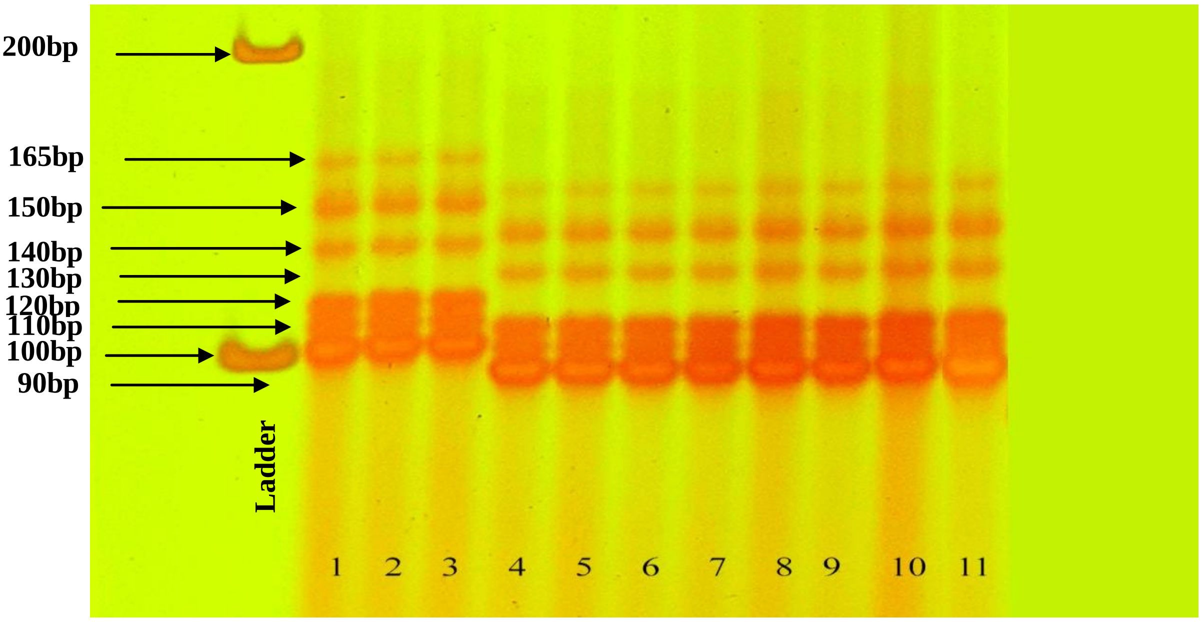


Figure 10: DNA profile of 11 wheat genotypes using TaXgwm294

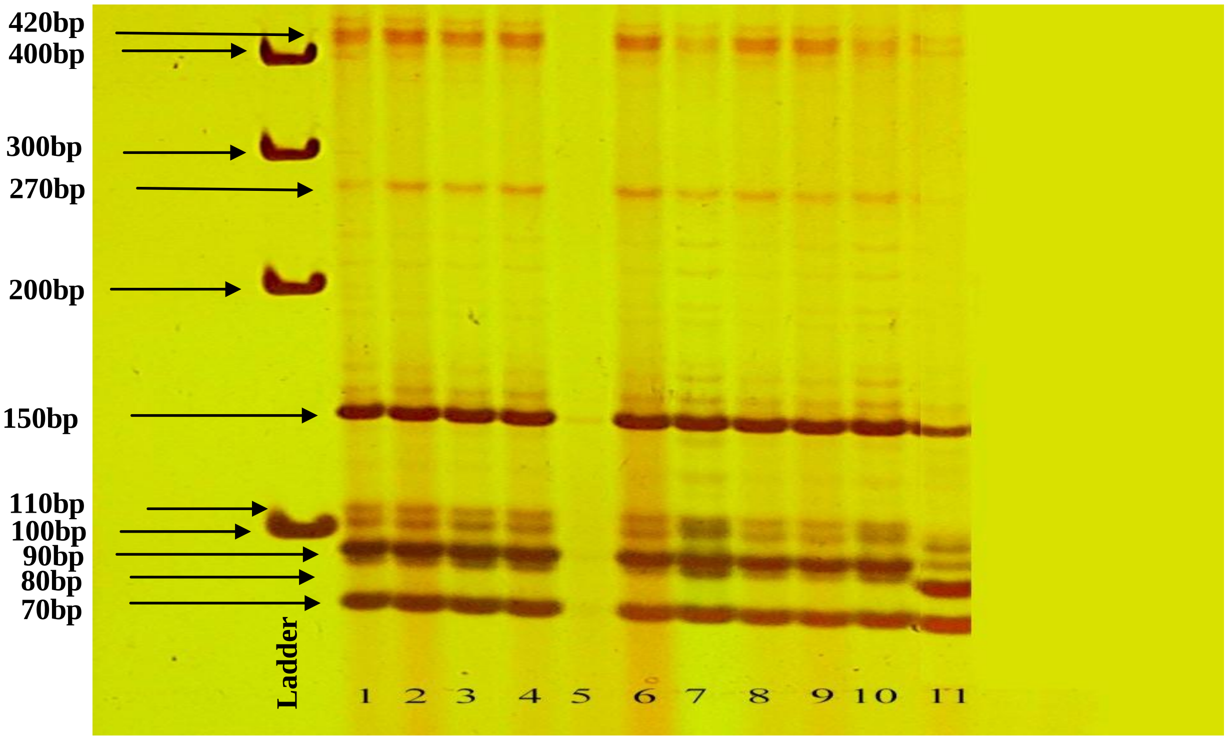


Figure 11: DNA profile of 11 wheat genotypes using TaBarc68

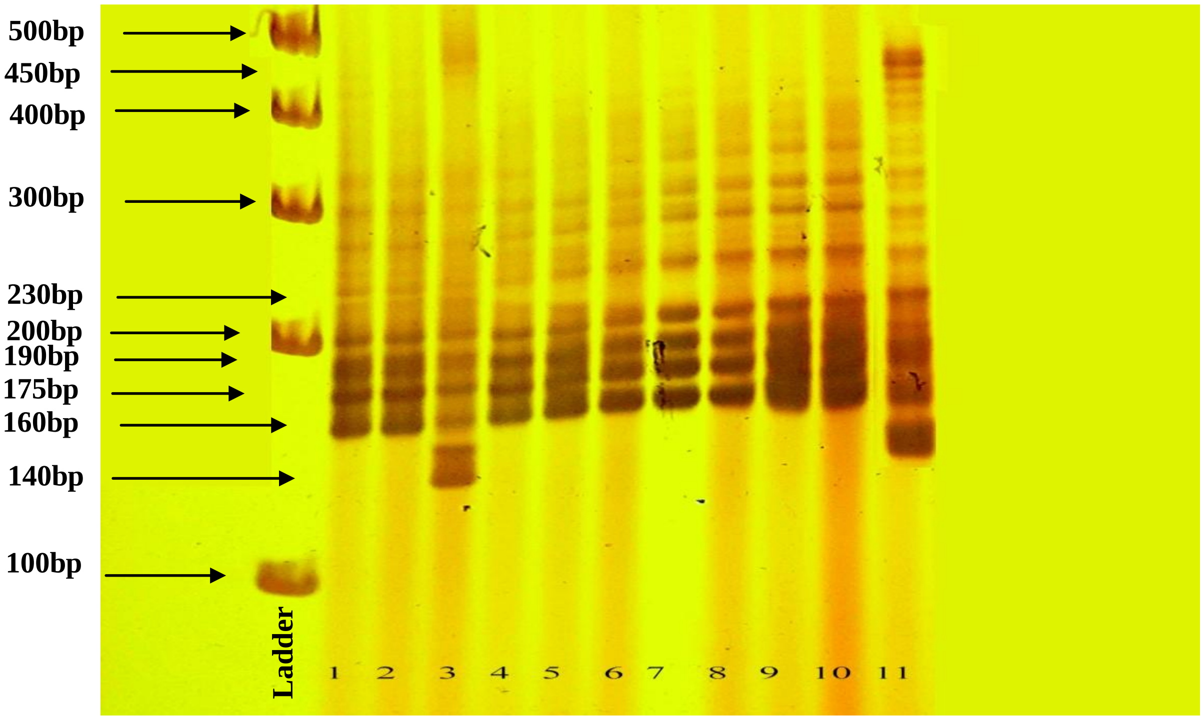


Figure 12: DNA profile of 11 wheat genotypes using Xgwm296

Table 18. Number of alleles, allele range, and PIC values of 4 SSR markers

Sl. No.	**SSR loci**	**Number of alleles**	**Range of allele size (bp)**	**PIC**
1	TaGwm291	5	170 – 270	0.8
2	TaXgwm294	8	90 – 165	0.86
3	TaBarc68	8	70 – 420	0.86
4	Xgwm296	7	140 – 450	0.82
Average		7		0.84

4.6 Population structure of 11 wheat genotypes

In case of population genetic structure analysis, Bayesian clustering modeling was executed in the STRUCTURE 2.3.4 software using 11 wheat genotypes data generated by SSR marker data (Figure 13 and 14). The clustering model presumes the underlying existence of K clusters, Ln (PD) derived ΔK was plotted against the K to determine the number of populations. Delta K shows only the uppermost clustering level and number of sub-populations in main population. According to Evanno *et al.* (2005) the highest log-likelihood was performed and yielded K=2 (Figure 13). This means that all populations represent two distinct clusters. The analysis of structure according to the geographical origin was performed by setting the range of possible number of subpopulations (K) from 1 to 12. In structure analysis, accessions were further categorized as pure or heterogeneous, accessions with more than 0.80 score were considered as pure and less than 0.80 as heterogeneous. Here, population I consisted (54.55%) of total genotypes i.e. 6 genotypes where all the genotypes (HSTUW4, HSTUW6, HSTUW7, HSTUW8, HSTUW9, and HSTUW10) were pure. In population II comprised of (45.45%) of total genotypes i.e. 5 genotypes where all the genotypes (HSTUW1, HSTUW2, HSTUW3, HSTUW5, and BARI Gom 32) were found pure. Phylogenetic dendrogram based on genetic distance revealed a similar trend to the population structure analysis, revealing two possible subpopulations using model-based STRUCTURE (Figure 14). Results revealed there are two possible divergent groups in tested representative wheat germplasms.

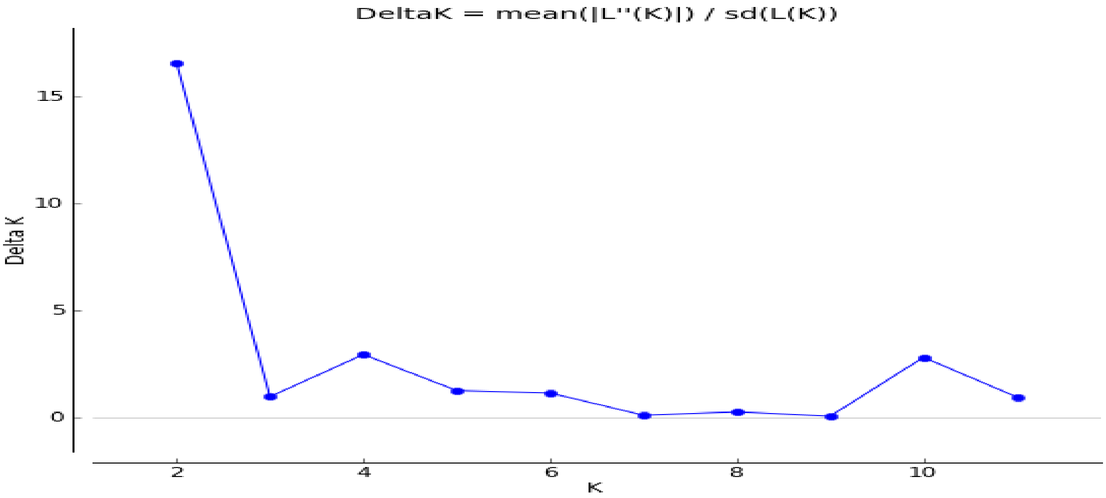
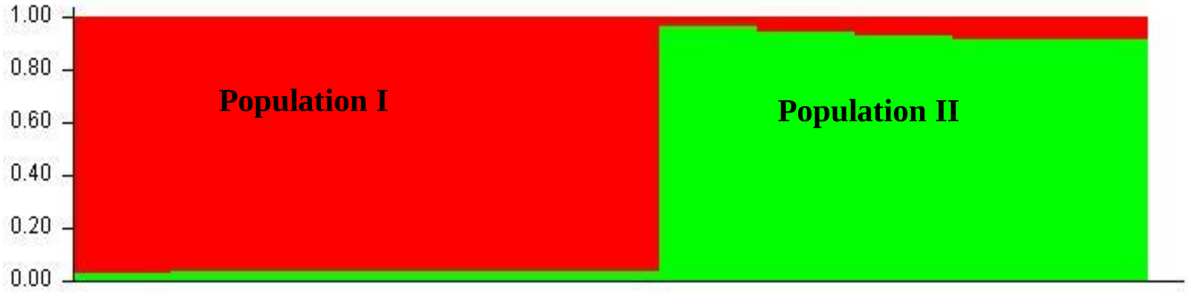
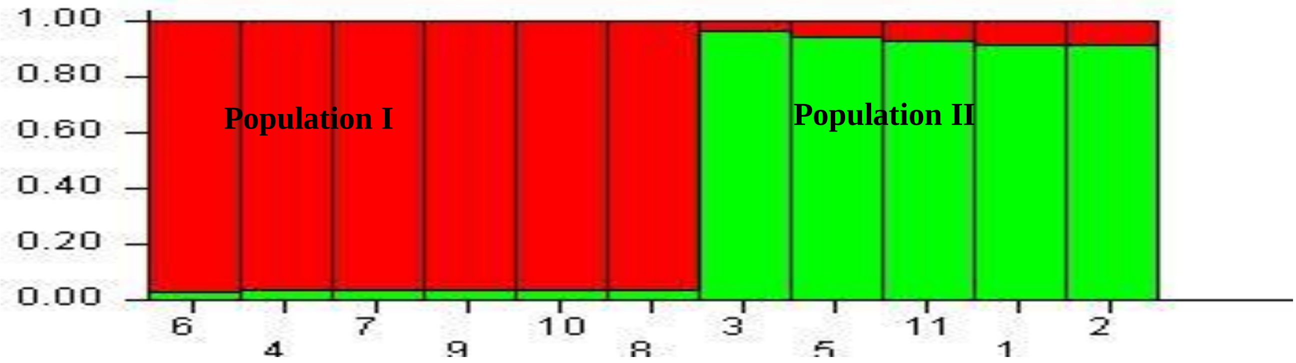


Figure 13: The best number of groups among location estimated by Evanno test methods



(A) Sort by Q



(B) Plot in multiple lines

Figure 14: Model-based population structure plot for each isolate with K=2, using structure with SSR markers data. Color codes are as follows: Population I red, Population II green (A). The code of each genotype (B) corresponds to the description in Table 2

4.7 Genetic variation among the 11 wheat genotypes

Cluster analysis of genetic similarity values for SSR alleles from all the wheat genotypes was based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) used to construct a dendrogram (Figure 15). These techniques have been applied in wheat breeding schemes by many scientists and obtained explanatory outcomes (Ahmed *et al.,* 2017; Salehi *et al.,* 2018). The cluster analysis revealed two major clusters (Group I and II) with a similarity coefficient varying between 0 and 5 indicating significant genetic variation among the wheat accessions studied. Cluster I comprised 6 genotypes and cluster II comprised 5 genotypes. In cluster I, the high genetic similarity was observed among HSTUW4, HSTUW6, HSTUW7, HSTUW8, HSTUW9, and HSTUW10 were less genetic distances and genetically identical to each other. In cluster II, the highest similarity was observed between HSTUW1 and HSTUW2 were less genetic distances and genetically identical to each other, while the lowest similarity was observed between HSTUW5 and BARI Gom 32 were more genetic distances and genetically diverse (Figure 15). Several studies using SSR had resulted in successful clustering of wheat cultivars. This type of marker was very effective in delineating diversity based on parental source by grouping cultivars (Ktavii *et al.,* 2014) as well as grouping based on agronomic characteristics and geographical origin (Naceur *et al.,* 2012). Depending on the degree of diversity, two (El-Bakatoushi, 2019) or three clusters (Wang *et al.,* 2017) can be formed following the UPGMA analysis. In addition, as high as 9 (Naceur *et al.,* 2012) and 13 clusters (Schuster *et al.,* 2009) have been reported in genetic diversity studies.

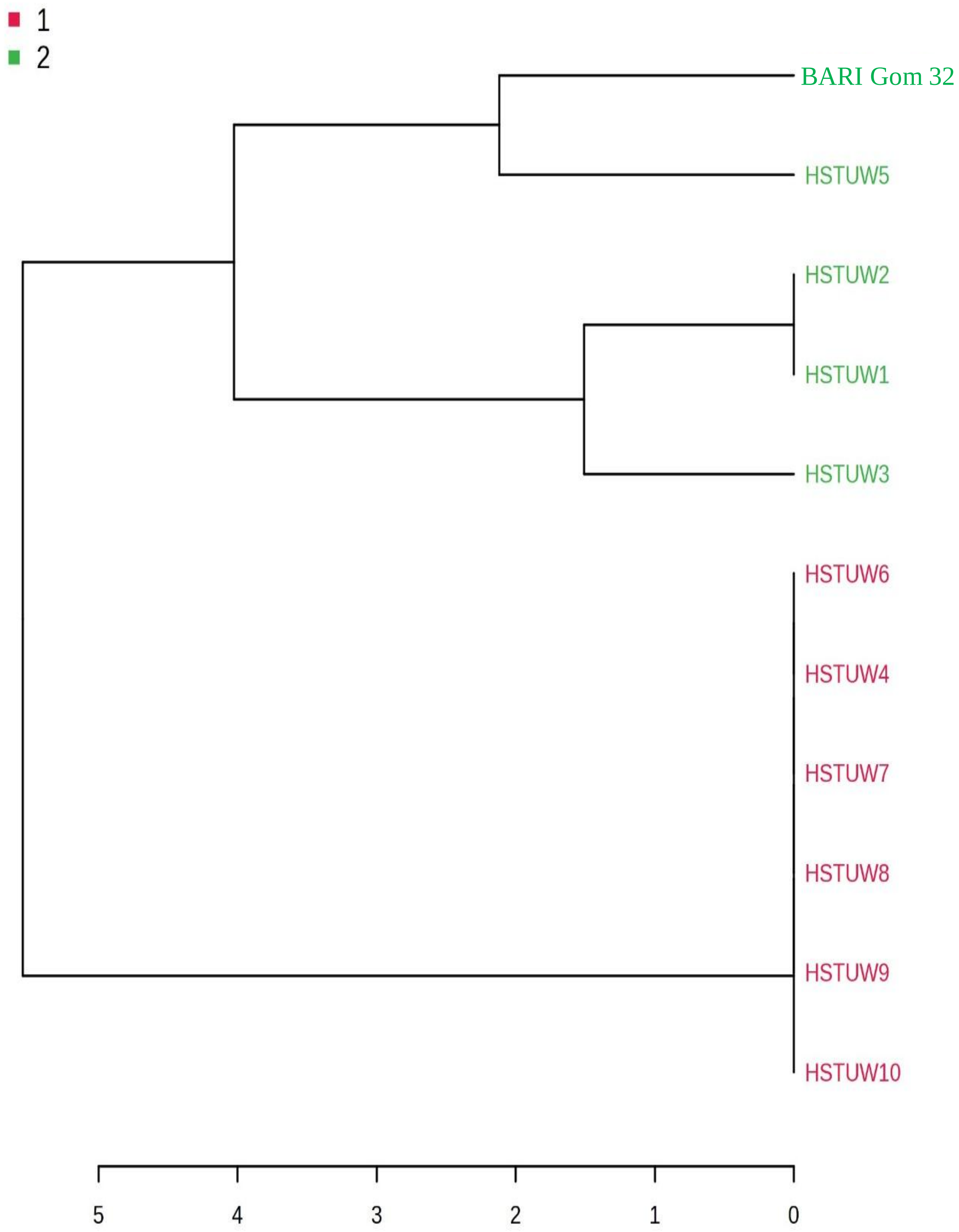


Figure 15: An UPGMA cluster dendrogram showing the genetic relationships between 11 wheat genotypes including 10 HSTU-developed advance lines and 1 check variety (BARI Gom 32) based on the alleles detected by 4 microsatellite markers

Table 19. Cluster groups and their containing 11 wheat genotypes name

Cluster	Control	
	Size	Genotypes
I	6	HSTUW4, HSTUW6, HSTUW7, HSTUW8, HSTUW9, and HSTUW10
II	5	HSTUW1, HSTUW2, HSTUW3, HSTUW5, and BARI Gom 32

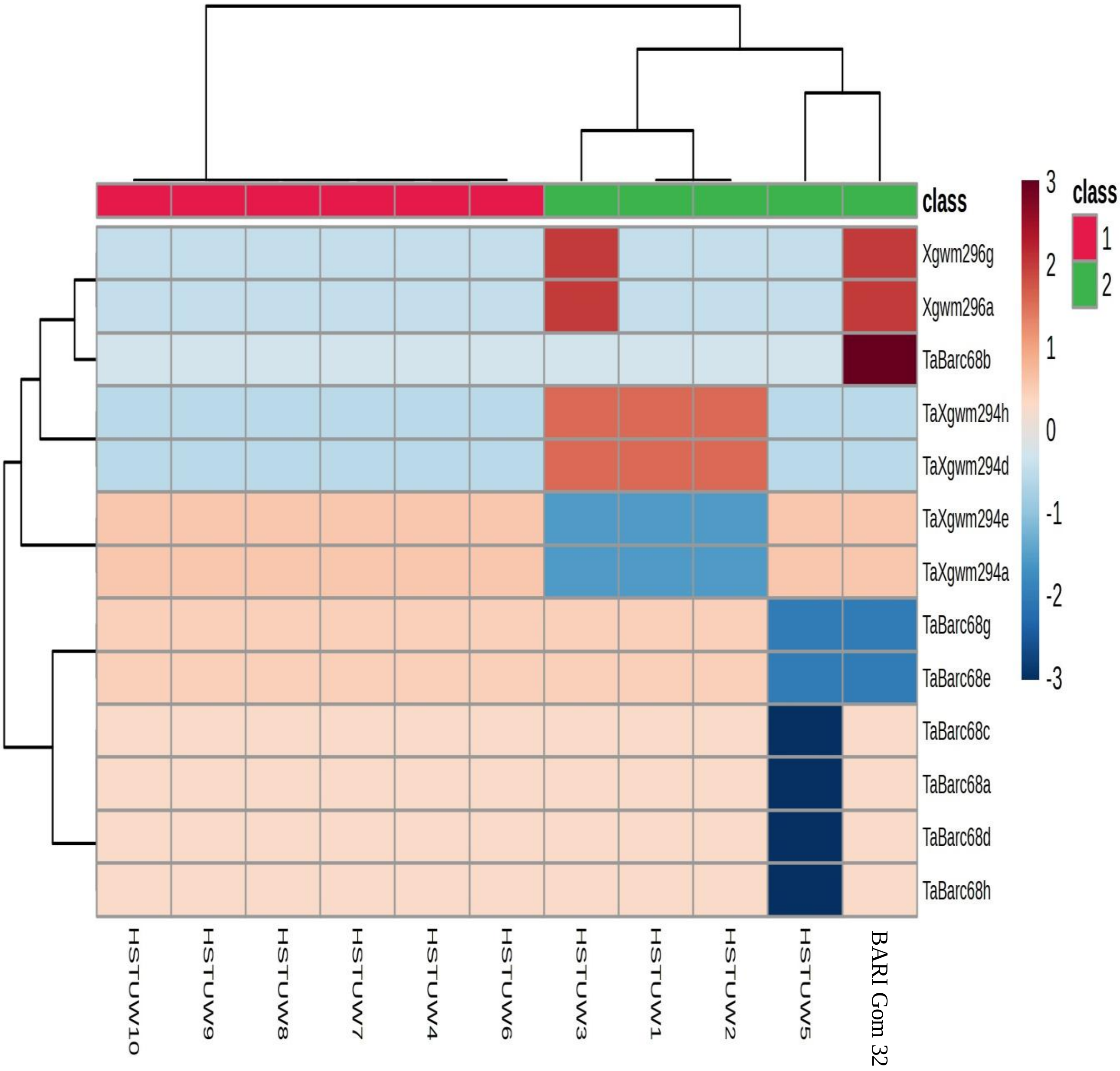


Figure 16: Heatmap visualization and hierarchical clustering analysis with MetaboAnalyst data annotation tools constructed based on the different genotypes for 28 SSR alleles. Rows: SSR markers; Columns: Wheat genotypes; Color key indicates genotype value, blue: Lowest, red: Highest

CHAPTER V

SUMMARY AND CONCLUSION

The present investigation was conducted with eleven (ten HSTU-developed advance lines and one check variety) wheat genotypes in RCBD design with three replications at Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh during Rabi season, 2022-2023. The objectives of the research work were to find heat-tolerant genotypes among the eleven wheat germplasms and to characterize the experimental materials through nine important yield and yield-related characters to assess the degree of divergence among the experimental wheat genotypes. Apart from the morphological analysis, the molecular markers (SSRs) were also applied to characterize the wheat genotypes at the DNA level. To propose elite plant type with desired characteristics i.e. mean performance, range, and stress-tolerant indices were also studied.

The observations were recorded on ten selected plants per genotype in each replication. The analysis of variance revealed highly significant differences among genotypes for the most traits. Again, treatment effects were no significant difference among most traits except days to maturity, number of spikes, and 1000-grains weight. The interaction between genotype and treatment revealed no significant difference among most traits except days to heading, days to maturity, number of grains per spike, and spike length**.** Mean values of days to heading ranged from 51.67 to 69.33 days under optimum sowing (OS) and 59.33 to 66.33 days under late sowing (LS); days to maturity 102.33 to 121.33 days (OS) and 94.00 to 106.33 days (LS); plant height 92.35 to 116.33 cm (OS) and 91.76 to 115.67 cm (LS); the number of grains per spike 30.93 to 45.20 (OS) and 34.27 to 47.90 (LS); spike length 8.58 to 10.73 cm (OS) and 9.25 to 10.05 cm (LS); number of spikes 351.67 to 505.67 (OS) and 332.33 to 440.00 (LS); grains weight per spike 1.32-1.88 g (OS) and 1.40-1.86 g (LS); 1000-grains weight 31.99 to 47.23 g (OS) and 30.23 to 47.04 g (LS); grains yield per plot 2087.2 to 2812.7 g (OS) and 2155.6 to 2751.0 g (LS). Overall study on the grain yield, yield contributing, phenological and physiological characters, it was observed that the genotypes HSTUW8, HSTUW1, and HSTUW4 ranked better category for maximum number of characters indicating their high tolerance to heat stress under heat-stressed environment.

The combined analysis of variance revealed highly significant differences among 11 wheat genotypes in most stress tolerant indices for grain yield and others yield related traits. In our studies in case of days to heading, the highest MP, GMP, and STI values were observed in HSTUW6 and the lowest SSI value was observed in HSTUW4. The highest RSI value was observed in HSTUW1. In days to maturity, the highest MP, GMP, and STI values were observed in HSTUW9 and the lowest SSI and highest RSI values were observed in HSTUW6. In plant height, the highest MP, GMP, and STI values were observed in HSTUW4 and the lowest SSI and highest RSI values were observed in BARI Gom 32. In number of grains per spike, the highest MP, GMP, and STI values were observed in HSTUW3 and the lowest SSI and highest RSI values were observed in HSTUW1. In spike length, the highest MP, GMP, and STI values were observed in HSTUW5 and the lowest SSI and highest RSI values were observed in HSTUW1. In number of spikes, the highest MP, GMP, and STI values were observed in HSTUW1 and the lowest SSI and highest RSI values were observed in HSTUW7. In grains weight per spike, the highest MP, GMP, and STI values were observed in HSTUW8 and the lowest SSI and highest RSI values were observed in HSTUW2. In 1000-grains weight, the highest MP, GMP, and STI values were observed in HSTUW8 and the lowest SSI and highest RSI values were observed in HSTUW10. In grains yield per plot, based on MP, GMP and STI genotypes the highest values of STI, GMP, and MP were recorded for HSTUW8 and the lowest SSI and highest RSI values were observed in HSTUW3. Overall study on the grain yield and stress indices, it was observed that the genotypes HSTUW8, HSTUW1, and HSTUW4 ranked better category for maximum number of stress indices indicating their high tolerance to heat stress under heat-stressed environment.

In marker study, 4 SSR markers were evaluated in 11 wheat genotypes for molecular characterization and identify the variation among the genotypes. The 4 SSR/microsatellite markers viz. TaGwm291, TaXgwm294, TaBarc68, and Xgwm296 were used. A total of 28 alleles were detected and number of alleles per locus ranged from 5-8 with an average of 7 alleles per locus. In population genetic structure analysis, population I consisted 54.55% of the genotypes (6 genotypes: HSTUW4, HSTUW6, HSTUW7, HSTUW8, HSTUW9, and HSTUW10) where all the genotypes were pure. In population II consisted 45.45% of the genotypes (5 genotypes: HSTUW1, HSTUW2, HSTUW3, HSTUW5, and BARI Gom 32) where all the genotypes were pure. In cluster I, the high genetic similarity was observed among HSTUW4, HSTUW6, HSTUW7, HSTUW8, HSTUW9, and HSTUW10 were less genetic distances and genetically identical to each other. In cluster II, the highest similarity was observed between HSTUW1 and HSTUW2 were less genetic distances and genetically identical to each other, while the lowest similarity was observed between HSTUW5 and BARI Gom 32 were more genetic distances and genetically diverse. The genotypes belonging to the same cluster were genetically more or less similar and the genotypes belonging to the different clusters were genetically different from each other.

In conclusion, seeds sowing in late period (20th December), heat stress was imposed on all the genotypes because the differential responses of genotypes to a heat-stressed condition indicate the heat tolerance ability of wheat genotypes. The results of the present studies indicated significant variation among the genotypes for most of the characters studied. The current genetic diversity analysis clearly differentiated genotypes into separate groups and indicated diverse genotypes.

- The effects of heat stress on HSTU-developed advance lines in wheat based on yield and yield attributing characters were significant in most of the lines.
- The genotypes HSTUW8, HSTUW1, and HSTUW4 might be identified as heat tolerant genotypes based on yield and others yield-related morpho-physiological traits under the heat-stressed condition.

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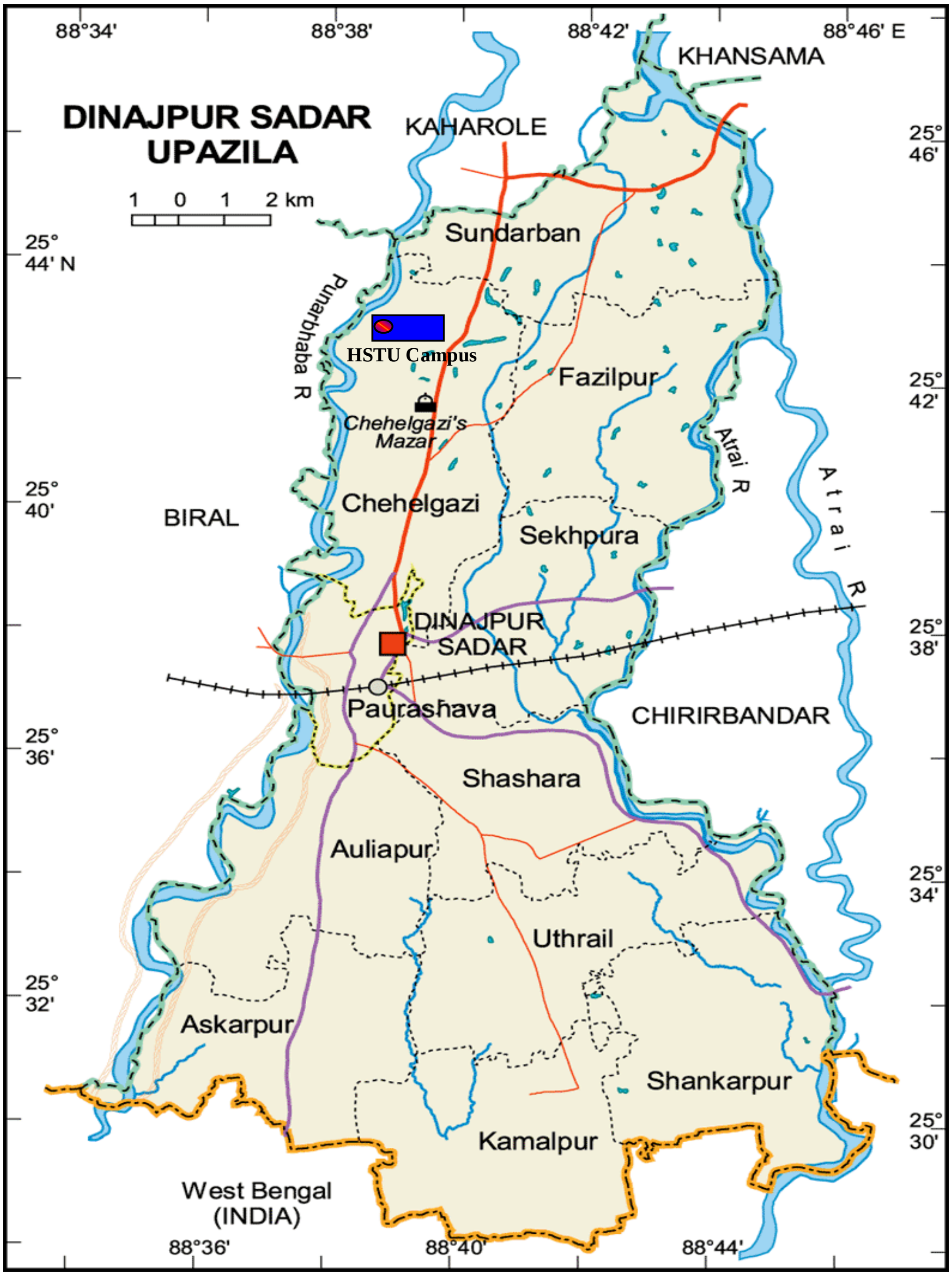
APPENDICES

Appendix I. Location of experimental site (map of Dinajpur Sadar Upazila showing the experimental area)



Appendix II: Some photographs of research work

Figure: A group photo during field visit

Figure: Showing vegetative growth phase

Figure: Recording 50% days to heading data

Figure: Recording standing plant height with meter scale

Figure: Showing harvesting on the optimum field

Figure: Showing an important discussion section during field visits

Figure: Field was (late sowing) visited by the seed certification agency, principal scientific officer & senior scientific officers from BWMRI, and our honorable teachers

Figure: Honorable supervisor and co-supervisor in the research field

Figure: Researcher in the research field

Figure: Showing samples dried in silica

Figure: Cutting of leaf for crushing

Figure: DNA quantification by NanoDrop

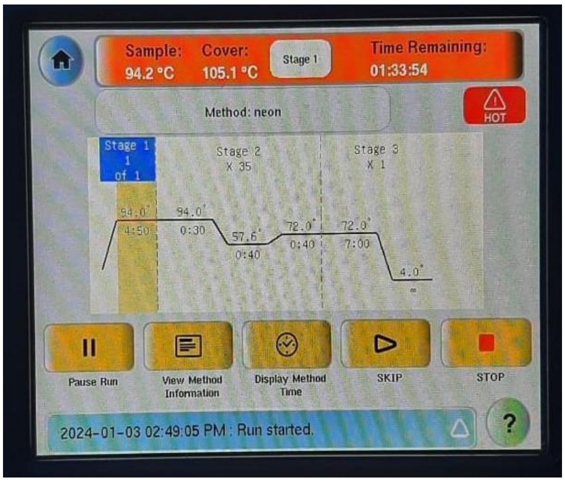
#	ng/µL	A260/A280	A260/A230
5	974.6	2.04	2.45
6	763.6	2.07	2.42
7	684.5	2.01	2.44
8	712.8	2.12	2.40
9	811.1	2.01	2.43

Figure: Results of DNA concentration

Sample: 86.1 °C
Cover: 104.9 °C
Time Remaining: 01:34:09
Method: neon
2024-01-03 02:49:05 PM : Run started

Figure: PCR samples

Figure: Giving the samples in PCR machine



Sample: 94.2 °C

Cover: 105.1 °C

Stage 1

Time Remaining: 01:33:54

Method: neon

2024-01-03 02:49:05 PM : Run started.

Figure: PCR process

Figure: Polyacrylamide gel electrophoresis process

Figure: AgNO$_3$ staining process after PAGE

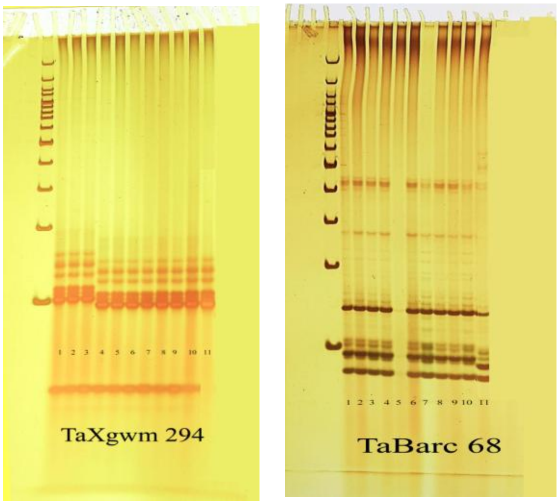


Figure: DNA fingerprint

Figure: Seeds treated with Provax 200 WP

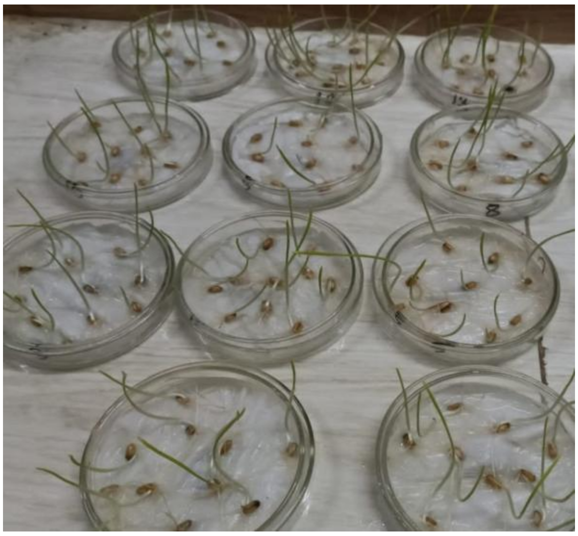


Figure: Seed germination test

HSTUW1

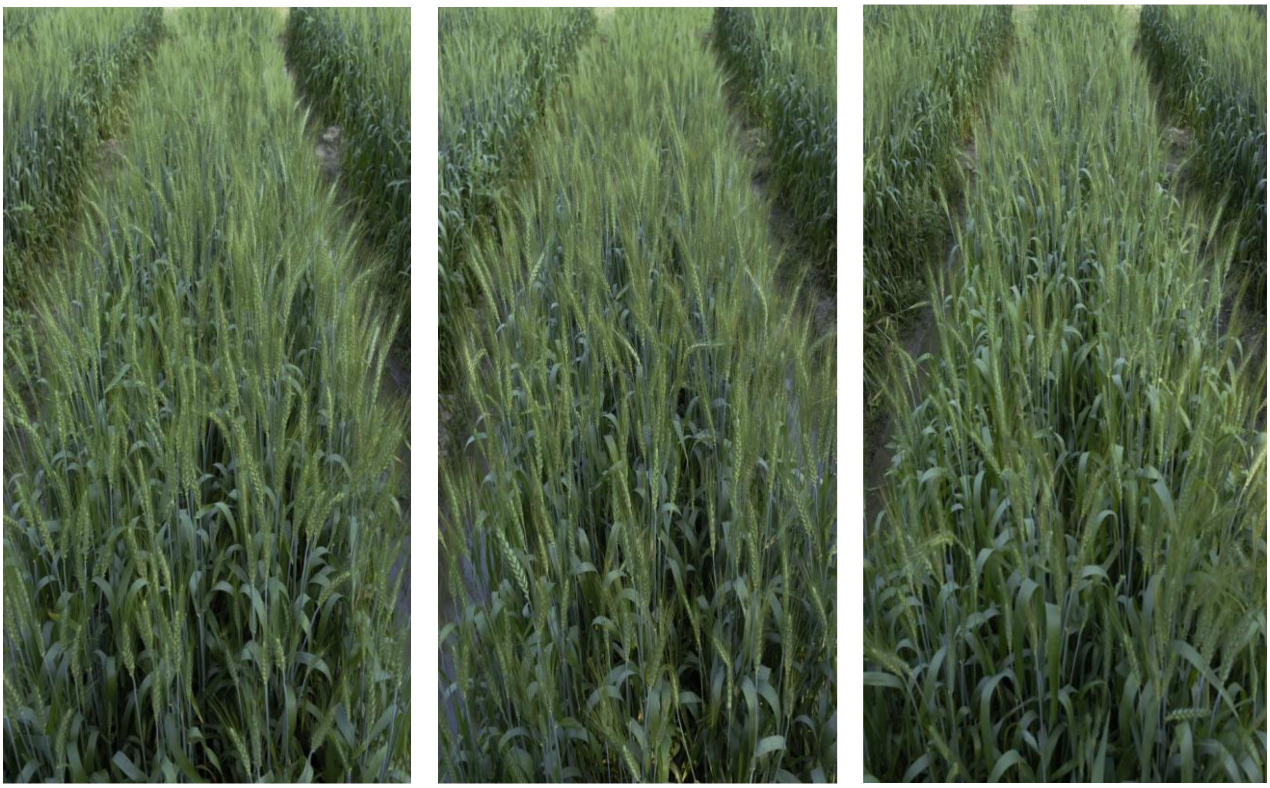
HSTUW2

HSTUW3

HSTUW4

HSTUW5

HSTUW6



HSTUW7 HSTUW8 HSTUW9

HSTUW10 BARI Gom 32

Figure: Showing individual plot of the wheat genotypes