CHAPTER I

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the leading cereals and is used as a staple food for 1.2 billion people worldwide (Afzal *et al.,* 2015; Iqbal *et al.,* 2018). To feed the increasing population, the global demand for wheat is expected to increase up to 40% by 2050 to meet food security (Rosegrant and Agcaoili, 2010; Abdelaal *et al.,* 2018; Jahan *et al.,* 2019). Wheat is the staple food of millions of people, being one of the three globally produced cereals (Maize and Barley being the other two). Wheat cultivation was started some 10,000 years ago, with its origin being traced back to southeast Turkey. Total worldwide wheat production was 778.2 million tons in 2021-22 and it was forecast that world wheat production in 2022-23 will be 781.1 million tons, up 2.91million tons from previous years, the current record outturn (FAO, 2022). China is the largest wheat producer and its average annual wheat production in 2022 was 138 million metric tons. The other top 5 wheat-producing countries in 2022 were the European Union (134.7 million metric tons), India (103 million metric tons), Russia (91 million metric tons), the United States (44.9 million metric tons), Canada (35.00 million metric tons). In 2022 an increase of 1.26 million tons or 0.16% in wheat production around the globe (USDA 2022).

Wheat consumption in Bangladesh has more than doubled in the past six years for changing food habits, lower prices than rice, and exports of bakery products. Some $ 4.33 lakh worth of bakery products were exported in 2015-16 but it has gone on to reach $ 21 crore last fiscal year. The grains imports have seen a 116 percent rise as its local cultivation failed to meet the demand. The country’s annual demand stands at around 77 lakh tones, 85 percent of which is met through imports, as per the National Board of Revenue (NBR) and the Department of Agricultural Extension (DAE). Some 67.34 lakh tones of it worth Tk 14,114 crore was brought over in the recently concluded fiscal 2019-20. It was just 31 lakh tones in fiscal 2014-15. Bangladesh's average production has been in the range of 10 to 12 lakh tones in the past six years (The Daily Star, 2020). About 10,85,368 tons were harvested from 3,28,924 hectares of land in 2020-2021. The highest yield 4.25 tons/ha was obtained in Meherpur district and the lowest yield 1.75 tons/ha in Moulovibazar and Sunamganj districts. The national average yield is 3.30 tons/ha in 2020-2021 (BBS, 2022).

The bread wheat (*Triticum aestivum* L., 2n=6x=42, AABBDD), the third-largest cereal food crop in the world, is ranked first in terms of cultivated area and second in terms of production

among cereal crops worldwide (FAO, 2020). With a projected total production of 765.53 million tons in the 2018/2019 cropping season, wheat was grown on approximately 218.22 million hectares across the world (USDA, 2022). To feed the anticipated 9.1 billion people by 2050, the production of cereals should increase by 1 billion tons per year, according to estimates (FAO 2015). Wheat of both the tetraploid (*Triticum durum* Desf.) and hexaploid (*Triticum aestivum* L.), is the most important cereal crop in Ethiopia, ranking third in total production (17%) next to maize (*Zea mays* L.) and tef (CSA, 2002). Wheat covers a total arable land of 110,434 ha with an average productivity of about 8.4 qt ha-1, which is below the national average (14.4 qt ha-1). To satisfy the rising demand for food supply, the current situation requires an increase in crop productivity (Iqbal et al., 2017). Water scarcity is a severe drawback that limits the area under cultivation and agricultural output in arid and semi-arid regions all over the world. Abiotic stresses have a significant impact on wheat grains' protein content, which alters the quality of baked goods (Zorb *et al.,* 2018).

Wheat is the second most important food crop showing greater genetic variability and diversity among the different genotypes in every corner in the world. India is the 2nd largest producer of wheat followed by China contributing 13.53% of the area to the total area of the world under wheat cultivation. The wheat production and productivity of India were 93.48 mt and 3.02 t ha-1 respectively in 2013–2014. Climate change is projecting perilous effects on agricultural production all over the world. In India, wheat production is to be reduced by about 6% and 18% by 2020 in timely sown irrigated and late sown wheat respectively as reported by Shetty et al., 2013. Short or long-term exposure to heat and drought stresses can significantly affect the growth and yield, particularly at sensitive growth stages (Prasad *et al.,* 2008). (Hoegh Guldberg *et al.,* 2018) estimated that due to climate change and 2-3°C rise in global temperature, losses of wheat yield will increase by up to 30% by 2050. As water availability is a major factor limiting crop production in many parts of the world, management practices focus on enhancing the water use efficiency of future crops (Majid *et al.,* 2017). The world's significant wheat exporters are the US, Canada, Australia, The Black Sea Region, Europe, and Argentina. These countries are expected to see minimal, or even negative, population growth towards 2050. In contrast, population growth will be strongest in the countries of the tropical and subtropical regions where little wheat is grown. It is believed that, even without projecting large imports by China, the world wheat trade will likely double by 2050, to 240 million tons or more (Weigand 2011).

In addition, Heat stress tolerance traits must be incorporated into crops anticipating the climate-changing scenario (EL-Shawy *et al.,* 2017). Because of global climate change, drought, salinity, and heat stresses are the major abiotic constraints for wheat productivity and adversely affect the yield and quality by altering the physiological activity (Kosina *et al.,* 2007; Otu *et al.,* 2018; Yassin *et al.,* 2019). Wheat is a monocot plant, that belongs to the tribe *triticeae*, under the grass family *poaceae*. Wheat grain has a high nutritional value with 70-75% starch, 14% water, 8-20% proteins, 2-3% non-starch polysaccharides, 2% lipids, 1.6% minerals, antioxidants, etc., and is the main staple food crop for a huge world population (Goesaert *et al*., 2005). To cope with climate change, the selection of suitable varieties, and optimization of irrigation water are needed to maintain sustainable food supplies (Bernardi, 2011).

Grain yield is a multigenic trait and evaluations of the inter association between grain yield & other yield traits and among themselves simplify effective selection outlines to expand the yield. Chances of succeeding improvement in any crop plant depend greatly on the scale of genetic variability. The magnitude of genotypic variability on yield and its constituent characters affect selection approaches to be accepted by the plant breeders as it is the heritable constitute the variability. In bread wheat varieties, many Agro-morphological characters (plant height, flag leaf length, flag leaf hairs on auricle, flag leaf waxiness of blade, flag leaf attitude, spike length, spikelet number, peduncle waxiness, spike density, peduncle length and awns presence) reveal main variability (Rehman *et al*., 2009).

Heritability is a quantity having an analytical role in determining phenotypic variability in the traits which can be ascribed to genetic differences in plant breeding experiments. The phenotypic data can be recorded directly whereas the genetic values are deliberated by the appropriate analyses. The amount of phenotypic value that will be inherited by the succeeding generation is estimated by genotypic values (Rehman & Alam 1994). Heritability shows a direct association with the response of selection known as genetic advance (GA) or progress. The higher the value of heritability, the simpler is selection event (Khan *et al.,* 2007). The evaluation of heritability depends on the environment along with the nature of the test population (Gemechu, 1996). Therefore, it is necessary to perform a more detailed examination using an analysis of the path coefficient, a statistical technique to decompose the correlation coefficient into direct and indirect effects, which facilitates the estimation of the contribution of each component (independent cause) to the grain yield (dependent variable) (Suleiman *et al.,* 2014). Path coefficient analysis was used by plant breeders to help identify traits that could be useful as a selection criterion for improving crop yield. The path coefficient divides

correlation coefficients into direct and indirect effects within the correlation system of traits. When there is a genetic correlation between two traits, the selection for one of them will produce a change in the other trait. In other words, the response of the correlation to the act of selection will take place Bishnoi et al., (2023).

The combination of high heritability and high genetic advancement provides an effective selection conditions (Larik *et al.*, 2000). The information on the correlation that occurs between significant characters may simplify the proper clarification of results and offer a basis for the development of more efficient breeding programs. Phenotypic correlation is the association between recorded two values or traits, while genotypic correlation is the natural association between characters. The information on the interrelationship of many yield constituents is of supreme significance for a plant breeder for making the results regarding range conditions. It is required to increase the productivity of wheat to fulfill the increasing demand for food. The knowledge of genetic variability, heritability, correlation coefficient, and other related parameters can help in further increasing the grain yield. Their understanding can direct the selection of yield-related traits and their relationship with the yield. The estimation of variability, heritability and correlation coefficient done in this study could be utilized for further increase in grain yield of the wheat genotypes (Verma *et al.,* 2019).

Using molecular markers for evaluation of the genetic diversity offers numerous advantages over traditional phenotypic traits. They are faster, cheaper (depending on the type of marker), could be detectable in all tissues and with a high level of polymorphism. So, microsatellites or simple sequence repeats (SSR) markers have been extensively used for genetic diversity study, genetic mapping and marker-assisted selection (Batool *et al.,* 2018). Microsatellites or simple sequence repeats (SSRs) provide an efficient tool in diversity studies for identifying the degree of genetic similarity. Due to their high rate of polymorphism, co-dominant character, selective neutrality, distribution across the genome and cost and labor efficiency, microsatellite markers are suitable for detecting allele frequency within the population and for assessing population structure (Khaled *et al.,* 2015).

The use of molecular markers for the evaluation of genetic diversity is very common. At present, SSR is one of the most promising molecular markers which can identify or differentiate genotypes within a species. SSRs are ubiquitously interspersed in eukaryotic genomes and can find applications as highly variable and multi-allelic PCR-based genetic markers. The high level of polymorphism and easy handling has made SSRs extremely useful for different

applications in crop improvement (Gupta *et al.,* 2009). Many researchers have extensively used microsatellites or simple sequence repeats (SSR) for the study of genetic diversity study, genetic mapping and marker-assisted selection (Ren *et al.,* 2012, Ahmed *et al.,* 2013, Batool *et al.,* 2018).

The study of the genetic diversity based on molecular markers and evaluation of morphological traits related to yield and quality traits may be helpful in the selection of appropriate parents for the development of economically useful new pedigrees with a broad genetic base, while evaluation of germplasm may be helpful to tap useful variation such as for grain yield, quality and rare alleles related to biotic and abiotic stresses resistance (Lopes *et al.,* 2015).

Across the globe, many researchers have explored the variability, correlation, path analysis and genetic diversity in wheat. Very few studies have investigated genetic variability and molecular characterization in wheat genotypes in Bangladesh. Here, phenotypical data were required for assessment of genetic variability correlation and path analysis respectively and as well as SSR markers are the best choice because of having some advantages over other markers to check the morpho-molecular divergence. Considering these facts, the research hypothesis might be the characterize the wheat cultivars and advanced lines using phenotypical data and SSR markers. This will produce suitable genes and genotypes together with diverse study for future breeding.

Therefore, the specific objectives of the present study were

i. To study the yield performance of newly developed advanced lines.
ii. To study the genotypic and phenotypic correlation among the traits and select the suitable yield contributing traits.
iii. To study the molecular level of variation among the advanced lines using SSR markers.

CHAPTER II

REVIEW OF LITERATURE

Review of related literature is a necessity in the sense that it provides scope for reviewing the stock of knowledge and information relevant to the proposed research. Wheat is an important cereal crop in many countries and has been an extensively studied crop for different aspects of improvement. It has a wealth of information on various aspects of crop breeding and research. Even though a few numbers of works have been done in Bangladesh and the whole world related to this research. However, the limited number of works so far published are mentioned here along with other related works. Some of the important findings relevant to the present study are reviewed below.

2.1 Germplasms found in the regions of the world

Germplasm exchange exerts a major influence on the genetic constitution of cultivated durum wheat and accounts for a large proportion of the genetic variability observed in many breeding programs (Rezgui *et al.,* 1993). Another study (Jaradat, 1991) reported a close relationship between genetic variability and agroecological characteristics (day length, rainfall, temperature) suggesting that landraces are adapted to specific environmental conditions.

Ethiopia is regarded as the center of origin of wild Emmer wheat, *Triticum turgidum* (L)

Thell. ssp. dicoccoides (Korn) Thell. Emmer is the tetraploid progenitor of cultivated durum *Triticum turgidum* ssp., *Turgidum* L. (Vavilov, 1951; Harlan, 1981).

Durum wheat is currently cultivated in the Middle East, North Africa, Europe, the Soviet Union, and the United States. More than eight million hectares of durum are currently grown with improved durum cultivars derived from International Maize and Wheat Improvement Center (CIMMYT) germplasm (Sharma *et al.*, 2021).

To avoid these potential problems, introducing favorable alleles from different gene pools into adapted germplasm is considered a successful strategy to enhance grain yield and other desirable agronomic traits (Spillane *et al.*, 2001). Individual plant selection in early segregating generations for grain yield and other agronomic traits has met with limited success. For highly inherited traits like kernel weight and plant height, early selection has proven successful in bread wheat (Jones *et al.*, 2005).

However, investigators do not agree with the value of prediction based on early-generation performance, particularly for quantitatively inherited traits. Several authors have attributed the lack of phenotypic selection for grain yield based on single plants in F_2 segregating populations to environmental- interactions and to the differential competition 5 between genotypes within populations (Sneep, 1977; McVetty and Evans, 1980). Wricke and Weber (1986) argued that the limited number of seeds available, spaced planting, and preponderance of dominance effects are the principal factors influencing the lack of efficiency of selection in F_2 or F_3 segregating populations; however, it was noted that delayed selection may seriously limit the genetic gain for quantitatively inherited traits such as grain yield (Sneep, 1977). This is due to the decrease in the frequency of high-yielding genotypes in advanced generations. Valentine (1979), working with barley, suggested that no opportunity for selection for grain yield in the earlier generation should be lost. He pointed out that selection for grain yield. Between families on a plot basis in F_3 and subsequent generations was more efficient than between individual plants in the F_2 generation. Nevertheless, he indicated some genetic advances in grain yield can be achieved by selecting plant height, kernel number, and kernel weight among spaced plants in the F_2 generation. Genetic diversity among parents is considered essential for the long-term improvement of grain yield in durum wheat. Abundant genetic variation for grain yield was found to occur among crosses between parental cultivars from different gene pools in durum wheat (De Pace *et al.,* 1985).

2.2 Environmental effects of wheat production

The environmental change affects the grain yield of wheat because the varieties of diversified origin are under cultivation at the commercial level by the farmers. The yields fluctuate widely as a result of their interaction with various environmental factors because yield is a complicated quantitative parameter and is the product of several contributing factors affecting grain yield directly or indirectly. Wheat production can be increased through the development of productive cultivars that better adapt to various agro-climatic conditions and also resist all types of biotic and abiotic stresses. Selection for grain yield improvement can only be effective if sufficient genetic variability is present in the breeding material. Individual plant selection in early segregating generations for grain yield and other agronomic traits has met with limited success. For highly inherited traits like kernel weight and plant height, early selection has proven successful in bread wheat (Islam *et al.,* 1985a). Evidence of environmental influence,

interplant competition, and compensation effects of yield components has been reported by several authors on wheat (McVetty and Evans, 1980).

2.3 Methods used in the breeding program

Several breeding methods have been used with cereals including the pedigree method, bulk populations, composite crosses, normal and reciprocal crosses, and single-seed descent. There has been little research to establish which one is the most effective method because strict comparisons are not easy to make and any conclusions would be subject to many conditions. A main difference between the methods is the generation in which selection begins. The pedigree procedure has been the most widely used method during segregating generations of self-pollinated crops. It is simply a family tree where inherited gene patterns are found transferred from parents to progenies. Besides, it can trace a faulty gene from where it is coming. Breeders of self-pollinated crops are confronted by two major problems: the first is identifying the best parental combinations that will result in the highest percentage of desirable progeny, and the second is the effectiveness of selection in early generations. A successful selection should maintain a high level of genetic variability among the lines of the population after successive generations. The pedigree method has commonly been found quite useful for handling segregating populations even with the presence of additive genes. The efficiency of selection depends not only on the selection method but also on the heritability of different traits. High heritability followed by high genetic advancement indicates the scope for their improvement through selection (Meitei *et al.*, 2014). Phenotypic correlations between all pairs of characters were calculated to establish any association that exists between them, and which might be useful in the planning of the breeding program. A normal cross is where a normal cross is done but, in a reciprocal, cross the sex of parents is reversed characters controlled by karyogenes are not affected. In the case of cytoplasmic inheritance, results are affected which helps to evaluate maternal inheritance. If there remains any faulty gene or a superior one, it will be carried into the next generations. In the pedigree method, selection for desirable combinations of characters starts in the generation. Progenies of the selected plants are grown and further selection is practiced in subsequent generations until or by which time homozygosity may be reached (Poehlman, 1959). The effectiveness of this method relies on the measurement or observation. The high amount of labor and land required is a disadvantage (Elliot, 1958). In contrast, in the bulk population and the single seed descent methods, selection

is delayed until an advanced such as (Hayes, Immer, and Smith, 1955). The generation chosen theoretically should depend on the no. of genes by which the parents differed but in practice, this is not known (Florell, 1929). The idea is to allow the population to become relatively homozygous before selection takes place.

The wheat variation and the variability of forms and cultivars, are significantly different for one or more traits and allow the cultivation of wheat in different regions of the world. Kosovo agroecological conditions are optimal for the production of wheat, while average yields brought 3.5 to 4 tons ha^{-1} (Zogaj, 2012). The cultivar is the basic unit in yield development, such a phenomenon 'scientists used to create different varieties of wheat, when they even created the opportunities for increased yields per unit area. The evaluation of genetic variation and identification of genotypes with high production capacity in F_3 generation, enable the beginning of the wheat breeding selection in early generations. The selection of genotypes of wheat in a generation is the initial stage of selection as a way of developing genotypes with desirable characteristics.

The variation of wheat for genetic production potential contributes to creating the genotypes with higher yields, if the selection is based on traits of the spike production capacity that have higher heritability (Larik *et al.,* 1999). carried an investigation of three wheat varieties (PKB Talas, BG Merkur, and PKB Lepoklasa) carried out at the experimental field and laboratory of the Institute PKB Agro-economic, for two years 2009 and 2010. Correlations between morphological and production traits of plants number of shoots, number of spike per 1 square meterlets per spike, number of grains per spike, 1000 grain weight, and grain weight per spike, were studied. Correlations were observed separately for three Institute PKB Agroeconomic varieties. Studies of associations between plant height and grain yield and yield components have produced mixed results. Small but positive associations between plant height and kernel per spike, kernel weight, and grain yield were computed from a collection of

868 genotypes of durum wheat from Jordan (Jaradat, 1991). Nevertheless, Bakheit *et al.,* (1989) found that plant height was positively associated with biological yield and number of spike per 1 square meterper plant but was negatively associated with kernel weight and harvest index in diallel crosses of eight durum cultivars. The association between plant height and grain yield, however, was inconsistent over generations.

Comparing near-isogenic semi-dwarf and tall lines of durum wheat, Joppa (1973) reported that semi-dwarf durum wheat is characterized by a greater tillering capacity but lower test weights than taller types.

Aycicek *et al.,* (2006) conducted the trials across two locations. Over two years, the correlation coefficients and path analysis were calculated between grain yield and yield components of 20 bread wheat genotypes. A positive and significant correlation was found between yield and plant density, plant height, grain number per spike, grain weight per spike, and 1000 kernel weight. Grain yield was negatively and significantly correlated with time to heading. Positive direct effect of plant height and grain weight spil.c.-1 and negative direct effect of time to heading associated with significant correlation with grain yield suggested that these yield components may be a good selection, criteria to improve the yield of wheat genotypes.

2.4 Yield and yield contributing traits of wheat cultivars

Wheat (*Triticum aestivum* L.) is cultivated worldwide primarily/mainly as a food commodity. It is one of the top dominant crops in the world as well as in Bangladesh. During recent years, many approaches have been made towards improvement in the yield potential/capacity of wheat crops. Planting time is one of the most critical considerations and agronomic factors involved in producing high yields of small grain cereal crops like wheat. Several studies documented the effect of plant date on wheat performance. Early or late sowing increases the risk of yield losses Ehdaie *et al.,* (2001). Similarly, biomass accumulation, grain yield, the number of spike per 1 square meterm2, and 1000 grain weight of wheat were increased with early sowing (early November) over late sowing (December) Aftab *et al.,* (2004). Like reproductive growth, the vegetative growth of wheat is also related to planting times. Sowing of wheat from 1st to 15th November produces maximum productive tillers m^{-2} Akhtar *et al.,* (2006). The short-growing season cultivars like Khyber-87 performed better in terms of grain yield in late sowing. These cultivars produce more productive tillers due to better germination and good stand establishment Sattar *et al.,* (2010). Another important and economic consideration for increasing wheat productivity is the effective use of nitrogen fertilization. Nitrogen fertilization is the most important factor in front of wheat agronomists for achieving high-yield targets. A sufficient supply of nitrogen at optimum planting time also resulted from good quality and vigorous seed. Different indicators are used for the measurement of seed quality and vigor like higher net assimilation, grain filling rates, and duration are mostly contributed to 1000 grain weight. Sowing dates and different nitrogen levels have also

produced a significant effect on 1000-grain weight. Sowing from 1st-15th November produced the highest 1000-grain weight Akhtar *et al.,* (2006). Similarly, nitrogen application increased seed development (grain filling rate and duration) which ultimately produced the highest grain Waraich *et al.,* (2007). Biological yield is also strongly linked with sowing dates and nitrogen levels. The previous research showed that early or normal sowing dates (1st-10th November) are more effective for biological yield as compared to late sowing e.g. December Aftab *et al.,* (2004).

Gholizadeh *et al.,* (2014) reported that the identification of effective yield-related traits is the main aim of each breeding program. In this research, the relationship between wheat seed yield and its components under saline conditions was investigated by using four statistical procedures including; simple correlation, multiple linear regression, stepwise regression, and path analysis. The experiment was conducted under saline field conditions at the research field of the National Salinity Research Center (NSRC) at Yazd, Iran based on a randomized complete block design with three replications. The electrical conductivity of irrigation water was 10 $ds.m^{-1}$. The multiple statistical procedures that have been used in this study indicated that biological yield, harvest index, and chlorophyll content were the most effective variables influencing seed yield. Based on the results, it seems that high yield of wheat plants under saline field conditions can be obtained by selecting breeding materials with high biological yield, harvest index, and chlorophyll content. This suggests that evaluation for salt tolerance among genotypes can be based on the genetic diversity in biological yield, harvest index, and chlorophyll content.

2.5 Importance of genetic diversity analysis of wheat

Wani *et al.,* (2022) studied that every successful crop development 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 challenges. 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 which prevalent in the wild wheat relatives into modern wheat in order to increase genetic gains and made agricultural systems climate resilient.

Begna *et al.,* (2021) reported that both agricultural improvement and the existence of crop plants in nature are based on genetic diversity. It was obvious that genetic diversity provides opportunities for cultivars to be improved with desired features, including both farmer and breeder-preferred traits. In the early days of agriculture, genetic variety was employed to provide enough food for subsistence. Since the climatic factors changed and negatively affected the natural growth and development of crop plants, plant breeders were concerned with developing climate-adapted cultivars. The presence of desired alleles was closely correlated with the presence of genetic diversity, which aids in the development of varieties that were climate-adaptable. Breeding strategies could broaden the genetic variety of stress tolerance and increase yield under stress by incorporating the adaptive natural genetic variations. The development of prospective variations such as resistant to new illnesses, insect pests, high heat, and extreme cold also heavily relied on genetic variation. The development of variations for particular qualities, such as the tolerance of abiotic and biotic stress and quality improvement was facilitated by genetic variety.

Swarup *et al.,* (2021) executed that multiple global concerns that impact food security, Production, accessibility and nutritional quality were faced by plant breeders. Climate change had a significant problem for plant breeders so it was important to create environmentally adaptable crop cultivars in response to quick climatic changes. To incorporate genetic variation into commercially available cultivars, plant breeders used a variety of crop genetic resources, breeding instruments, and techniques. Breeders exploited genetic variety to create novel cultivars with enhanced agronomics, such as increased yield and resistance to biotic and abiotic stress, as well as to enhance the nutritional value of foods for a population that was expanding globally. The crucial task of strategic integration of new genetic diversity was carried out by plant breeders while maintaining crucial economic traits of individual crops, such as relative maturity (for corn, *Zea mays L.*), fruit type (for tomatoes, *Lycopersicon esculentum Mill.*), plant type (for lettuce, *Lactuca sativa L.)* and habitat type (for canola, *Brassica napus L.)* that were highly specialized for particular consumer preferences or market needs. Genetic diversity incorporated crop enhancement and also produced new cultivars for upcoming breeding programs.

Mourad *et al.,* (2020) carried out an important crop such as wheat *(Triticum aestivium L.)* grown all over the world and had a complex genome. Understanding the genetic diversity among worldwide wheat genotypes was crucial for finding the parents with relevant agronomic traits that might be exploited in the various breeding projects. Understanding genetic variation

was also helpful in breeding research like genome-wide association studies (GWAS), marker-assisted selection (MAS), and genomic selection.

Mukhopadhyay *et al.,* (2016) carried out a study of genetic diversity to categorize an individual or population concerning other individuals or populations. It measured the degree of genetic variation within a population, a key component of biodiversity. The presence of various alleles in the gene pool and consequently various genotypes within populations were reflected in the genetic variation among individuals. From an individualistic and population standpoint, genetic variety was very important. All phenotypic plasticity is dependent on an organism's genetic diversity, which also enables it to adapt and evolve under various environmental stressors. There were primarily three lines of evidence that support the impact of ecology on genetic diversity. For the creation of relevant methods in conservation biology as well as many other applicable domains, knowledge regarding genetic diversity was required. Genetic variety was seen to be essential for a species' ability to evolve from a fundamental evolutionary perspective.

Bhandari *et al.,* (2017) reported that the foundation for plant survival in nature and crop enhancement is dependent on genetic diversity. Plant breeders could create new and improved cultivars with desirable qualities including farmer and breeder-preferred attributes (high yield potential, large seed, pest and disease resistance photo-sensitivity, etc.) by using genetic diversity and plant genetic resources. The presence of genetic diversity which might be found in wild species, closely related species, breeding stocks, mutant lines, etc., may act as a source of beneficial alleles and help the plant breeders to create varieties that are more tolerant to climatic change. There was found genetic variability within and between crop plant species. So, breeders could choose superior genotypes to either utilize as parents in hybridization programs or to use directly as new varieties. To achieve heterosis and produce transgressive segregants, two parents must have genetic diversity. Breeders could create varieties for specific traits like quality enhancement and resistance to biotic and abiotic challenges with the help of genetic diversity. Additionally, it was made easier to generate new lines for non-traditional purposes such as bio-fuel types of sorghum, maize, etc. Diversity was crucial for crop plants' ability to adapt to many habitats, particularly those with shifting climatic circumstances.

Govindaraj *et al.,* (2015) studied that plant genetic resources (PGRs) which were used to develop crops in order to meet future global concerns relating to food and nutritional security could be used to capture and conserve plant genetic variation. Plant breeders created new and improved cultivars with desirable qualities including traits favored by both farmers and

breeders. Crops must have genetic diversity in order to continue to progress since it gives breeders alternatives for creating new kinds and hybrids. The ability of populations and species to endure across evolutionary time in the face of shifting environments dependent on genetic diversity. Breeders can more efficiently use genetic resources with less pre-breeding activity by knowing about the population structure, allelic richness, and diversity parameters of germplasm.

Mujeeb-Kazi *et al.,* (2013) studied that three *Triticeae* gene pools contained genetic diversity that was essential for the improvement of every crop. 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 was 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.

Caliskan *et al.,* (2012) concluded that genetic diversity was crucial for the survival of a species since it allowed for the essential adaptability to the current biotic and abiotic environmental conditions as well as the ability to change the genetic makeup to adapt to environmental changes. The genetic diversity in plants showed how much genetic variation was there in plant populations. A complete analysis and evaluation of plant genetic variation as well as the identification of genes impacting commercially significant features were now made possible by the growing accessibility of PCR-based molecular markers.

2.6 Dependability on heritability for successful breeding

The heritability of a trait within a population is the proportion of observable differences in a trait between individuals within a population that is due to genetic differences. Factors including genetics, environment, and random chance can all contribute to the variation between individuals in their observable characteristics. Heritability measures the fraction of phenotype variability that can be attributed to genetic variation. High heritability values indicate the genetic relationship between parents and progeny. The heritability of a character describes the

extent to which it is transmitted from one generation to the other generation. Information on heritability parameters and the evaluation of the relationship between important quantitative traits is very useful in any effective wheat breeding program. It is well well-known fact that quantitative characters depending on the nature of gene action are differently influenced by environmental variation. Moreover, the extent of heritability of quantitative traits is negatively correlated with the environment. Fasoulas (1973) attempting to improve response to selection for grain yield in wheat, proposed adjustment of individual F_2 plant data to neighboring plants.

Populations were superior to Fasoulas's approach. They concluded that an opportunity exists to select for grain yield under spaced-planted conditions that were conducive to higher single-plant yield and a greater range of variability for this trait. Recently, Hill et al. (1991), argued that F_3 row performances can be predicted from F2 data in oats using adjusted single plant data. However, they found that unadjusted plant observation was more reliable in predicting F3 rows, particularly for highly inherited traits such as plant height. Most of the results ascribed the observed genetic variability to additive gene action where a high frequency of transgressive segregation was obtained for the components of yield. However, both additive and dominant types of gene action were found to control the expression of grain yield.

However, investigators do not agree with the value of prediction based on early-generation performance, particularly for quantitatively inherited traits. Several authors have attributed the lack of phenotypic selection for grain yield based on single plants in F_2 segregating populations to environmental- interactions and to the differential competition 5 between genotypes within populations (Sneep, 1977; McVetty and Evans, 1980). Wricke and Weber (1986) argued that the limited number of seeds available, spaced planting, and preponderance of dominance effects are the principal factors influencing the lack of efficiency. of selection in F_2 or F_3 segregating populations; however, it was noted that delayed selection may seriously limit the genetic gain for quantitatively inherited traits such as grain yield (Sneep, 1977). This is due to the decrease in the frequency of high-yielding genotypes in advanced generations. Valentine (1979), working with barley, suggested that no opportunity for selection for grain yield in the earlier generation should be lost. He pointed out that selection for grain yield. Between families on a plot basis in F_3 and subsequent generations was more efficient than between individual plants in the F_2 generation. Nevertheless, he indicated some genetic advances in grain yield can be achieved by selecting plant height, kernel number, and kernel weight among spaced plants in the F_2 generation.

Ethiopia is regarded as the center of origin of wild Emmer wheat, *Triticum turgidum* (L)

Thell.ssp. dicoccoides (Korn) Thell. Emmer is the tetraploid progenitor of cultivated durum *Triticum turgidum* ssp. *turgidum* L. cony. durum (Desf). (Vavilov, 1951; Harlan 1981). Durum wheat is currently cultivated in the Middle East, North Africa, Europe, the Soviet Union, and the United States.

Individual plant selection in early segregating generations for grain yield and other agronomic traits has met with limited success. For highly inherited traits like kernel weight and plant height, early selection has proven successful in bread wheat (Islam et al., 1985a).

Tahmasebi *et al.,* (2013) grouped fifteen wheat lines with one wheat cultivar based on agro-morphological characters to assess the genetic diversity and interrelationship of traits in some of the promising wheat lines and determine tlik.; traits effective on grain yield, The field experiments were carried out in 2009-2010 at the Research Station located at Shirvan Chardavol, Ilam. Analysis of variance showed significant differences between all traits except grain filling period and number of grains per spike. Among all traits, higher genetic coefficient variation and phenotypic coefficient variation were observed for grain yield, number of spike per 1 square meters, and 1000-grain weight. Correlation analysis showed the 1000-grain weight, plant height and number of spike per 1 square meter had a positive and significant relationship with grain yield. In regression analysis (stepwise method), 1000 grain weight, plant height, and number of spike per 1 square meter remained in the final model (R2- 0.73). Cluster analysis based on squared Euclidean distance and Ward's method categorized the lines into three groups. The information on diversity and relationships among the agro-morphological traits will be helpful to breeders in constructing their breeding populations or lines and implementing selection strategies.

Zarei *et al.*, (2013) investigated to identify agronomic and morphophysiological traits related to drought tolerance in 410 F_5 families of durum wheat under rainfed conditions. The relationships between the durum grain yield and the related traits under drought conditions were evaluated using several multivariate analyses, including simple correlation, path-coefficient analysis, stepwise regression, factor analysis, and cluster analysis. For path coefficient analysis, traits were partitioned into two groups: traits with the primary effects on grain yield and traits with the secondary effects on grain yield via their effect on the primary traits. Path coefficient analysis indicated that at the primary level biomass had the highest positive direct effect on grain yield (0.584). In contrast, at the secondary level, the highest

direct effect on the number of seeds per spike (0.517) belonged to the spike length (0.517), and the mean grain weight (0.218) was related to peduncle length. Factor analysis revealed four factors. The first factor accounted for about 0.2735 of the total variation NV as strongly associated with the number of spike per 1 square meterpc; plant, the number of tillers per plant, biomass, and grain yield. Principal component and cluster analysis exhibited strong relationships between grain yield, above-ground biomass, the number of tillers per plant, and the number of spike per 1 square meterper plant.

2.7 The Yield performance analysis of wheat

Zerga *et al.,* (2017) evaluated that In Ethiopia, several improved bread wheat *(Triticum aestivum L.)* varieties have been released by different research centers to see the adaptability and performance of different bread wheat genotypes. However, nothing has been done at Gurage Zone and therefore a total of twenty-five bread wheat *(Triticum aestivum L.)* genotypes were evaluated for adaptability and performance at Gurage Zone in two different environments. The genotypes were grown in a randomized complete block design. Data were collected on 13 agronomic characters. Based on the mean separation, the highest grain yield (4941.70kg/ha) was recorded from Hoggana, while the lowest yield (1983.30 kg/ha) was obtained from Kakaba and Sofumar at Fereziye. At Kotergedra, the highest grain yield of (5366.7 kg/ha) was also recorded from Hoggana, and the lowest yield of (3166.7 kg/ha) was obtained from Kakaba. The highest above-ground biomass was also obtained from Hoggana at both locations 10850.00 kg/ha and 16992.00 kg/ha at Fereziye and Kotergedra respectively. Statistically, the variety Hoggana gave the highest tillers per plant and spikes per plant at both locations those are positive contributions to grain yield.

Hazari *et al.,* (2017) conducted a study in that field experiment undertaken to study the Performance of wheat genotype under different irrigational approaches in the Terai agro-ecological condition during the *rabi* season of 2016. The experiment was conducted at the experimental field of Uttar Banga Krishi Viswavidyalaya with six different wheat genotypes under two irrigation management practices i.e. irrigated and restricted irrigated condition, in Factorial Randomized Block Design comprising three replications. The result of the experiment revealed that the highest economic yield was found in HD 2967 under both the irrigated and restricted irrigation conditions (47.1 q ha-1 & 26.8 q ha-1 respectively). Significant differences were found in all the growth and yield attributing characters in two irrigational conditions. Water stress was the key reason for the reduction of yield. By observing

SPAD values HD 2967 and DBW 39 can maintain their greenness than other genotypes in later growth stages. It can be a very useful character for screening wheat genotypes for drought tolerance.

Laghari *et al.,* (2010) conducted a study in which Comparative yield performance studies of fourteen new advanced lines of wheat (*Triticum aestivum* L.) were conducted along with two local check varieties (Sarsabz and Khirman). Some morphological (grain yield, plant height, 1000-grain weight, spike length, number of spike per 1 square meterlets/spikes, number of grains/spikes, main spike yield) and phenological data (days to ear emergence, maturity period, and grain filling period) were studied. To determine the possible relationship of yield-associated traits with grain yield, correlation coefficient studies were also performed. Results revealed that two genotypes BWM-3 and MSH-36 could mature earlier within 126 days than check varieties; hence suitable for late planting. Genotypes showed different responses for various agronomic traits. NIA-8/7, MSH-3 and MSH-5 had higher 1000 grain weight (>46.0g) whereas, BWM-3, BWQ-4, and MSH-3 produced significantly higher grain yields (4898, 4897, and 4686 kg/ha respectively) than other entries. Two genotypes BWQ-4 and BWS-78 had a greater number of grains (>70) per spike. A positive correlation of grain yield was observed with 1000-grain weight and main spike yield.

Arain *et al.*, (2018) conducted a study that was undertaken to comparatively analyze the extent of genetic diversity for various quantitative traits among the wheat material exotic to Pakistan, received from CIMMYT (The International Maize and Wheat Improvement Center), Mexico. Nineteen advanced lines from the Semi-Arid Wheat Yield Trial (SAWYT) were studied along with a local cultivar, considered a control (NIA-Amber). Data were recorded on nine important agro-morphic traits. The compared genotypes differed significantly ($p \leq 0.05$) in the studied traits, where line V6 produced the highest mean grain yield (6,049 kg ha-1) and maximum 1,000- grain weight (45.0 g). Other lines, V19, V17, and V2, also showed superiority in yield (5,723, 5,150, and 5,067 kg ha-1, respectively). Days to heading established a significant positive association with days to maturity ($r = 0.7995$), plant height ($r = 0.3168$), spike length ($r = 0.2696$), and spikelets per spike ($r = 0.4391$). The important yield associated trait, 1,000-grain weight, had a highly significant positive correlation ($r = 0.6833$) with grain yield. Cluster analysis for various quantitative traits showed important information about genetic diversity for the studied traits among wheat genotypes.

2.8 The variability and path analysis of wheat

Bishnoi *et al.,* (2023) conducted a study that was undertaken to Direct phenotypic selection in wheat improvement programs requires preliminary knowledge of traits association degrees. In this study, a field experiment was conducted on the wheat crop in two different conditions (irrigated and drought), To determine the degree and direction of the association between grain yield and its attributing characters. The experimental findings indicated that correlation coefficients showed a highly significant and positive association between grain yield and harvest index followed by above-ground biomass. However, other traits have a significant indirect impact on grain yield through the harvest index and above-ground biomass. According to this, choosing genotypes with higher yields would be more effective if selection were based on these traits. The minimum yield reduction irrigated conditions were observed for the genotypes WH1127, WH1164, WH1105, WH1080, IC498438, EC609554, and EC609575. In the light of the fact that these genotypes have a higher yield potential under moisture stress conditions and could be utilized as donors in bread wheat improvement programs for drought tolerance.

Nukasani *et al.,* (2013) evaluated that Hundred fourteen pre-breeding lines of wheat were evaluated for variability. High values of GCV and PCV were recorded for tillers meter-1 and grain yield meter-1, whereas moderate values were for the rest of the traits. High heritability coupled with high genetic advance was recorded for traits plant height, tillers m-1, grain yield m-1, grain wt spike-1, and spike length indicating that these characteristics are governed by additive gene effects and directional selection for these traits would be more effective. Correlation and path analysis studies revealed that, three characters, tiller number m-1 >grain weight spike-1 >number of grains spike-1 in that order are the most important as these exhibited positive and strong association and maximum positive direct effects on grain yield. Therefore, while imparting the selection in wheat characters tiller number m-1, grain weight spike-1 and number of grains spike-1 must be given preference.

Anwar *et al.,* (2009) evaluated that Correlation coefficients were computed for grain yield plant-1, tillers plant-1, spikelets spike-1, 1000 grain weight, spike length, days to heading, days to maturity, and plant height from the F1 crosses developed from four lines and three testers including their parents. The results revealed that grain yield plant-1 was positively and significantly correlated with the number of tillers plant-1 and days to maturity at the genotypic level but non-significantly correlated at the phenotypic level. Days to maturity had a positive

genotypic correlation with grain yield plant-1, number of tillers plant-1, and 1000-grain weight. Days to maturity and tillers plant-1 had a positive direct effect on grain yield plant-1 also. Therefore, more days to maturity and more tillers plant-1 would be important selection criteria for improved grain yield plant-1 in the breeding material studied.

Bhardwaj *et al.,* (2023) conducted a study that was undertaken to indicate that grain yield per plant(g) has a positive and highly significant correlation with biological yield per plant (g), number of productive tillers per plant, harvest index (%), number of grains per spike. Path analysis identified biological yield per plant and number of productive tillers per plant as important direct components for grain yield per plant (g). As per the analysis of variance, variations due to blocks and checks were found to be significant for all the traits. Ten clusters were formed according to Non- hierarchical Euclidean cluster analysis and the maximum inter-cluster was recorded between clusters 6 and 8 (86.478), followed by clusters 4 and 8 (83.180). Early maturing genotypes were contained in cluster 1 whereas cluster 4 contained the genotypes which gave the maximum grain yield per plant. High-yielding genotypes identified were: DBW- 187, DBW-303, DBW-222, HD-3226, and HS-240.

Sethi *et al.,* (2023) evaluated that High heritability along with high genetic advance as a percent of the mean was recorded for grains/spike, grain yield/plot (g), number of effective tillers per meter, spike length (cm), biological yield per plot(g/plot) and harvest index (%). Correlation analysis explained that grain yield per plothad a significant and positive correlation with the number of effective tillers/meters, spike length (cm), number of spike per 1 square meterlets/spikes, number of grains/spikes, 1000-grain weight (g), biological yield/ plot (g) and harvest index (%). Harvest index, spike length, days to heading, and 1000-grain weight (g) were found directly correlated with grain yield (g/plot). Path analysis showed that harvest index and biological yield had a positive effect on grain yield followed by the direct effect of spike length, days to 50% heading, and 1000-grain weight thereby indicating that these were the main contributors to the grain yield.

Verma *et al.,* (2019) evaluated that Phenotypic data was recorded for nine characters *viz*. plant height, number of effective tillers per meter, spike length, number of grains per spike, number of spike per 1 square meterlets per spike, 1000-grain weight, grain yield per plot, and biological yield per plot. The highest value for GCV and PCV was observed for biological yield per plot(18.09 & 19.18 % respectively). Heritability for broad sense (h2) and genetic advance as 5% of mean were highest for harvest index (96.50 %) and biological yield (35.17%),

respectively. Grain yield per plotwas highly and significantly correlated with all the traits except plant height and grain yield per plot. Path analysis revealed that biological yield per plothad the maximum direct positive effect on grain yield per plotand harvest index, whereas, all other traits except plant height contributed indirectly towards grain yield per plotvia biological yield per plot.

Mohanty *et al.,* (2016) reported that the Wide spectrum variability was revealed for all the characters studied which could provide scope for improvement through selection. High Phenotypic Coefficient of Variation (PCV), Genotypic Coefficient of Variation (GCV), heritability, Genetic advance (GA), GA (%) of mean was observed in the characters viz., proline content, no. of tillers plant-1, no. of spikes plant-1, no. of grains spike-1 and yield plant-1 under late heat stress condition. The genotypes viz. GW 2011-403, WSM 135, GW 2008-153, RAJ 4358, RAJ 4362, J-07-47 & UP 2783 were found to be high yielders that may exploit heat tolerance. Yield plant-1 was found to have significant positive association with plant height, no. of tillers plant-1, no. of spikes plant-1, spike length, no. of spikelets spike-1, no. of florets spike-1, no. of grains spike-1, floret fertility and grain weight spike-1 at both genotypic and phenotypic levels; while a significant negative correlation was observed with chlorophyll-a and total chlorophyll content at both the levels and chlorophyll-b at genotypic level only.

2.9 Molecular marker-assisted wheat breeding

Kumar *et al.,* (2021) evaluated that in crop plants, the use of molecular markers had been widespread for assessing genetic diversity, examining population structure, and examining marker-trait associations (MTAs) for critical features. Without the creation and application of molecular markers, crop plant genetic improvement would not been conceivable.

Rana *et al.,* (2021) reported the major reasons why molecular markers had been widely used in wheat breeding programs and the reason was linkage and QTL maps. Major genes and quantitative trait loci (QTLs) were shown on chromosomal areas using linkage maps. Since they were becoming more widely available, molecular markers linked to numerous efficient resistance genes have been successfully applied in numerous wheat breeding programs. Molecular markers identified important traits that were important for the plant breeding program. Because of their reliability marker systems like SSR, STS, SCAR, DArT, and SNPs were frequently utilized.

Al-Ashkar *et al.,* (2020) explained the use of molecular marker technologies in genetic diversity studies, marker-assisted selection (MAS), paternity analysis, quantitative trait loci mapping (QTL), cultivar identification, phylogenetic connection analysis, and genetic mapping during the evolution of wheat. Identifying polymorphisms was largely dependent on DNA fingerprinting markers. Molecular markers can be effectively employed for phylogenetic identification and variation.

Randhawa *et al.,* (2013) studied numerous wheat cultivars and made them available for commercial production in Canada, molecular markers were used to help select qualities such as disease resistance, agronomic suitability, and quality. For rust resistance (leaf, stem, and stripe rust), orange wheat blossom midge, high grain protein concentration, *Fusarium* head blight, and common bunt marker-assisted breeding were frequently utilized in most wheat breeding programs. In order to test durum wheat ergot resistance markers were also used. A marker for the over-expression of the Bx7 high molecular-weight glutenin component was used to select for increased gluten strength. The disease TTKS (Ug99), which poses a severe danger to the world's wheat crop, was a set of stem rust races that were related, and resistance genes were being pyramided against them via marker-assisted breeding. Shortly, more efficient molecular breeding of wheat would be possible with tightly linked diagnostic markers and high-throughput genotyping.

Kesawat and Das Kumar (2009) reported that molecular markers were the most widely used genetic markers because of their relative abundance. Molecular markers have grown in significance during the last few decades in biotechnology and genetics research. Molecular markers provided important information for crop plant development in studies of genetic variability and genetic interactions among many accessions of different plant species. The use of molecular markers has become a valuable technique for describing genetic material.

2.10 Importance and uses of SSR markers in wheat

Swetha *et al.,* (2022) reported 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 several 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 identify 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.

Mallick *et al.,* (2022) studied a tetraploid *T. turgidum var. durum* cultivar with *LrTrk/YrTrk* resistance genes that were transferred using SSR markers in a marker-assisted backcrossing operation.

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

Shafi *et al.,* (2021) reported 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.

Jamil *et al.,* (2020) reported that numerous crops, including helianthus, barley, soybean, wheat, date palm, rice, and maize, can be fingerprinted using SSRs.

Sönmezoğlu *et al.,* (2018) explained that SSR markers were beneficial, trustworthy, and useful in genetic characterization investigations of bread wheat drought resistance. Additionally, it found that microsatellites were useful for genotype identification, genetic resource characterization, and genotyping related to drought.

Kumar *et al.,* (2016) conducted a study for determining the degree of genetic similarity in diversity research, 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.

Tomar *et al.,* (2016) After conducting a correlation analysis of morphological and agronomic traits under drought stress, it was discovered that 31 wheat genotypes have a phylogenetic link through SSR markers.

Faheem *et al.,* (2015) studied that SSR markers were being used in genome-based genetic diversity research to examine drought tolerance.

Ramya *et al.,* (2015) studied 24 current wheat genotypes with physiological and genetic characteristics that had been documented for use in breeding research to test their resistance to heat and drought.

Bousba *et al.,* (2012) reported that SSR was a good molecular marker for genetic characterization research in wheat due to its multialleleic nature, chromosomal specificity, high polymorphism ratio, and widespread distribution throughout the wheat genome.

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.

Guichoux *et al.,* (2011) reported that because of their high mutation rates, high polymorphism levels, and outstanding reproducibility, SSR were extensively employed markers in population genetics, functional genomics, association mapping, diversity analysis, comparative mapping, and gene tagging research. Since SSR markers were co-dominant, they could reveal whether the targeted locus is homozygous or heterozygous. They were used in population genetic analyses across the board because they were present in every eukaryotic genome. Golabadi *et al.,* (2011) identified QTLs with yield-trait and competent traits, such as 1000 grain weight and harvest index, using microsatellite markers.

Chapter III

MATERIALS AND METHODS

The experiment was conducted at the experimental farm and also at the laboratory of the Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200. The materials and methods of this study are presented in this chapter under the following sub-headings.

That experiment was carried out at Optimum sowing time (15th November 2022)

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 May 2022-2023.

3.2 Climate

The experimental field belongs to the subtropical climate where the rainfall is heavy in the Kharif season (March–August) and scantily 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

The experimental field was 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 is sandy loam under the order Inceptisol. The experimental field had a well-organized irrigation and drainage system.

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 9. During the soil sample 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

The experiments were laid out in Randomized Complete Block Design (RCBD) with three replications. The total land area was 17.5m ×18m (315m^2) shown in figure 1.

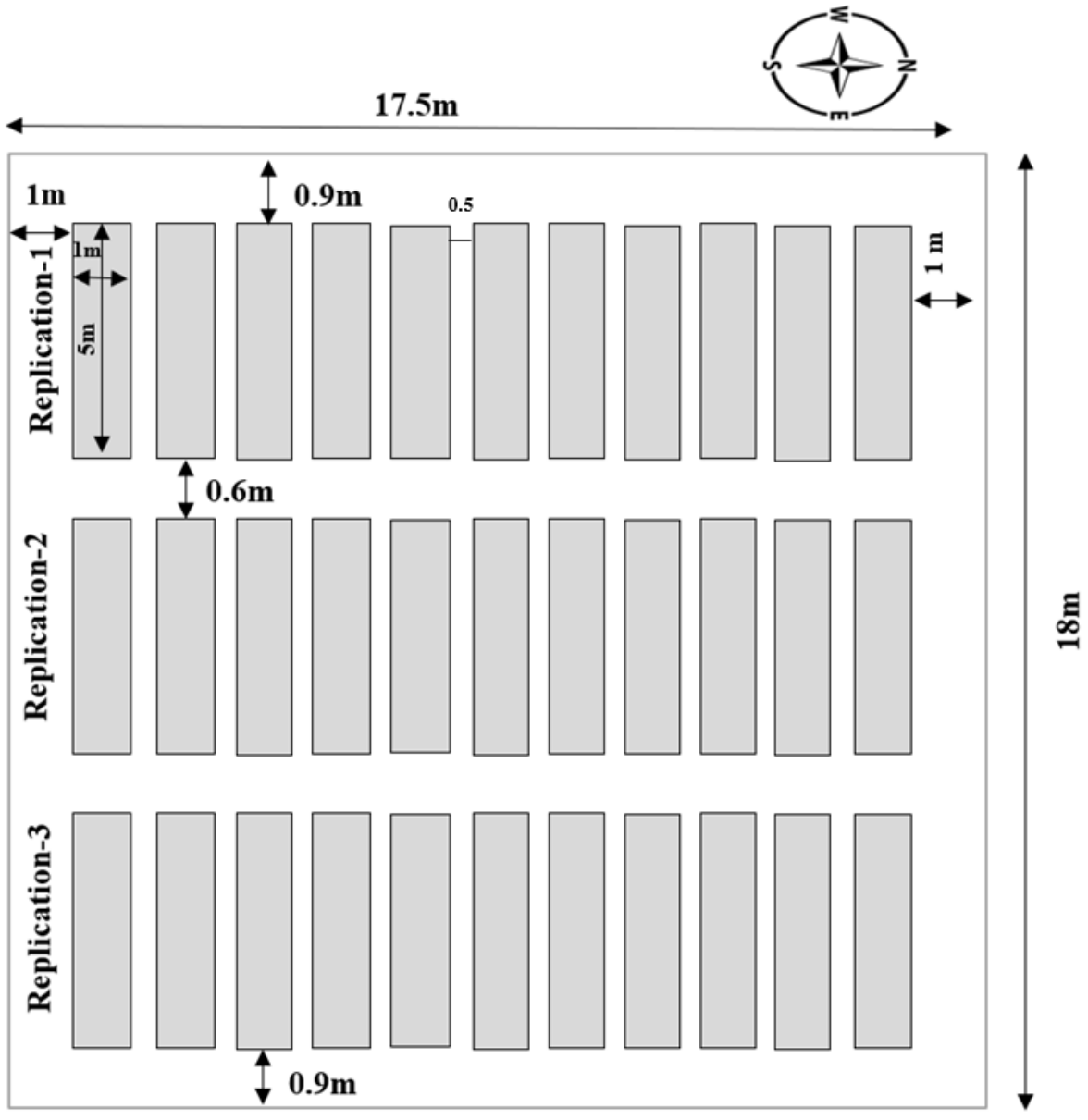

Figure 1: Field experimental layout of an experiment of wheat for the Rabi season 2022-2023

3.6 Experimental materials

In the experiment, have experimental materials which 10 advanced lines of wheat including 1 check varieties a total of 11 lines were used for the preliminary yield trial conducted at both the Hajee Mohammad Danesh Science & Technology University, Dinajpur field, and Molecular Breeding Laboratory of Genetics and plant breeding department were used as experimental materials and given in Table 1.

Table 1: Plant genetic materials with their name used in this experiment

Sl No.	Entry Name	Source
01	HSTUW 1	Heat tolerance advance line, The Department of Genetics and Plant Breeding, HSTU, Dinajpur.
02	HSTUW 2	
03	HSTUW 3	
04	HSTUW 4	
05	HSTUW 5	
06	HSTUW 6	
07	HSTUW 7	
08	HSTUW 8	
09	HSTUW 9	
10	HSTUW 10	
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, 12g seed is required per square meter as followed 120kg/ha seed rate. Since the experimental plot was 5 m^2 and 5 lines for each plot. So, the seed was required per plot 5×12= 60gm per plot. A total of 180 gm seeds were required for 3 replications of each line respectively.

3.8 Layout of the Experiment

The experiment was laid out in a randomized complete block design (RCBD) with 3 replications. The layouts of the experiment were prepared 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 row-to-row distance was maintained at 0.25m. The area of the individual experimental plots was 5 square meters.

Figure 2: The experimental layout of wheat genotypes after germination period

3.9 Experimental duration

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

3.10 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 crossed-plowed several times followed by laddering to obtain a good tilth. Weed and stable were removed and finally the desired soil tilth for planting wheat seed. The experimental plot was partitioned into unit plots in accordance with the design of the experiment mentioned earlier. The recommended doses of well-rotten cow dung, Dolomite, and also Dhaincha were

incorporated as green manure, and chemical fertilizers as indicated in the next were mixed with the soil of each unit plot.

3.11 Sowing of seeds

The seed was sown on the field at 2-2.5cm depth and sown in the field on 15th November, 2022. The seeds were sown singly for each line and maintained a distance of 12 cm row to row, plot to plot was 0.5 m, and replication to replication was 0.6 m respectively.

3.12 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/315m2 of N, P, K, S, Zn, Mg, and B, respectively. The doses and method of application of fertilizer are shown in Table 2.

Table-2. Doses and methods of application of fertilizers in a wheat field

Manure and fertilizers/ Manure	Doses (kg/432m^2)	Method
Cow dung	150	Basal application
Dolomite	31	Basal application
Urea	7.90	3 split doses (1/3rd during FLP and rests are top dressed, 1/3rd at 21 DAS, and 1/3rd not applied yet)
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 sulfate	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)

3.13 Seed treatments

Seeds were treated with a fungicide Provax 200 wp @ 3g/kg to protect emerging seedlings from soilborne fungal disease.

3.14 Planting of Seeds in the Field

The wheat seed was planted in lines, row to a row distance of 12 cm, and seeds were planted in the well-prepared plot on 15th November 2022. When the seedling started emerging in beds, it was carefully observed.

3.15 Intercultural operation

After the emergence of the seedling, various inter-cultural operations were accomplished for better growth and development of the Wheat seedling as follows:

3.15.1 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 the 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.

Figure 3: The wedding operation is conducted in the experimental field

3.15.2 Irrigation

Irrigation was provided at the knee stage, pre-flowering stage, and milking stage at 20, 27, 35, 45, 55, and 65 days after planting (DAP) for six times of proper growth and development of the plant in each block.

3.16 Harvesting, threshing, and cleaning

The crops were harvested when 50% maturity was obtained. At this stage, the spike turned into a brownish color, and the photosynthate never goes up to the spike as far. The maturity of crops was determined when 80% of the wheat seeds became physiologically mature. The plants were harvested separately. The seeds were collected separately for future research and other plants were harvested generally for yield. The harvested crop was bundled, properly tagged, and then brought to the threshing floor. The yield of grain was recorded after thoroughly drying in the sun.

Figure 4: Morphological maturation in the experimental field

3.17 Measurement of morphological traits

Randomly ten (10) wheat plants from each plot were select through eye estimation. After randomly selected plant were used for data recorded per genotypes in three replications on 9 morpho-physiological characters viz., Days to 50% heading, Days to 50% maturity, Average grain weight per spike (g), Average grain per spike (g), 1000 grain weight (g), Number of spike

per 1 square meter per 1 square meter, Average spike length (cm), Average plant height (cm), Yield per plot(g) consist of 1 square meter, 4 square meters, and 5 square meters.

A brief outline of nine (9) morpho-physiological data recording procedures is given below:

3.17.1 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.

3.17.2 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 centimeters (cm).

3.17.3 Days to 50% Maturity

Days to 50% maturity were calculated by spike color turning into a brownish color. The photosynthate was not able to go up to the grain. The grain became harder and produced a stony sound when it was crushed.

3.17.4 Number of spike per 1 square meter per 1 square meter

Spikelets containing any food material or not were counted as total spikelets per panicle.

3.17.5 Spike length (cm)

Panicle length was recorded from the basal node of the rachis to the apex of each panicle.

3.17.6 The average 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 recorded for further study purpose.

3.17.7 Average grain weight per spike

Ten (10) number of dried and matured spikes (g) from selected plants in randomly were collected from each plot. Then average grain weight per spike was weighed by using an electrical balance for further study purpose.

3.17.8 1000 grain weight (g)

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

3.17.9 Yield per plot(g)

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

- 1 square meter
- 4 square meters
- Total yield (5 square meters)

3.18 Study of molecular diversity utilizing Microsatellite/SSR markers

11 Lines including 10 advance lines and 1 check varieties were used for PCR-DNA based assay by using SSR markers was conducted at both BWMRI molecular breeding laboratory and molecular breeding laboratory of Genetics and Plant Breeding Department of HSTU. 4 different markers were employed in the experiment of genomic DNA extraction. The markers were illustrated with their sequence and phenotypic characters are given in the following Table 3.

Table 3: Microsatellite/Simple Sequences Repeat (SSR) Markers

SI	Primer name		Sequence	Annealing Temperature ◦C
1	TaBarc101	F	GCTCCTCTCACGATCACGCAAAG	
		R	GCGAGTCGATCACACTATGAGCCAATG	62
2	Xwmc112	F	TGAGTTGTGGGGTCTTGTTTGG	
		R	TGAAGGAGGGCACATATCGTG	58
3	Barc20	F	GCGATCCACACTTTGCCTCTTTTACA	
		R	GCGATGTCGGTTTTCAGCCTTTT	59
4	Gwm495	F	GAGAGCCTCGCGAAATATAGG	
		R	TGCTTCTGGTGTTCCTTC G	56

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

3.18.1 Microsatellite/Simple Sequences Repeat (SSR) Markers

A total of 4 microsatellite (SSR) markers primer pairs (Sigma Aldrich, Germany) were selected for the genetic diversity analysis of the 11 wheat genotypes from HSTU-developed heat-tolerant advance lines and 1 check variety from BWMRI as shown in Table 1. 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, chromosomal position, and other relevant information to these markers are published on the Grain Genes website (http://www.wheat.pw.usda.gov). The annealing temperature and primer sequences to SSR markers are shown in Table 3.

3.18.2 Data collection for molecular characterizations

Data collected from the young wheat leaves; the collection procedures are given below.

3.18.3 Sampling and lyophilization of leaves

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

3.18.4 Genomic DNA extraction

3.18.4.1 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 5: Crushed the leaves in porcelain mortal

3.18.4.2 DNA extraction using modified CTAB method

The leaf sap was collected into a 2ml centrifuge tube and 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 before warming). The tubes were taken out of the water bath and left them 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 aqueous phase was removed with a wide-bore pipette. The aqueous phase was transferred to a

clean tube 1.5 ml then add 2/3 or 1 volume of isopropanol and mixed gently to precipitate the nucleic acids. At this stage, the samples were stored at -4°C overnight. 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. 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 6: DNA extraction procedure using modified CTAB method

Table 4: Chemicals for DNA extraction

Sl. No	Component	Amount
1.	CTAB	800 µl
2.	Chloroform	600 µl
3.	Isopropyl alcohol	200 µl
4.	Ethyl alcohol	70%

Table 5: Chemicals for CTAB buffer Preparation

SL No	Components	Amount
1.	D-sorbitol (1M)	36.436g
2.	Tris-HCl (1M)	24.23g
3.	EDTA	148.9g
4.	NaCl (1M)	58.44g
5.	CTAB (Hexadecyl trimethyl-ammonium bromide)	20ml
6.	Sarcosin	20ml
7.	ddH_2O	--

¾ pieces of leaves were collected in the Eppendorf tube and dried for 2-3 days in silica gel.

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

800 µl of warmed CTAB buffer was added to each tube and vortex thoroughly. Incubated the samples at 65^0C for 45 minutes and every 10 minutes mixed them gently by inversion.

Took the tubes out of the water bath and left them at room temperature for 5 minutes.

Added 600 µl of chloroform phenol/ chloroform isoamyl alcohol (24:1)

Mixed them by inversion for about 2 minutes (100 times) gently until two layers are mixed.

Centrifuged them for 4000 rpm at room temperature for 20 minutes.

The aqua phase was removed with a wide-bore pipette.

The aqua phase was transferred to a clean tube (1.5 ml) then 2/3 or 1 volume of cold isopropanol was added and mixed gently to precipitate the nucleic acids. At this stage, we stored the samples at -4^0C overnight.

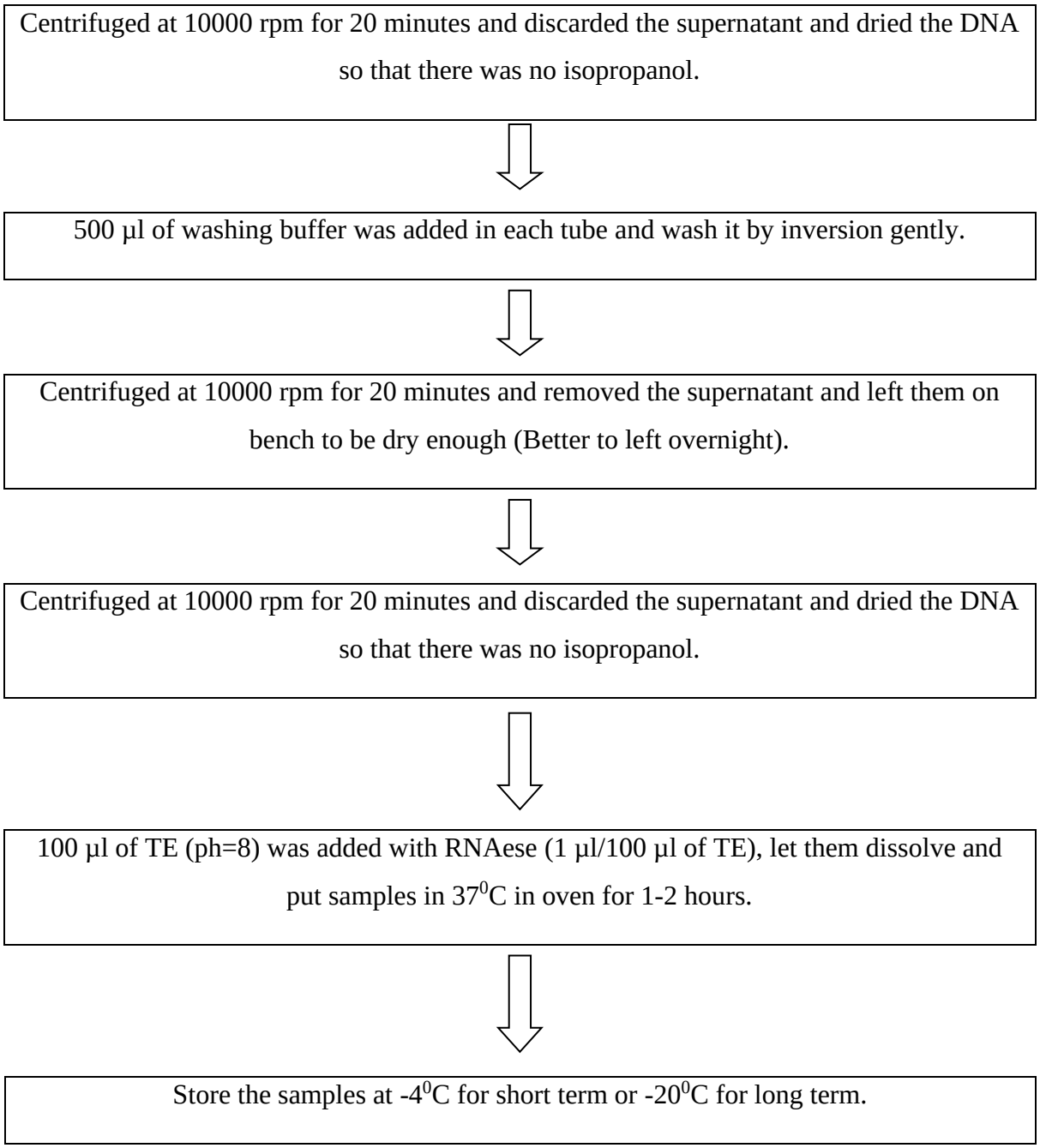

Figure 7: Showing the protocol for DNA extraction (Modified CTAB method)

3.18.4.3 DNA quantification

The quality of the extracted DNA samples was also checked prior to PCR amplification through (Thermo Fisher Scientific, USA).

Figure 8: DNA Quantification using a NanoDrop^TM1000 Spectrophotometer

3.18.4.4 DNA dilution

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

3.18.4.5 PCR amplification

PCR amplification was performed in 10µl volume using a variety of Thermal Cycler. The reaction mixture contained 10 m/M/l Tris-HCL, 50m/M/L KCL, 2 m/M/L MgCl2, 200umol/l of each dNTPs, 250 n/M/l of each primer, 20 to 40 mg genomic DNA, and 0.25 U Taq DNA polymerase. The PCR amplification was as follows: one cycle of 95^0C for 3 minutes,35 cycles of 94^0C for 1 minute, 50 to 60^0C for 1.5 minutes and 72^0C for 1 minute, and a final extension at 72^0C for

10 min. Reaction products were mixed with one-fifth volume of loading buffer (100 Mm/l EDTA Ph 7.0, 10 Mm/l Tris–HCL Ph 7.5, 5% Ficoll 400, 0.05% bromophenol 0.05% xylene cyanol) and 8µl were loaded vertically for electrophoresis 8% denaturing polyacrylamide gels

in 1×TBE (90 mm/l Tris borate pH 8.3, 2 mm/l EDTA) at 50 Ma for 2 to 3 h. Gels were then silver stained and photographed. 4 selected SSR markers were used in this study

Table 6: Chemical Reagents used for PCR, based on SSR markers of wheat genotypes

Name	Amount	Origin	Observation
1. Acrylamide	500g	Bio Basic Canada INC	
2. Ammonium persulfate (APS)	100g	SISCO Research Laboratories Pvt Ltd. (SRL)	
3. N, N Methylene-bis acrylamide (BIS)	100g	Lobal chemic	
4. TEMED	100ml	ROTH	
5. BORIC ACID	500g	ROTH	
6. TBE Buffer	1L	SIGMA	
7. EDTA Buffer		SIGMA	
8. Ethanol+ Acetic Acid+ water	200ml+10ml+2000ml		Washing for 15 minutes
9. $AgNO_3$ + water	2gm+1000ml		Washing in a dark mood for 15 minutes
10. NAOH+ formaldehyde +water	30gm+8ml+2000ml	Sisco research laboratories	Washing for 5 minutes depends on color
11. Tris (hydroxymethyl aminomethane	500g	Merck	

12. Deionized water	
13. DNA ladder	2µl
14. Loading dye	1µl
15. Master mix	8µl
16. Mineral oil	3µl

3.18.4.6 Electrophoresis separation and silver nitrate staining

The reaction products were then run into polyacrylamide gels in 1×TBE buffer (90 mM Trisborate pH 8.3, 2 mM EDTA) at 50mA for 2 to 3 h (Wang *et al.*, 2007). The polyacrylamide gel 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 with $AgNO_3$ (0.2%) solution for 15 min. After a brief rinse (10 sec) with 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 the reaction.

Table 7: Chemicals used for preparation Polyacrylamide gel (8%)-1000 ml

SL NO	Component	Amount
1.	Acrylamide (40%)	200 ml
2.	BIS (2%)	107 ml
3.	10X TBE	100 ml
4.	dd H2O	593 ml

3.18.4.7 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 I th allele.

The population structure analysis was done using the Bayesian clustering method in structure software version 2.3.4 (Pritchard *et al.,* 2000). The population structure was inferred through the model-based program, STRUCTURE 2.3.3, and was implemented with a burn-in period of 10,000 and run length of 100,000 Markov Chain Montecarlo number (MCMC) replications for a population range from *K* = 2 to 14 (Pritchard *et al.,* 2000). The final *K* value was determined using the Evannos Δ*K* method and Ln probability data was used to detect the presence of genetically distinct populations using a graphical approach (Evanno *et al.,* 2005). Every band was considered a single locus. All the scorable Loci were considered for the generation of a bivariate 1-0 data matrix and genetic distances (GD) among the genotypes were estimated using an 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) Chong and Xia (2018).

3.19 Statistical analyses

Data was entered in Microsoft Excel (Ms-excel) 2016 and was analyzed using R-Studio 4.2.2 (Strimmer K, 2022), STAR (Gulles *et al.,* 2014), OPISTAT (Sheoran *et al.,* 1998) and AGRISTAT software. ANOVA was calculated through STAR software (Gulles *et al.,* 2014); correlation analysis was performed with R software using the 'Agricolae' package (De Mendiburu, 2015), and mean performance and genetic parameters were estimated *via* the package "Variability". Path coefficient analysis was conducted by OPISTAT (Sheoran *et al.,* 1998), and lastly, combining ability and heterosis analysis done by AGRISTAT developed by Dr. N. Manivannan.

3.19.1 Equations of Analysis

The following formulas were used to calculate different parameters of the experiment:

$$\textbf{Yield (t/ha)} = \frac{\textbf{Adj.field wt.} \times 10{,}000 \times (100 - \textbf{MC}) \times 0.8}{\textbf{Plot area} \times 85}$$

Where,

MC= Moisture content and

0.8= Shelling percent

Adjusted field weight = CF× Plot yield$\mathbf{CF= \frac{M-0.3\times(N)}{M-N}}$

Where,

M= Optimum number of plants,

N= No. of missing plants,

0.3= Constant and

CF= Correction factor

3.19.2 Analysis of variance

The analyses of variances for most of the characters under consideration were performed by the F variance test. The significance of the difference among the means was evaluated by Tukey's test for interpretation of results. Here the mean values of all the characters were evaluated and analysis of variance was performed by the 'F' test. To test the differences between genotypes Tukey's test was performed.

Table 8: The structure of ANOVA

Sources of Variation	Degrees of freedom (df)	Mean sum of squares (MS)	Expected MS
Replication (r)	(r-1)	Mr	
Genotype (g)	g-1	Mg	$\sigma e^2 + r.\sigma g^2$
Error	(r-1) (g-1)	Me	σe^2
Total	(rg-1)		

Where, r = number of replications, g = number of genotypes

3.19.3 Estimation of genotypic and phenotypic variances

Genotypic and phenotypic components of variance were computed according to the formula given by Lush (1940) and Chaudhary and Prasad (1968).

Genotypic variance (V_g) = $\frac{G.MSS-E.MSS}{r}$

Error variance (V_e) =E.MSS/r

Phenotypic variance (Vp) = V_g+V_e

Where,

G.MSS= Mean sum of squares for genotypes

E.MSS= Mean sum of squares for error

r= Replication

3.19.4 Genotypic and Phenotypic Coefficient of variation

Genotypic and phenotypic coefficients of variation were computed according to the formula given by Burton and Devane (1953).

Genotypic coefficient of variation (GCV) = $\sqrt{\frac{Vg}{\bar{x}}} \times 100$

Phenotypic coefficient of variation (PCV) = $\frac{\sqrt{Vp}}{\bar{x}} \times 100$

Where,

Vg = Genotypic variance

Vp = Phenotypic variance

$\bar{x}$ = General mean of the character

GCV and PCV were classified low, moderate or high following Desmukh *et al.* (1986) as shown below:

0-10% : Low

10-20% : Moderate

20% and above : High

3.19.5 Estimation of heritability

Broad sense heritability was estimated as the ratio of genotypic variance to the phenotypic variance and was expressed in percentage (Hanson *et al.,* 1956).

Heritability (h^2) = $\frac{Vg}{Vp} \times 100$

Where,

Vg= Genotypic variance

Vp = Phenotypic variance

The heritability percentage was categorized by the following scale given by Robinson *et al.* (1949).

0-30% : Low

30-60% : Moderate

60% and above : High

3.19.6 Estimation of genetic advance (GA)

Genetic advance (GA) was computed according to the formula given by Johnson *et al.* (1955) which is as follows:

Genetic advance (GA) = $i.h^2.\sqrt{Vp}$

Where,

i = Selection differential (2.06) at 5 percent selection intensity

h^2 = Broad sense heritability

The genetic advance (GA) as percent of mean was categorized as low, moderate or high as suggested by Johnson *et al.* (1955) which is as follows:

0-10% : Low

10-20% : Moderate

20% and above : High

3.19.7 Genetic advance over mean (GAM)

Genetic advance over mean was computed by the formula given below:

GAM = $\frac{GA}{\bar{x}} \times 100$

Where, $\bar{x}$ = mean of the population

3.19.8 Estimation of genotypic and phenotypic correlation coefficients

The genotypic and phenotypic correlation coefficients between yield and its different contributing characters were estimated by the following formula (Johnson *et al.*, 1955).

Genotypic correlation coefficient, $r_{g1.2} = \frac{Cov.g_{1.2}}{\sqrt{\sigma^2 g_1 \times \sigma^2 g_2}}$

Where,

$Cov.g._{1.2}$ = Genotypic covariance between the variables x_1 and x_2.

$\sigma^2 g_1$ = Genotypic variance of the variable X_1

$\sigma^2 g_2$ = Genotypic variance of the variable X_2

Phenotypic correlation of coefficient, $r_{p\ 1.2} = \frac{Cov.ph_{1.2}}{\sqrt{\sigma^2 ph_1 \times \sigma^2 ph_2}}$

Where,

$Cov.ph_{1.2}$= Phenotypic covariance between the variable X_1 and X_2

$\sigma^2 ph_1$ = Phenotypic variance of the variable x_1

$\sigma^2 ph_2$ = Phenotypic variance of the variable x_2

3.19.9 Estimation of path coefficient

Estimation of path co-efficient Correlation co-efficient components of different yield attributes with grain yield were portioned in to components of direct and indirect effects by path co-efficient analysis followed by Singh and Chaudhary (1985); and Dabholkar (1992). Assuming 12 independent (X_1, X_2, ..., and X_{12}) and one dependent variable (Y), path co-efficient can be represented as follows:

$r_1y = P_1.\ y + r_{1.2}\,P_2.\ y + r_{1.3}\,P_3.\ y + r_{1.4}\,P_4.\ y + r_{1.5}\,P_5.\ y + r_{1.6}\,P_6.\ y$ ..... $r_{1.12}\,p_{12}.y$

$r_2y = r_{2.\,1}P_1.y + P_{2.}\,y + r_{2.3}P3.\ y + r_{2.4}\,P_4.\ y + r_{2.5}\,P_5.\ y + r_{2.6}\,P_6.\ y$ ..... $r_{2.12}\,p_{12}.y$

$r_3y = r_{3.1}P_1.y + r_{3.2}\,P_2.\ y + P_{3.}\,y + r_{3.4}\,P_{4.}\,y + r_{3.5}\,P_{5.}\,y + r_{3.6}\,P_6.\ y$ ..... $r_{3.12}\,p_{12}.y$

$r_4y = r_{4.1}P_1.y + r_{4.2}\,P_2.\ y + r_{4.3.}\,y + P_4.y + r_{4.5}\,P_5.\ y + r_{4.6}\,P_6.\ y$ ..... $r_{4.12}\,p_{12}.y$

$r_5y = r_{5.1}P_1.y + r_{5.2}\,P_2.\ y + r_{5.3}\,P_3.\ y + r_{5.4}\,P_4.\ y + P_5.\ y + r_{5.6}\,P_6.\ y$ ..... $r_{5.12}\,p_{12}.y$

....

$R_{13}y = r_{13.1}P_1.y + r_{13.2}\,P_2.\ y + r_{13.3}\,P_3.\ y + r_{13.4}\,P_4.\ y + r_{13.5}\,P_5.\ y + r_{13.6}\,P_6.\ y$ ..... $p_{12}.y$

Where, $P_1.\ y$, $P_2.\ y$, $P_3.\ y$, ..., $P_{12}.y$ = Path co-efficient of the variables x_1, x_2, x_3, ..., x_{12} on the variable Y respectively.

The indirect effect of a particular character through other characters is worked out by multiplying direct paths and particular correlation coefficients between those characters, respectively.

CHAPTER IV

RESULTS AND DISCUSSION

The experiment was conducted during the Rabi season from October 2022 to April 2023. The experiment's outcome was obtained to analyze the variances, mean comparison, correlation, and path analysis of 11 genotypes viz. 10 advance line and 1 check variety (BARI Gom 32). The analyses were done on yield and yield contributing characters viz. days to 50% heading, days to 50% maturity, grain weight per spike, average grain per spike, 1000 grain weight, number of spike per 1 square meter, average spike length, average plant height, yield per plot.

However, many studies on the Analysis of variance (ANOVA), Mean performance, Genetic diversity, Genotypic and Phenotypic Correlation, Genotypic and Phenotypic Path analysis, and Direct and indirect effect of Path coefficient analysis, with selected heat tolerant advance lines in wheat. Preliminary yield trial of newly developed heat-tolerant advance line in wheat studied in the Genetics and Plant Breeding department research, Hajee Mohammad Danesh Science and Technology University, Dinajpur from 2022 to 2023. The results have been presented and discussed under the following headings.

4.1 Analysis of variance for different quantitative characters

The analyses of variance (ANOVA) revealed that there were highly significant differences among the genotypes ($p \leq 0.01$) for all the traits under study *viz.*, Days to 50% heading, Days to 50% maturity, Grain weight per spike, Average grain per spike, 1000 grain weight, Number of spike per 1 square meters, Average spike length, Average plant height, yield per plot indicating the presence of adequate genetic differences among the selected advanced lines. The ANOVA (Table 9) glimpses for different traits revealed that the mean sum of squares due to genotypes was significant for all the characters under study, indicating substantial variability in the material taken under study. This variability indicates ample scope for improvement of traits under study. Many workers, including Drikvand *et al.,* (2013), Bhutto *et al.,* (2016), and Khan *et al.,* (2017), and Phougat *et al.,* (2016) reported high variability for different traits in wheat. Variations across genotypes were identified owing to changes in the genes carried by various genotypes and the interaction of distinct gene combinations owned by different genotypes with the environment to which the genotypes were exposed.

The results of ANOVA for the quantitative traits of the tested genotypes are presented in Table 9. Thus, there is a good opportunity to select a better-advanced line to improve the grain yield for further multilocation yield trials. The mean squares against three replications were non-significant for all the characters studied except average plant height, number of spike per 1 square meter, and yield per plot. The mean squares of all the advanced wheat genotypes were found significant for all the characters, the studied coefficient of variation is simply a measure of the dispersion of the variable.

4.2 Mean performances of different yield and yield contributing traits in wheat genotypes

The mean values having the same letter(s) did not significantly differ at a 5% probability level. Analyses of the mean performances of nine yield contributing characters of the data are presented in Table 10 to Table 12.

Table 9: Analysis of variance (Mean squares) derived from RCBD one-factor model on morphological characters in Wheat genotypes

Sl. No	Character	Source of variation with the mean sum of square			Coefficient of Variation (%)
		Replication (df 2)	Genotype (df 10)	Error (df 20)	
01	Days to heading	0.576	99.024 ***	3.242	2.84
02	Days to maturity	0.091	76.691 ***	1.091	0.94
03	Average plant height	156.82 *	200.92 ***	4.82	2.11
04	Grain weight per spike	0.070	0.156 ***	0.022	9.04
05	1000 grain weight	15.475	80.487 ***	9.019	7.17
06	Number of spike per 1 square meter	4020.2	8907.6 ***	1014.5	7.73
07	Average spike length	0.692	1.412 **	0.283	5.53
08	Average grain per spike	4.485	62.971 ***	6.641	6.43
09	Yield per plot	1195376 **	157678 ***	25145	6.44

*, **, *** indicates significance at 5%, 1%, 0.1% Level of Probability and NS= non-significant respectively

Table 10: Mean performance of morphological character of wheat genotypes

Genotype	Days to heading	Days to maturity	Grain weight per spike
HSTUW 1	51.67e	102.33d	1.34c
HSTUW 2	55.67d-e	105.67c	1.32c
HSTUW 3	65.33a-b	107.33c	1.41b-c
HSTUW 4	69.33a	113.00b	1.75a-c
HSTUW 5	69.00a	112.00b	1.88a
HSTUW 6	68.33a	111.67b	1.85a-b
HSTUW 7	68.00a	113.00b	1.84a-b
HSTUW 8	62.00b-c	112.67b	1.80a-b
HSTUW 9	64.33a-c	121.33a	1.84a-b
HSTUW 10	65.00a-c	113.00b	1.74a-c
BARI Gom 32	60.00c-d	107.00c	1.42b-c
Range	**51.67 – 69.33**	**102.33 – 121.33**	**1.32 – 1.88**
Mean	**63.52**	**110.82**	**1.65**
SE (±)	**1.04**	**0.60**	**0.09**
HSD	**5.31**	**3.08**	**0.44**

Here, SE= Standard error, HSD= honestly significant difference,

4.2.1 Days to heading

The days to 50% heading (Days) were significant for all the characters (Figure 9). However, the average range of Days to heading was found at 51.67 (HSTUW 1), to 69.33 (HSTUW 4) where the mean was 63.52±1.04 shown in Table 10. Days to 50% heading and plant height also contributed directly to the development of grain yield per plant but indirect effects through other traits made these associations non-significant, therefore these traits would not be nominated for effective selection in grain yield improvement (Anwar *et al.,* 2009).

4.2.2 Days to maturity

The average range of days to maturity (Days) was depicted at 121.33 (HSTUW 9) to 102.33 (HSTUW 1) with a mean value of 110.82±0.60 (Table 10). However, the higher number of tillers per plant and more days taken to 50% maturity were the nominated traits for effective selection of genotypes for grain yield improvement. Similar to the result of Anwar *et al.,* (2009).

4.2.3 Grain weight per spike

In the case of Grain weight per spike (g), The range of the mean values of grain weight per spike was 1.32 g for HSTU-developed advanced line HSTUW 2 to 1.888 g for HSTUW 5 (Figure 13). The Mean values of the parameter were 1.65 g with the SE value ±0.09 shown in Table 10. A similar result was reported by Ojha *et al.,* (2018).

Table 11: Mean performance of morphological character of wheat genotypes (Contd.)

Genotype	Average plant height	Average spike length	Average grain per spike
HSTUW 1	94.57e-f	8.58c	34.13b-c
HSTUW 2	92.35f	9.10b-c	39.43a-b
HSTUW 3	94.08e-f	8.80c	44.50a
HSTUW 4	116.33a	9.90a-c	39.57a-b
HSTUW 5	114.73a-b	10.73a	45.03a
HSTUW 6	109.73b-c	9.84a-c	41.63a-b
HSTUW 7	104.98c-d	9.85a-c	43.57a
HSTUW 8	105.50c-d	9.57a-c	38.33a-c
HSTUW 9	107.17c	10.58a-b	45.20a
HSTUW 10	107.55c	9.71a-c	38.57a-b
BARI Gom 32	99.40d-e	9.05b-c	30.93c
Range	**92.35 - 116.33**	**8.58 – 10.73**	**30.93 – 45.20**
Mean	**104.22**	**9.61**	**40.08**
SE (±)	**1.27**	**0.31**	**1.49**
HSD	**6.47**	**1.57**	**7.60**

Here, SE= Standard error, HSD= honestly significant difference.

4.2.4 Average plant height

A wide range of variations were recorded for average plant height. With a mean value of 104.22±1.27, the range of plant height was 92.35cm - 116.33cm (Figure 10). The tallest genotype was HSTUW 4 (116.33cm). On the contrary, the shortest genotype was HSTUW 2 (92.35cm). The genotypes appeared taller than those shown in Table 11.

4.2.5 Average spike length

The average spike length is estimated at the mature stage of the plant. The average range of average spike length was measured from 8.58 cm to 11.94 cm with a mean value of 9.61±0.31 (Figure 11). HSTUW 5 (10.73 cm) showed the highest average spike length followed by HSTUW 9 (10.58 cm), HSTUW 4 (9.90 cm), HSTUW 7 (9.85 cm), HSTUW 6 (9.84 cm). The lowest average spike length HSTUW 1 was revealed at 8.58 cm (Table 11).

4.2.6 Average grain per spike

A wide variation was also recorded for the average grain per spike which ranged from (30.93 to 45.20) with a mean value of 40.08±1.49 (Figure 13). The highest average grain per spike was found in HSTUW 9 (45.20), followed by HSTUW 5 (45.03), HSTUW 3 (44.50), HSTUW 7 (43.57), HSTUW 6 (41.63), and the lowest number of spike per 1 square meter per panicle was found in BARI Gom 32 (30.93) shown in Table 11.

Table 12: Mean performance of morphological character of wheat genotypes (Contd.)

Genotype	Number of spike per 1 square meter	1000 grain weight	Yield per plot
HSTUW 1	505.67a	39.45a-c	2812.73a
HSTUW 2	477.00a-c	33.09b-c	2087.22d
HSTUW 3	435.33a-d	31.99c	2125.11c-d
HSTUW 4	377.00d	45.43a	2665.91a-b
HSTUW 5	359.00d	41.98a	2490.78a-d
HSTUW 6	409.33b-d	45.55a	2576.14a-c
HSTUW 7	351.67d	43.38a	2468.77a-d
HSTUW 8	373.33d	47.23a	2671.95a-b
HSTUW 9	385.00c-d	41.22a-b	2243.07b-d
HSTUW 10	378.00d	45.46a	2450.05a-d
BARI Gom 32	483.33a-b	46.01a	2493.52a-d
Range	**351.67 - 505.67**	**31.99 - 47.23**	**2087.22 - 2812.73**
Mean	**412.24**	**41.89**	**2462.30**
SE (±)	**18.39**	**1.73**	**91.55**
HSD	**93.94**	**8.86**	**467.68**

Here, SE= Standard error, HSD= Honestly significant difference.

4.2.7 Number of spike per 1 square meter

The number of spike per 1 square meter was estimated after harvesting the plant (Figure 14). The number of spike per 1 square meter significantly differed from 351.67 - 505.67 with a mean value of 412.24±18.39 (Table 12). HSTUW 7 (351.67) produced the lowest number of spike per 1 square meter whereas HSTUW 1 (505.67) produced the highest number of spike per 1 square meter followed by BARI Gom 32 (483.33), HSTUW 2 (477.00), HSTUW 3 (435.33) and HSTUW 9 (385.00).

4.2.8 1000-grain weight

In the 1000 grain weight sown in Figure 15, the average seed index was 41.89±1.73 g, and the range of 1000 grain weight was estimated at 31.99 to 47.23 consisting of HSTU developed advanced line HSTUW 8 followed by BARI Gom 32, HSTUW 6, HSTUW 10 and HSTUW 4 respectively shown in table 12. That result indicated a high scope to isolate a good genotype for this character in the present materials.

4.2.9 Yield per plot

However, the yield per plot were shown in Figure 16. The average range of yield per plot was 2087.22 g to 2812.73 g, with a mean value of 2462.30±91.55 with standard error (Table 12). The Highest yield per plot was recorded in HSTUW 1 (2812.73g) followed by HSTUW 8 (2671.95g), and HSTUW 4 (2665.91g) respectively. and the lowest value was found in HSTUW 2 (2087.22g) followed by HSTUW 3 (2125.11g), and HSTUW 9 (2243.07g) indicating that there was a high scope to isolate good genotypes or lines for this character in the present observation. The results agree with the findings of Osundare *et al.*, (2017).

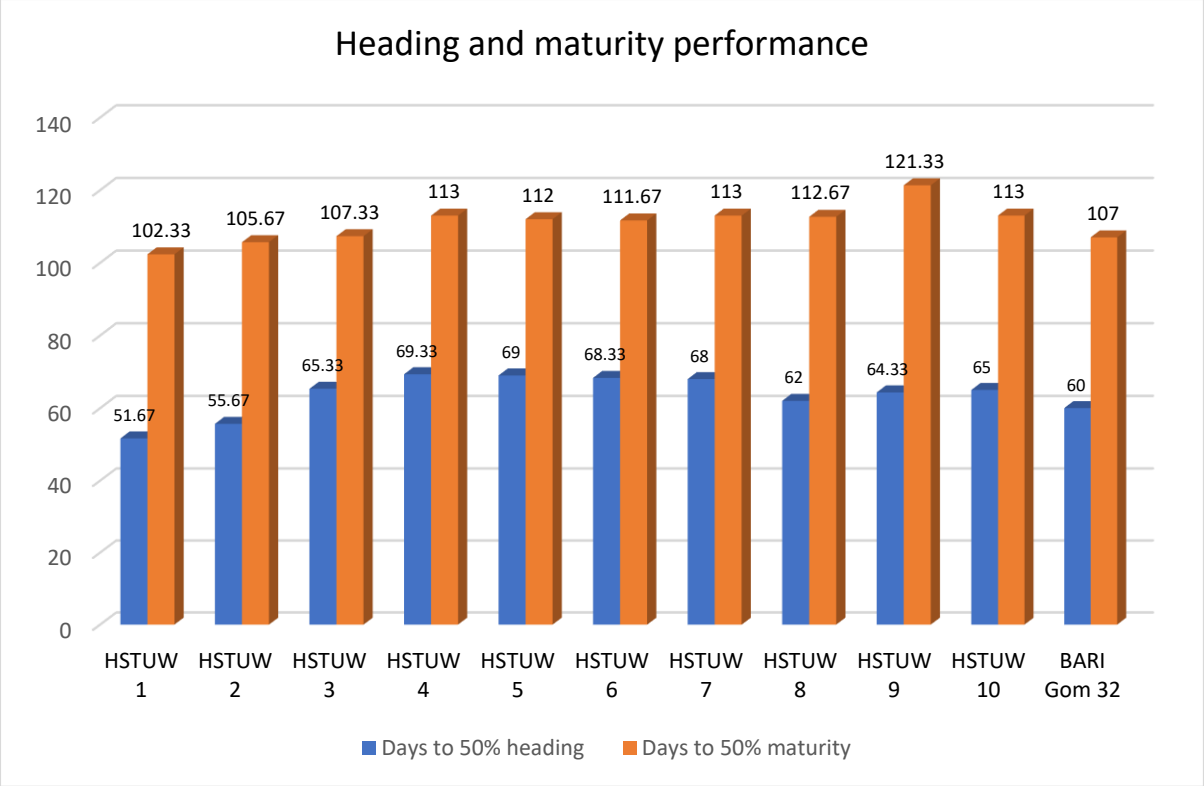

Figure 9: Mean performance of days to heading and days to maturity of wheat genotypes

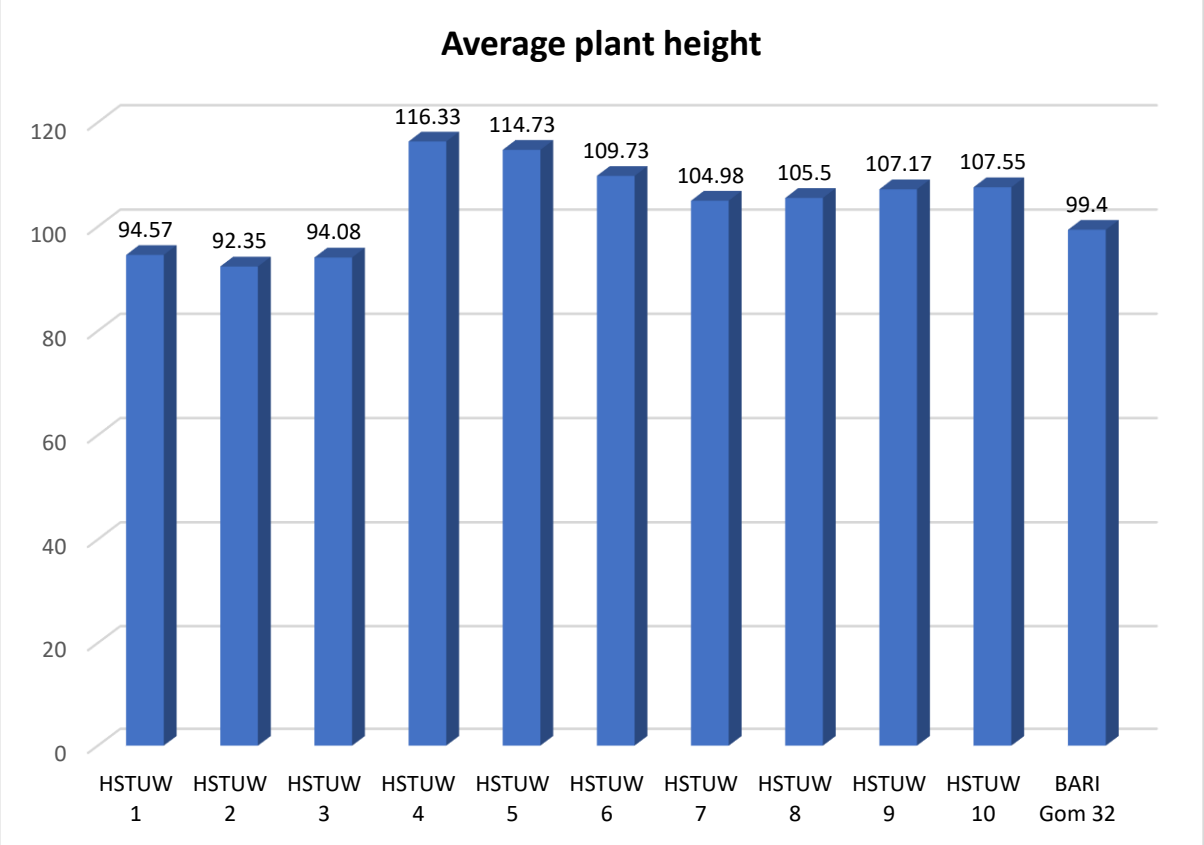

Figure 10: Mean performance of average plant height of wheat genotypes

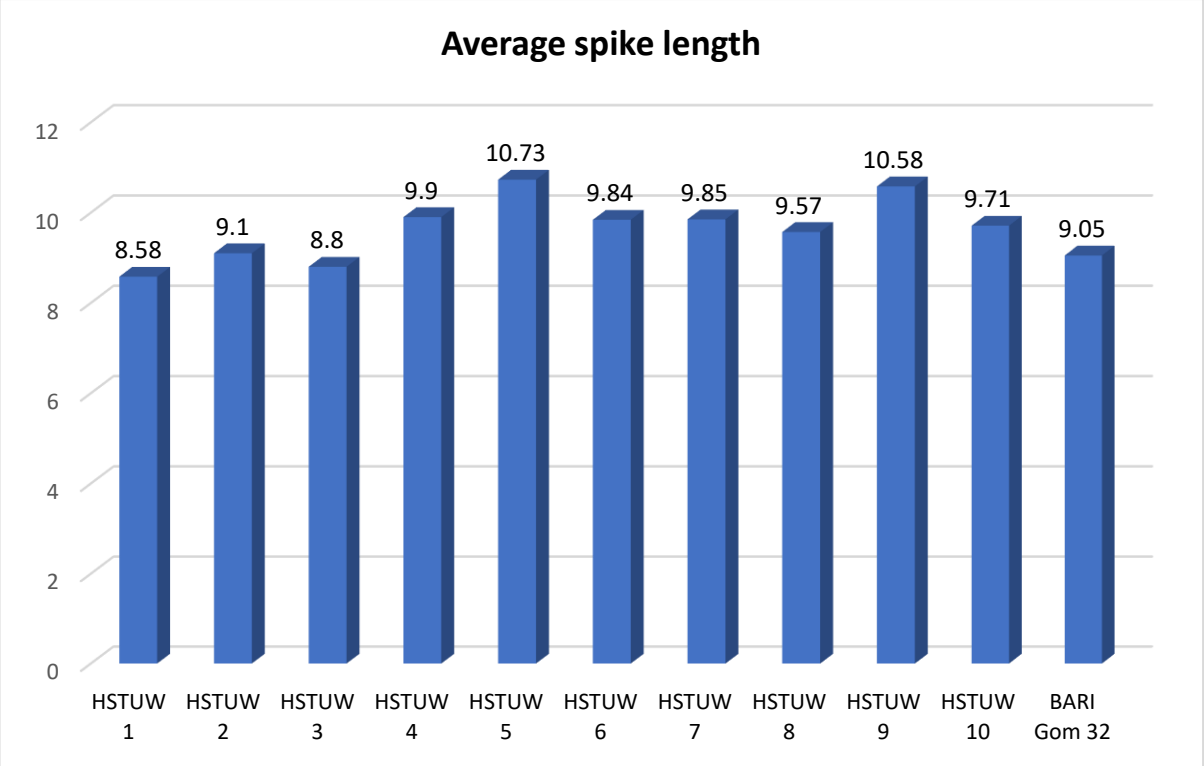

Figure 11: Mean performance of average spike length of wheat genotypes

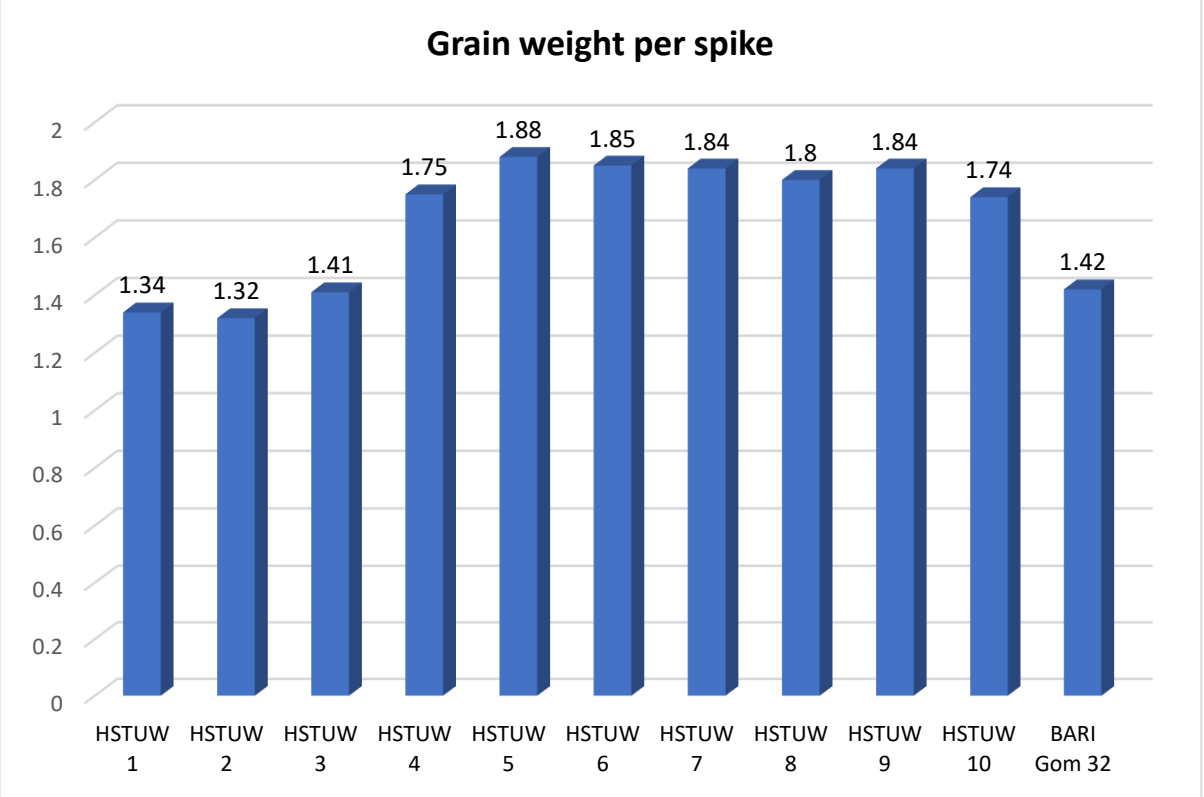

Figure 12: Mean performance of grain weight per spike of wheat genotypes

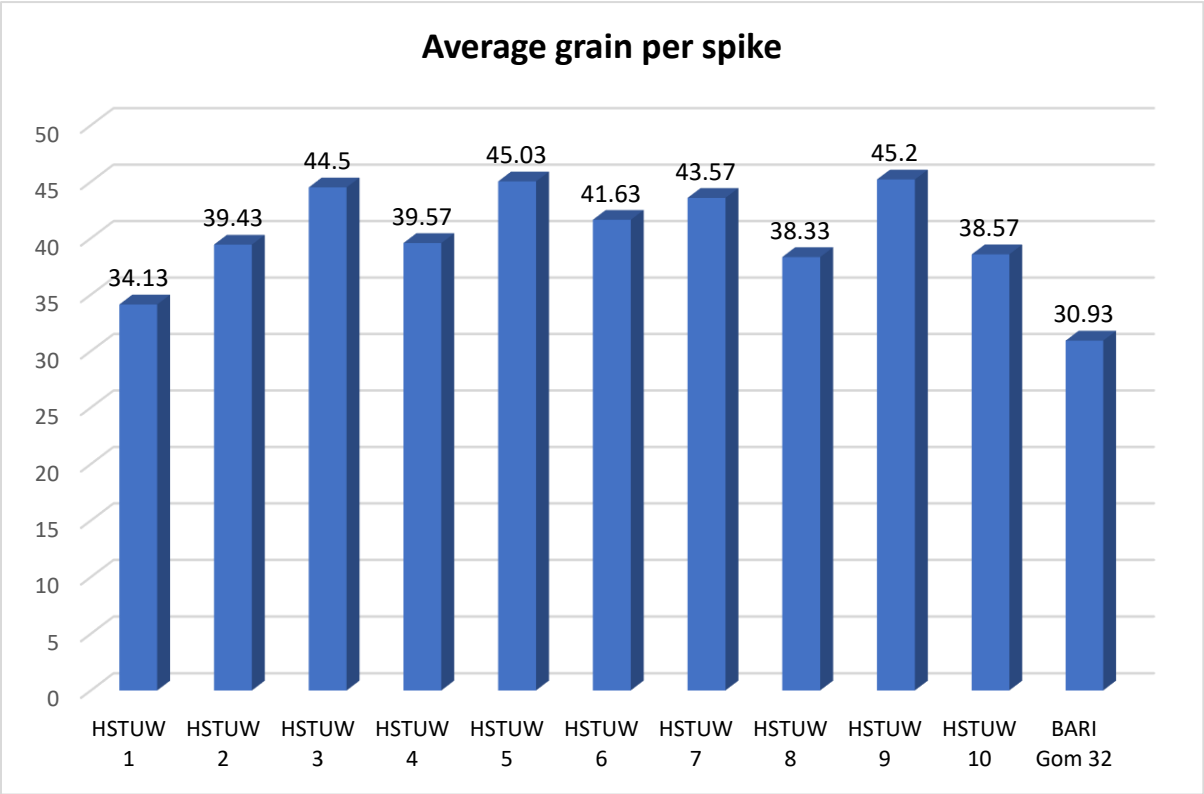

Figure 13: Mean performance of average grain per spike of wheat genotypes

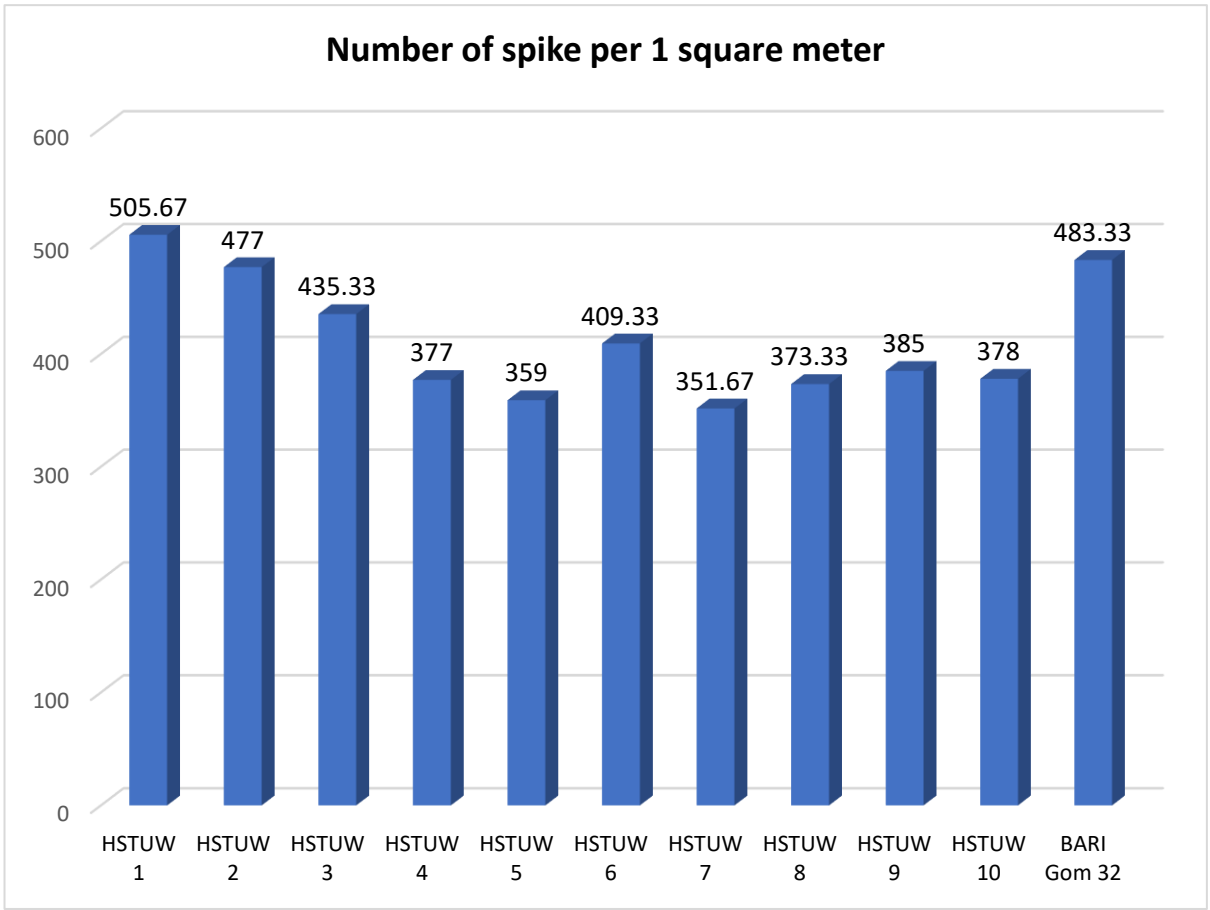

Figure 14: Mean performance of the number of spike per 1 square meter of wheat genotype

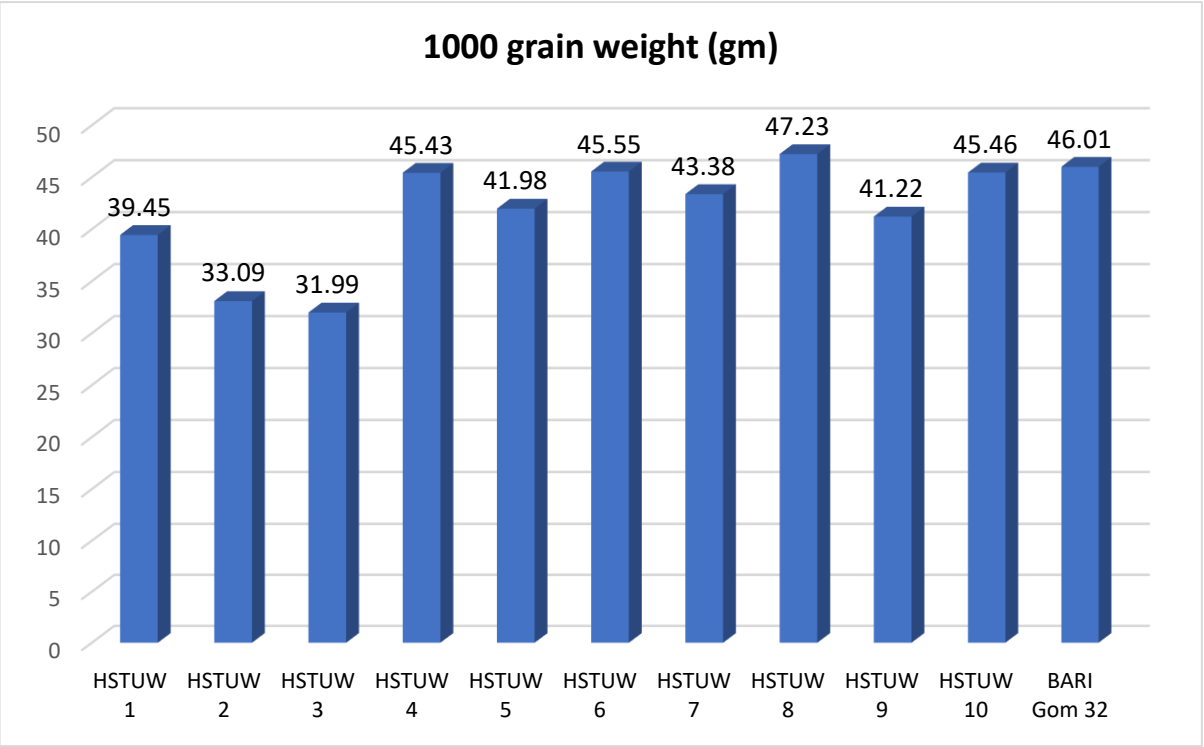

Figure 15: Mean performance of 1000 grain weight of wheat genotypes

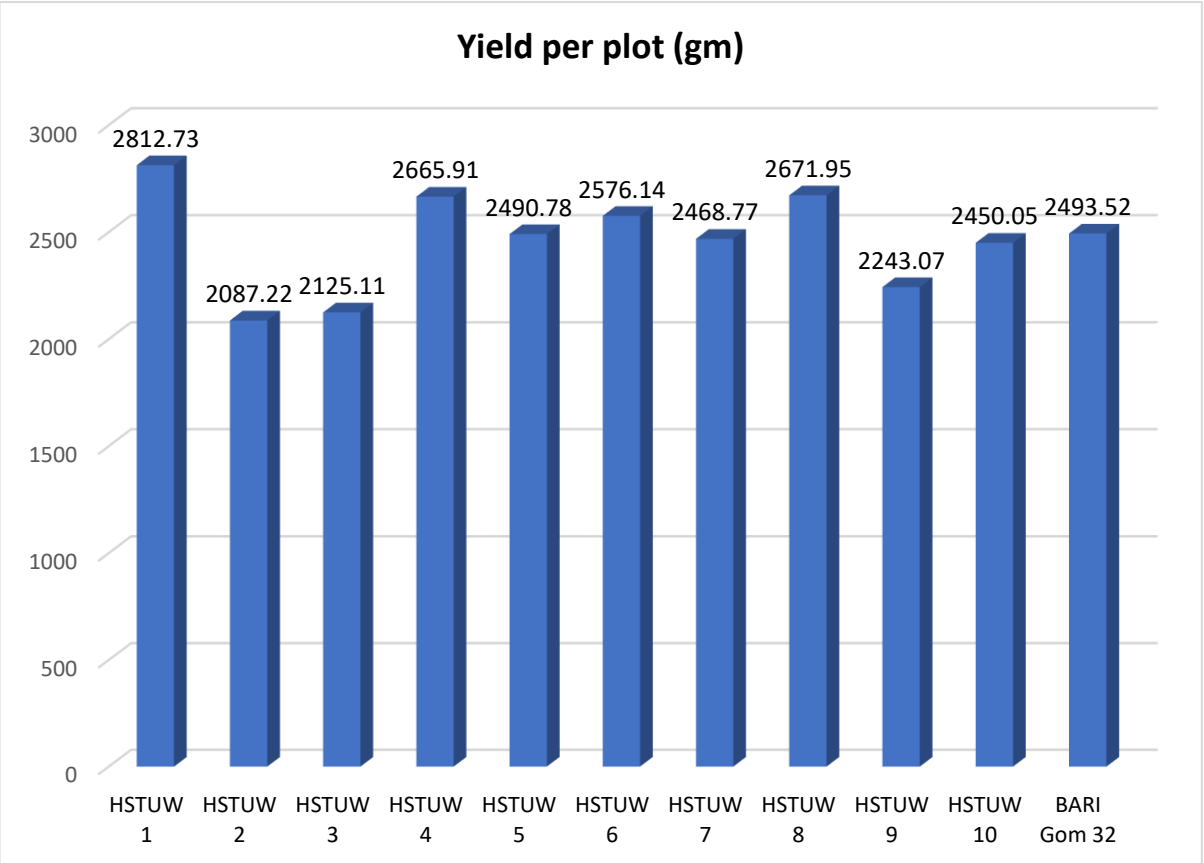

Figure 16: Mean performance of yield performance of wheat genotypes

4.3 Estimation of simple correlation coefficient

The experiment's outcome was obtained from correlation coefficients of 11 genotypes. The analyses were done on 9 yields and yield contributing characters viz. days to 50% heading, days to 50% maturity, grain weight per spike, average grain per spike, 1000 grain weight, number of spike per 1 square meter, average spike length, average plant height, yield per plot.

Yield is a complex quantitative character so information about the contributing characters deserves considerable attention. Selection of superior genotypes for yield can be effective only if the association of yield with attributing traits is known and considered during the process. The correlation studies estimate the degree and direction of the relationship between two or more variables and provide information about yield contributing characters. In breeding, it can be used to identify the component characters that can be used for selection for improvement of yield and can also aid in selecting elite genotypes from diverse populations (Johnson *et al.*, 1955).

However, the Pearson's correlation denoted that, there are present two colors for indicating the positive and negative direction of correlation between the pointed character. The dark blue color pointed the highly positive then the light blue. Similarly, the dark red color represents a highly negative relation to the light red color shown in Figure 17.

The correlation between all the characters with yield per plot was positively significant with 1000 grain weight (0.70). Additionally, the rest of all the characters showed non-significant negative and positive correlations with yield per plot namely NOS (-0.07), GWPS (0.25), APH (0.39), DTH (-0.04), DTM (-0.12) and AGPS (-0.46) respectively. The case of the number of spike per 1 square meter showed a very strong negatively significant correlation between all the characters viz. GWPS (-0.92), DTH (-0.84), ASL (-0.81), APH (-0.79), DTM (-0.79) and AGPS (-0.65) respectively. That denoted that when the number of spike per 1 square meter was increased then the presented character should decrease, when all yield contributing characters were decreased then the number of spike per 1 square meter should be increased. Whenever the rest of the characters showed a strong to lightly weak positively significant correlation between all the characters presented in Figure 17.

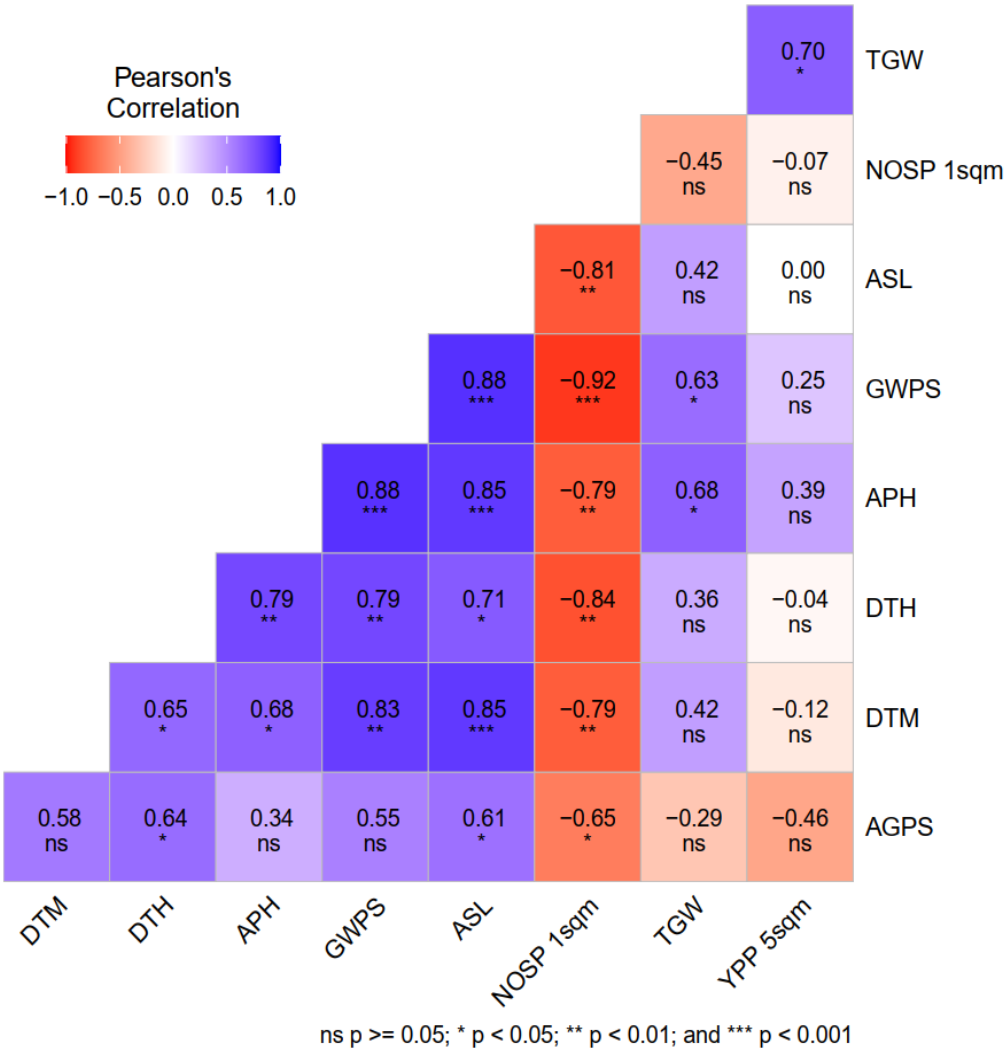

Figure 17: Simple correlation coefficient among morphological characters of wheat genotypes

Here, DTH= Days to heading, DTM= Days to maturity, APH= Average plant height, NOS = No of spike, ASL= Average Spike length, AGPS= Average Grains per spike, GWPS= Grain weight per spike, TGW = 1000 grain weight, YPP= Yield per plot.

4.3.1 Genotypic Correlation coefficients among traits of wheat genotypes

The genotypic correlation coefficients of 11 wheat genotypes among a total of 9 yields and yield contributing characters showed that the yield per plot had a very strong positive significant correlation with 1000 grain weight (0.8292) at a 5% level of probability. However, yield-contributing characters showed positive non-significant correlations with average plant height (0.4214), average grain weight per spike (0.3554), and negative non-significant correlations with Days to heading (-0.0393), Days to maturity (-0.1188), Number of spike per 1 square meter (-0.1795), average spike length (-0.0584), and average grain per spike (-0.4879) (Table 13). That result represents that when the 1000 grain weight increases then the yield per plot will increase. Average grain per spike has a positive significant correlation with Days to heading (0.6858), Days to maturity (0.6175), and Average spike length (0.6729) respectively. However, several spike (-0.6576) showed a negative significant correlation with the average grain per spike. That result depicts that when the number of spike per 1 square meter was increased that case average grain per spike decreased. And the negative non-significant association with 1000 grain weight (-0.3247). Similarly, the number of spike per 1 square meter has a strong negative significant correlation with grain weight per spike (-0.9873), Days to heading (-0.8947), Days to maturity (-0.8599), and Average plant height (-0.8554) respectively. However, the strength of association is less and strength is weak. But the number of spike per 1 square meter has a negative non-significant association with 1000 grain weight (-0.5039) That means there was no significant relation between the number of spike per 1 square meter with 1000 grain weight. 1000 grain weight is also an important trait and exhibited positive and significant association with average plant height (0.7478), While a moderate positive significant correlation with grain weight per spike (0.6154). As regards the grain weight per spike it has a strong positive significant association with average plant height (0.9616), Days to maturity (0.9039), and Days to heading (0.9034) respectively. Finally, average plant height has a strong positive significant correlation with Days to heading (0.8002), with a moderate association with Days to maturity (0.6996). Finally, the character Days to maturity have a moderate positive significant association with days to heading (0.6692) the traits are moderately significant and strength is weak.

4.3.2 Phenotypic Correlation coefficients among traits of wheat genotypes

The phenotypic correlation coefficients of 11 wheat genotypes among a total of 9 yield and yield contributing characters showed that the yield per plot had a very strong positive significant correlation with 1000 grain weight (0.5199) was similar to the genotypic correlation while the moderate positive significant association with average plant height (0.3459), But the character yield per plot exhibited a moderate negative significant association with average grain per spike (-0.4269). That result depicts that the relation between yield per plot and average grain per spike is vice versa i.e. When yield per plot increases that time average grain per spike must be decreased similarly if the average grain per spike increases that time yield per plot must be decreased (Table 14). In that case, yield per plot also showed that non-significant association with the number of spike per 1 square meter (0.0868) and average spike length (0.0471) respectively. 1000 grain weight exhibited a significant strong positive association with grain weight per spike (0.6483), average plant height (0.5852), and moderate association with days to maturity (0.372). Similarly, average grain per spike (AGPS) exhibited a strong significant positive association with average spike length (0.557), Days to heading (0.5587), grain weight per spike (0.5554), and the strong negative association with number of spike per 1 square meter (-0.6428) respectively. However, Average spike length (ASL) exhibited a powerful positive association with average plant height (0.7386), Days to maturity (0.6544), grain weight per spike (0.6246), and Days to heading (0.5981) and showed a strong negative significant association with number of spike per 1 square meter was (-0.6702). Cases of other strong positive significant association showed Days to maturity (DTM) with Days to heading (0.6274), average plant height (APH) with days to heading (0.7602), and Days to maturity (0.6517), grain weight per spike (GWPS) with days to heading (0.6173), Days to maturity (0.7226) and average plant height (0.7412) respectively. While the strong negative significant association showed days to heading (-0.7468), Days to maturity (-0.6876), average plant height (-0.6972), grain weight per spike (-0.8256), and 1000 grain weight (-0.3769) respectively.

Chandra *et al.,* 2004 got a significant correlation between yield per plant with plant height, grains per spike, and Spikes per plant but *Sohail et al.,* 2018 got no significant correlation at the genotypic and phenotypic level in yield per plant with plant height and hundred-grain weight. Meena *et al.,* 2021 have got significant correlation at genotypic and phenotypic levels in grain yield with plant height and 1000 grain weight at genotypic and phenotypic levels. Meena *et al.,* 2021 have suggested that for improving the wheat grain yield under terminal heat stress, genotype should be selected based on bold grains or high grain weight per spike, a greater number of tillers /sq.m. Kumar *et al.,* 2020 have suggested that a high thousand kernel weight could be used for selecting genotypes under heat stress.

Sharma *et al.,* 2006; Ahmed *et al.,* 2007; Monpara and Kalariya, 2009; Singh *et al.,* 2010, Sakhare and Ghawat, 2011 and Singh *et al.,* 2012 reported stronger or weaker association of yield with yield component traits in wheat. They suggested that wheat crop grain yield potential can be effectively improved by obtaining the maximum expression of yield contributing characters showing a stronger association in the desirable direction.

Table 13: Genotypic Correlation coefficients among traits of wheat genotypes

Character	DTH	DTM	APH	GWPS	TGW	NOS	ASL	AGPS	YPP
DTH		0.6692 *	0.8002 **	0.9034 **	0.4273 NS	-0.8947 **	0.787 **	0.6858 *	-0.0393 NS
DTM			0.6996 *	0.9039 **	0.4478 NS	-0.8599 **	0.9879 **	0.6175 *	-0.1188 NS
APH				0.9616 **	0.7478 **	-0.8554 **	0.9122 **	0.3686 NS	0.4214 NS
GWPS					0.6154 *	-0.9873 **	1.0858 **	0.5491 NS	0.3554 NS
TGW						-0.5039 NS	0.5549 NS	-0.3247 NS	0.8292 **
NOS							-0.9151 **	-0.6576 *	-0.1795 NS
ASL								0.6729 *	-0.0584 NS
AGPS									-0.4879 NS
YPP									

*, ** significant at 5% and 1% level of probability respectively, and NS= non-significant respectively

Here, DTH= Days to heading, DTM= Days to maturity, APH= Average plant height, GWPS= Grain weight per spike, TGW = 1000 grain weight, NOS= No of spike, ASL= Average Spike length, AGPS= Average Grains per spike, YPP= Yield per plot.

Table 14: Phenotypic Correlation coefficients among traits of wheat genotypes

Character	DTH	DTM	APH	GWPS	TGW	NOS	ASL	AGPS	YPP
DTH									
DTM	0.6274 **								
APH	0.7602 **	0.6517 **							
GWPS	0.6173 **	0.7226 **	0.7412 **						
TGW	0.2559 NS	0.372 *	0.5852 **	0.6483 **					
NOS	-0.7468 **	-0.6876 **	-0.6972 **	-0.8256 **	-0.3769 *				
ASL	0.5981 **	0.6544 **	0.7386 **	0.6246 **	0.2212 NS	-0.6702 **			
AGPS	0.5587 **	0.5191 **	0.3066 NS	0.5554 **	-0.2508 NS	-0.6428 **	0.557 **		
YPP	-0.0416 NS	-0.1123 NS	0.3459 *	0.0935 NS	0.5199 **	0.0868 NS	0.0471 NS	-0.4269 *	

*, ** significant at 5 and 1 percent respectively, and NS= non-significant respectively

Here, DTH= Days to heading, DTM= Days to maturity, APH= Average plant height, GWPS= Grain weight per spike, TGW = 1000 grain weight, NOS= No of spike, ASL= Average Spike length, AGPS= Average Grains per spike, YPP= Yield per plot.

Table 15: Direct (Diagonal) and indirect effect of Genotypic path coefficients analysis among eleven traits of wheat genotypes

Character	DTH	DTM	APH	GWPS	TGW	NOS	ASL	AGPS	YPP
DTH	**-0.234**	-1.241	-0.534	3.228	-1.395	2.196	0.943	-3.003	**-0.039 NS**
DTM	-0.157	**-1.854**	-0.467	3.230	-1.461	2.110	1.184	-2.704	**-0.119 NS**
APH	-0.187	-1.297	**-0.668**	3.436	-2.441	2.099	1.093	-1.614	**0.421 NS**
GWPS	-0.212	-1.676	-0.642	**3.573**	-2.008	2.423	1.301	-2.404	**0.355 NS**
TGW	-0.100	-0.830	-0.499	2.200	**-3.264**	1.237	0.665	1.422	**0.829 ****
NOS	0.209	1.594	0.571	-3.528	1.645	**-2.454**	-1.096	2.879	**-0.179 NS**
ASL	-0.184	-1.831	-0.609	3.880	-1.811	2.246	**1.198**	-2.946	**-0.058 NS**
AGPS	-0.160	-1.145	-0.246	1.962	1.060	1.614	0.806	**-4.379**	**-0.488 NS**

Residual 0.0184

Here, DTH= Days to heading, DTM= Days to maturity, APH= Average plant height, GWPS= Grain weight per spike, TGW = 1000 grain weight, NOS= No of spike, ASL= Average Spike length, AGPS= Average Grains per spike, YPP= Yield per plot.

Table 16: Direct (Diagonal) and indirect effect of Phenotypic path coefficients analysis among eleven traits of wheat genotypes

Character	DTH	DTM	APH	GWPS	TGW	NOS	ASL	AGPS	YPP
DTH	**-0.409**	-0.215	0.706	-0.620	0.284	-0.142	-0.021	0.376	**-0.042 NS**
DTM	-0.256	**-0.343**	0.605	-0.726	0.412	-0.131	-0.023	0.349	**-0.112 NS**
APH	-0.311	-0.224	**0.929**	-0.745	0.648	-0.132	-0.026	0.206	**0.346 ***
GWPS	-0.252	-0.248	0.689	**-1.008**	0.718	-0.157	-0.022	0.374	**0.093 NS**
TGW	-0.104	-0.128	0.544	-0.651	**1.108**	-0.072	-0.008	-0.169	**0.520 ****
NOS	0.305	0.236	-0.648	0.830	-0.418	**0.190**	0.024	-0.433	**0.087 NS**
ASL	-0.244	-0.225	0.686	-0.628	0.245	-0.127	**-0.035**	0.375	**0.047 NS**
AGPS	-0.228	-0.178	0.285	-0.558	-0.278	-0.122	-0.020	**0.673**	**-0.427 ***

Residual 0.1134

Here, DTH= Days to heading, DTM= Days to maturity, APH= Average plant height, GWPS= Grain weight per spike, TGW = 1000 grain weight, NOS= No of spike, ASL= Average Spike length, AGPS= Average Grains per spike, YPP= Yield per plot.

4.4 Direct (Diagonal) and Indirect Effect of Genotypic Path Coefficients Analysis among nine Traits of wheat Genotypes

Genotypic Path coefficient analysis splits the correlation coefficient into direct (Diagonal) and indirect (Off diagonal) effects. It measures the direct and indirect contribution of independent variables to dependent variables i.e. yield per plot in the present study. Data presented (Table 15) revealed that character Grain weight per spike (GWPS) exhibited the highest positive direct effect on yield per plot (3.573) and non-significant association with grain yield per plot (0.355). Singh *et al.,* 2009 also reported similar results in wheat. Average spike length also exhibited a maximum positive direct effect on yield per plot (1.198) and exhibited a negative non-significant association with grain yield per plot (-0.058), similar results were reported by Sharma *et al.,* (2006) and Ahmed *et al.,* (2007). reported that the association of grain yield with tiller number, grains spike-1, 1000 grain weight, and plant height was found to be positive and significant under different environments.

Important trait 1000 grain weight exhibited a positive direct effect on yield per plot however magnitude is high and it has a positive indirect effect with DTH, DTM, APH, GWPS, and ASL while negative indirect effect with NOS and AGPS on YPP. However, the number of spike per 1 square meter has a positive indirect effect with YPP and also negative indirect effect *via* DTH, DTM, APH, GWPS, ASL, and AGPS on YPP. magnitude is less and it has a negative indirect effect with DTH, DTM, APH, GWPS, TGW, AGPS, and ASL Sharma *et al.* (2006) reported similar results in wheat. Present findings are in confirmation with Cifci *et al.*, 2012.

Plant height had a Positive direct effect on grain yield per plot, while Days to heading, Days to maturity, average grain weight per spike, average plant height, 1000 grain weight, average grain per spike, and average spike length had a positive indirect effect on grain yield per plot although the magnitudes are very high. Similar findings were reported (Kashif and Khaliq, 2004) in wheat for plant height, spikelets spike-1, and 1000 grain weight.

The residual factor value was found to be 0.0184 indicating that there are some other factors influencing the grain yield per plot, which were not being included in the study.

4.5 Direct (Diagonal) and Indirect Effect of Phenotypic Path Coefficients Analysis among nine Traits of wheat Genotypes

Phenotypic Path coefficient analysis splits the correlation coefficient into direct (Diagonal) and indirect (Off diagonal) effects. In that case, it also measures the direct and indirect contribution of independent variables to dependent variables i.e. yield per plot in the present study. Data presented (Table 16) revealed that character 1000 grain weight exhibited the highest positive direct effect on yield per plot (1.108) and a strong positive significant association with grain yield per plot (0.520). Kamboj *et al.,* 2010 also reported similar results in wheat. Average grain per spike also exhibited a maximum positive direct effect on yield per plot (0.673) and exhibited a negative moderate significant association with grain yield per plot (-0.427), similar results were reported by Singh *et al.,* (2009) reported that the positive and significant association was found to be under different environments of grain yield with tiller number, grains spike-1, 1000 grain weight, and plant height.

Important trait 1000 grain weight exhibited a negative direct effect on yield per plot however magnitude is less and it has a positive indirect effect with the number of spike per 1 square meter it has also negative indirect effect *via* DTH, DTM, APH, GWPS, ASL, and AGPS on YPP, on grain yield. Sharma *et al.,* (2006) reported the similar results in wheat. The direct effect of the number of spike per 1 square meter on grain yield per plot is negative, however, its indirect contribution was positive for all the yield-contributing characters on yield per plot. Present findings are in confirmation with Cifci *et al.*, 2012.

Plant height had a negative direct effect on grain yield per plot, while Days to heading, Days to maturity, average grain weight per spike, and average spike length had a negative direct effect on grain yield per plot although the magnitudes are very small. Similar findings were reported (Kashif and Khaliq, 2004) in wheat for plant height, spikelets spike-1, and 1000 grain weight.

The residual factor value was found to be 0.4134 indicated that there are some other factors influencing the grain yield per plot, which were not being included in the study.

4.6 Genetic parameters on the selected characters in Wheat genotypes

The study of different parameters such as genotypic variance (σ^2g), phenotypic variance (σ^2p), genotypic coefficient of variation (GCV %), phenotypic coefficient of variation (PCV %), heritability (h^2b), genetic advance (GA) and genetic advance as percent of the mean (GAM) of 9 characters were estimated to observe the variability existed among the characters. The results presented in (Table 17) revealed that the phenotypic variances for all the characters were higher than the genotypic variances for all the characters. It was observed earlier that there was significant variation among the wheat genotypes/lines for all the characters. The variation among the wheat genotypes /lines was judged at phenotypic and genotypic levels.

The values of phenotypic and genotypic co-efficient of variation higher than 20% are regarded as high, whereas values less than 10% are considered low and values between 10% and 20% are medium (Tahir *et al.,* 2002).

Heritability values of more than 80% are very high, values from 60% to 79% are moderately high, values from 40% to 59% are medium, and values less than 40% are low (Singh, 2001).

Johnson *et al.,* (1955) classified genetic advance as a percentage of the mean (GAM); values from 0-10% are low, 10-20% are moderate, and 20% and above are high.

The highest genotypic and phenotypic variances were recorded with Yield per plot (44177.68 and 69323.02), Number of spike per 1 square meter (2631.04 and 3645.52), Average plant height (65.36 and 70.18), and Days to heading (31.93 and 35.17), respectively (Table 17). The low values of phenotypic and genotypic variances were recorded with the character Grain weight per spike (0.04, 0.07), and Average spike length (0.38, 0.66), respectively.

PCV was higher than the corresponding GCV for all the traits indicating that there was an influence on the environment. The genotypic coefficient of variances (GCV) ranged from 4.53% for the Days to maturity to 12.73% for Grain weight per spike (Table 17). The genotypic coefficient of variation was more than 10% and less than 20% values are recorded in Grain weight per spike (12.73), Number of spike per 1 square meter (12.44), 1000 grain weight (11.65), and Average grain per spike (10.81). which are considered as medium genotypic coefficient of variation. The phenotypic coefficient of variances (PCV) ranged from 4.63% for the Days to maturity to 15.62% for the Grain weight per spike. The phenotypic coefficient of variation (PCV) was more than 10% in Grain weight per spike (15.62), Number of spike per 1 square meter (14.65), 1000 grain weight (13.68), Average grain per spike (12.58), and Yield

per plot (10.69) respectively which are considered as the medium phenotypic coefficient of variances (PCV), in Table 17. Similar findings were made by (Tahir *et al.,* 2002). During the research, no higher genotypic coefficient of variation was found, and the rest of the findings are considered as low genotypic coefficient of variation.

This study shows that phenotypic variances (σ^2p) and phenotypic coefficient of variances PCV were higher than their relative genotypic variances σ^2g and genotypic coefficient of variances GCV for all the characters studied, indicating that the environment influenced the expression of these characters.

The Heritability (Broad sense) estimates the relative contributions of differences in genetic and non-genetic factors to the total phenotypic variances in the population. It is an important concept in quantitative genetics, particularly in selective breeding. The heritability estimation varied from 57.10% to 95.85% for the average spike length and Days to maturity respectively. Among 9 characters, 3 characters showed higher heritability (>80%) which was Days to maturity (95.85%), Average plant height (93.13%), and Days to heading (90.78%) respectively. This means those characters could be easily improved by selection. Most of the characters Average grain per spike (73.87%), 1000 grain weight (72.54%), Number of spike per 1 square meter (72.17%), Grain weight per spike (66.47%), and Yield per plot (63.73%) showed values from 60% to 79% are moderately high heritability (Table 17). Present findings are in confirmation with Monpara, 2011 and Singh *et al.*, 2012.

This suggested most likely that heritability is due to the additive genetic effects and selection could be effective in early segregating generations for these traits and the possibility of improving wheat grain yield through direct selection for hundred-grain weight. This finding was similar to reports earlier (Dwivedi *et al.,* 2002).

Table 17: Genetic parameters on the characters of Wheat genotypes

SL. NO	Characters	Genotype Variance (σ2g)	Phenotype Variance (σ2p)	GCV (%)	PCV (%)	Heritability (%)	GA	GAM (%)
01	Days to heading	31.93	35.17	8.90	9.33	90.78	11.09	17.46
02	Days to maturity	25.20	26.29	4.53	4.63	95.85	10.12	9.14
03	Average plant height	65.36	70.18	7.76	8.04	93.13	16.07	15.42
04	Grain weight per spike	0.04	0.07	12.73	15.62	66.47	0.35	21.38
05	1000 grain weight	23.82	32.84	11.65	13.68	72.54	8.56	20.44
06	Number of spike per 1 square meter	2631.04	3645.52	12.44	14.65	72.17	89.77	21.77
07	Average spike length	0.38	0.66	6.38	8.45	57.10	0.95	9.94
08	Average grain per spike	18.78	25.42	10.81	12.58	73.87	7.67	19.14
09	Yield per plot	44177.68	69323.02	8.54	10.69	63.73	3345.65	14.04

Here, GCV (%) = Genotypic coefficient of variation, PCV (%) = Phenotypic coefficient of variation, GA = Genetic advance, GAM= genetic advance as percent of mean

Similar heritability in broad sense in hundred-grain weight (HGW) (69.10-76.49%) was obtained by Chandra *et al.*, 2004. Sohail *et al.,* 2018 also got a similar result in 1000 grain weight (83%) but medium heritability in the broad sense in HGW (55%) is obtained by Ghuttai *et al.,* 2015. In yield per plant, Sohail *et al.,* 2018 got a similar result (68%) also Chandra *et al.,* 2004 got a similar result (70%) but Koujalagi *et al.,* 2017 got higher heritability (81.68%).

Genetic advance (GA) under selection refers to the improvement of characters in genotypic value for the new population compared with the base population under one cycle of selection at a given selection intensity (Wolie *et al.,* 2013). The high value of GA was revealed in the Yield per plot (3345.65) and the low in Grain weight per spike (0.35). Genetic advance as percent mean expected GAM had a general range between 9.14% for the Days to maturity to 21.77% for No. of spike. The high values of GAM (>20%) were recorded for Number of spike per 1 square meter (21.77%), Grain weight per spike (21.38%), and 1000 grain weight (20.44%) presented in Table 17. However, most of the character's values from 10% to 20% are namely Average grain per spike (19.14%), Days to heading (17.46%), average plant height (15.42%), and Yield per plot (14.04%) respectively similar to Johnson *et al.,* (1955).

That result indicated a high scope to isolate a good genotype or lines for these characters in the present materials.

4.7 Molecular characterization utilizing Microsatellite/SSR markers

This present study was conducted with 11 wheat genotypes. A total of four SSR/microsatellite markers were used to identify the variation among the genotypes. The SSR/microsatellite markers viz. Gwm495, Barc20, TaBarc101, and Xwmc112 were found polymorphic. A total of 31 alleles were detected and the number of alleles per locus ranged from 6 to 9 with an average of 7.75 alleles per locus (Table 18). This was similar to 2 - 12 (mean = 5.63) alleles per locus reported by Asmamaw *et al.,* (2019) in wheat who used 70 polymorphic SSR loci. Above mentioned markers showed different polymorphic patterns among the studied (11) wheat genotypes.

The 4 polymorphic markers were, Gwm495, Barc20, TaBarc101, and Xwmc112 Ladder used in this experiment was 100bp. Gel pictures of PCR-amplified fragments using those SSR markers are shown in Figures 18, 19, 20, and 21.

Initially, DNA has 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 polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis (PAGE) analysis (PAGE).

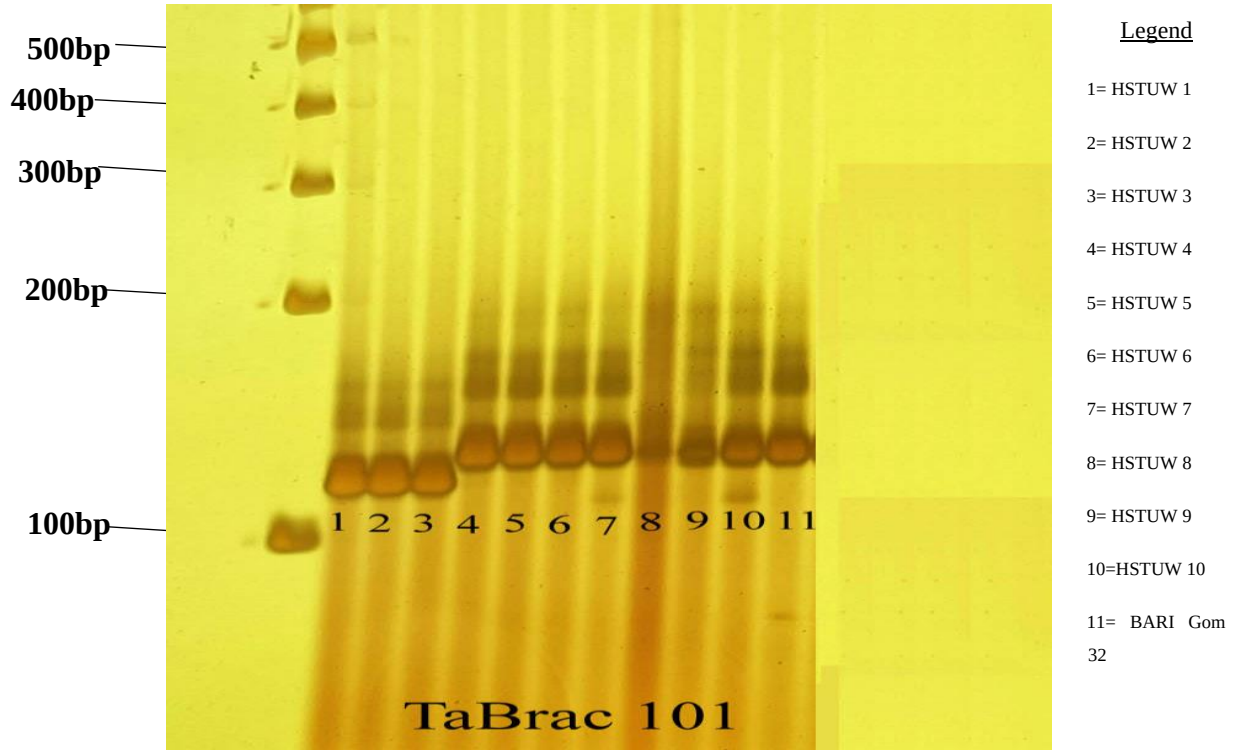

Figure 18: The DNA profile of wheat genotypes using **TaBarc101**

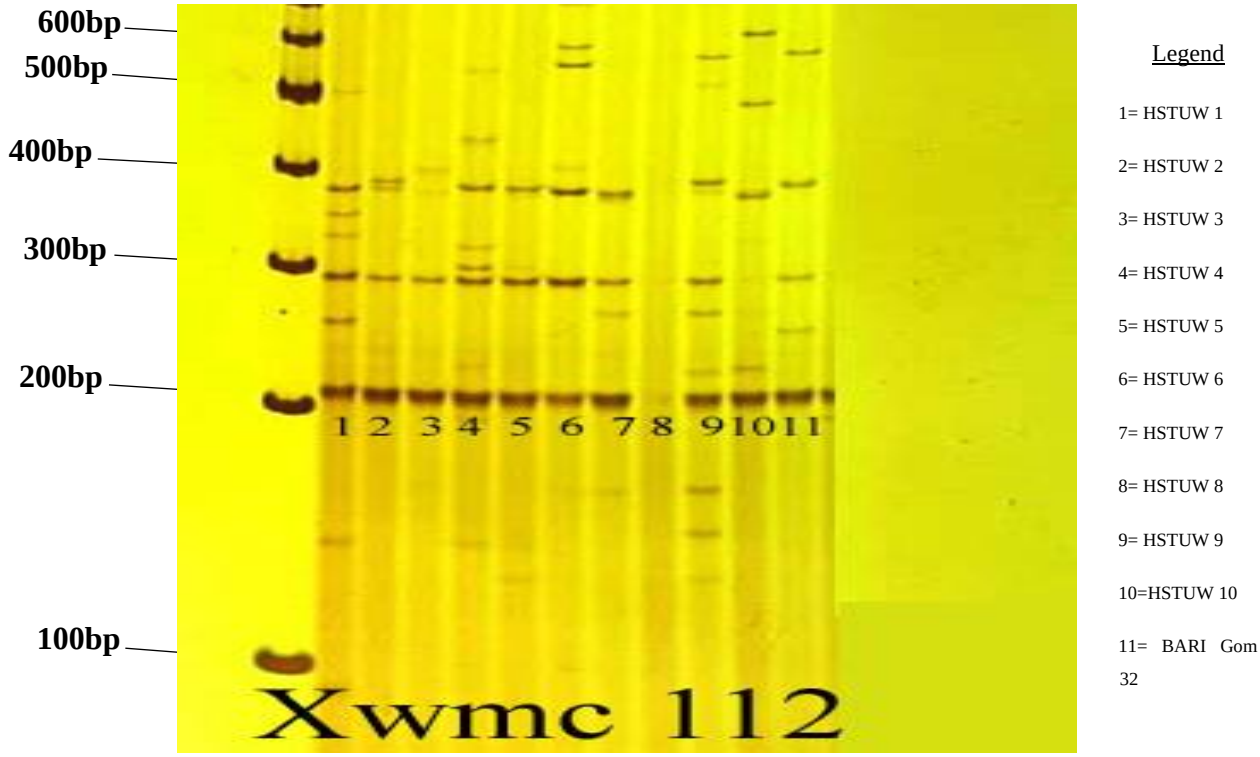

Figure 19: The DNA profile of wheat genotypes using **Xwmc112**

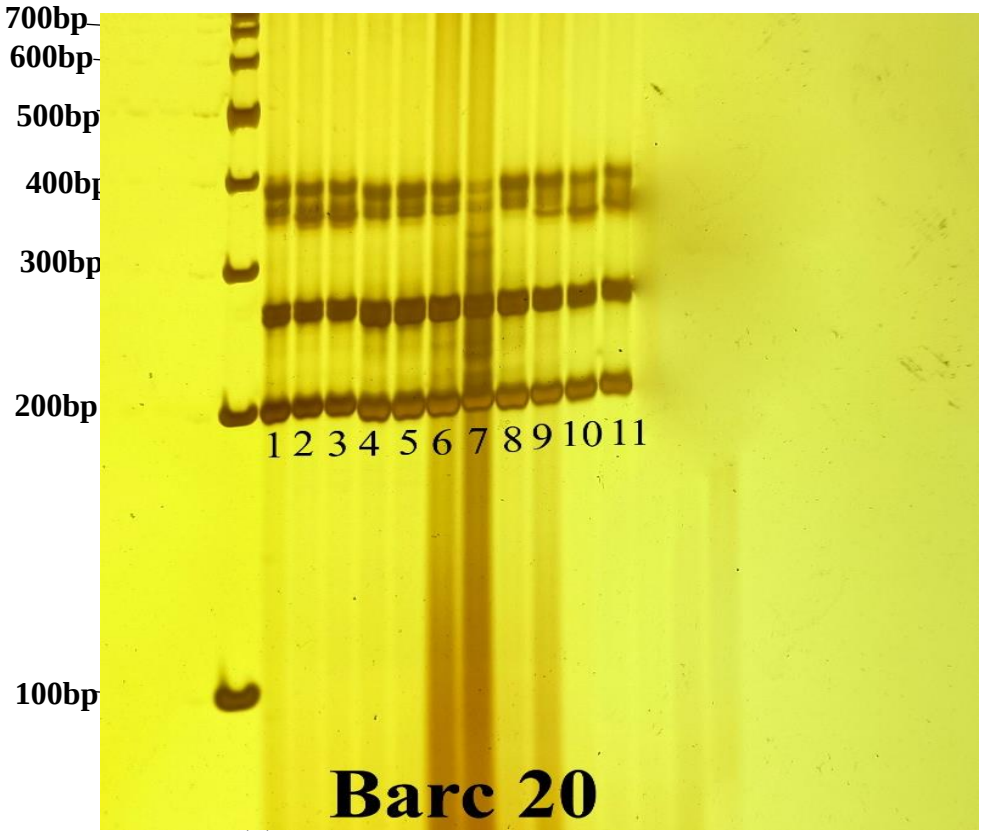

Figure 20: The DNA profile of wheat genotypes using **Barc20**

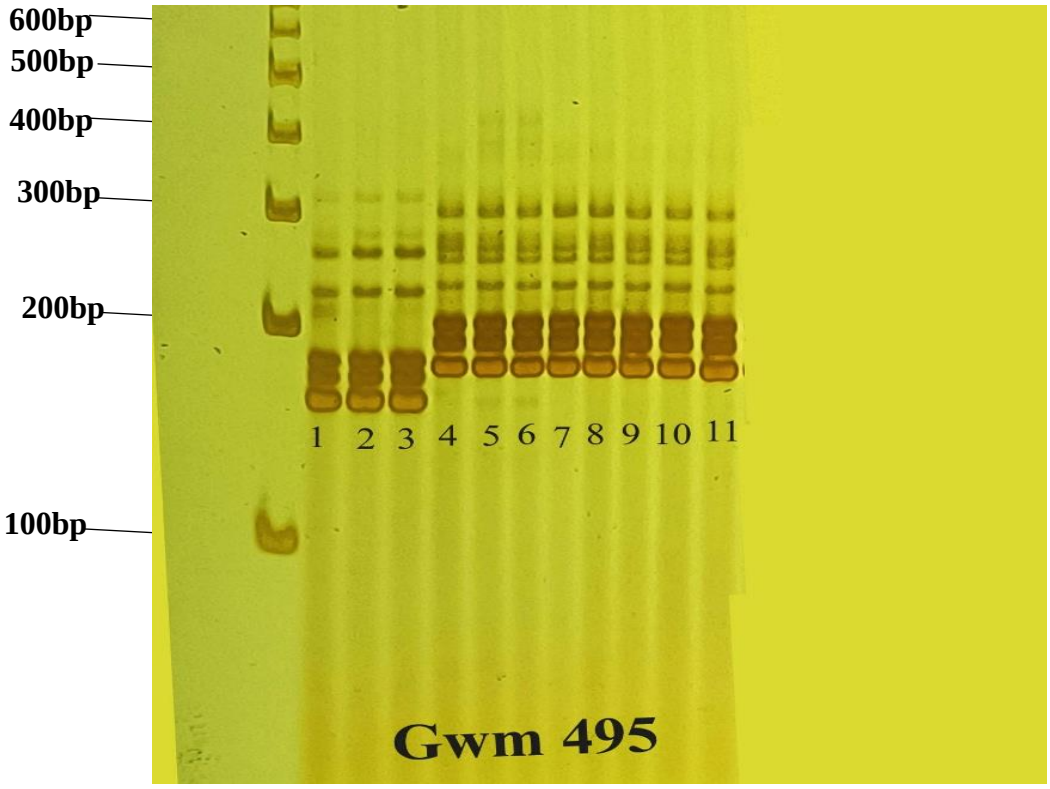

Figure 21: The DNA profile of wheat genotypes using **Gwm495**

4.7.1 Assessment of Polymorphism from SSR Profiles

The genetic diversity was measured by the estimation of Polymorphic information content (PIC). The most polymorphic microsatellite markers were Xwmc-112 and Gwm-495 with 9 alleles followed by Barc-20 with 7 alleles and TaBarc-101 with 6 alleles respectively (Table 18). The loci polymorphism was considered high, medium, or low if PIC > 0.5, 0.5 > PIC > 0.25, and PIC < 0.25 respectively (Vaiman *et al.,* 1994). The PIC values for the analyzed SSR markers ranged from 0.82 for TaBarc-101 to 0.87 for Gwm-495, with an average of 0.84 per marker (Table 18). The highest number of alleles per locus Gwm-495 and the highest PIC value 0.87 were estimated. Out of the 4 SSR markers used in the present study, all the markers had a PIC value greater than approximately 0.5. These markers appeared to be highly informative/polymorphic and could therefore be utilized in marker-assisted selection of wheat genotypes because they are capable of distinguishing between the genotypes. The PIC values which are recorded in this study are significantly higher than the PIC values reported in the other studies of Ahmed *et al.,* (2020), Salehi *et al.,* (2018), and Mardi *et al.,* (2011). In this study, the polymorphic bands revealed differences among the genotypes and thus would be used to examine and establish systematic relationships among genotypes. Earlier reports of Haque *et al.,* (2021) and Thungo *et al., (*2020) supported our findings.

Table 18: Number of alleles, allele range, and PIC values of 4 polymorphic markers

Serial	SSR loci	Number of alleles	Range of allele size (bp)	PIC
1	TaBarc-101	6	120-185	0.82
2	Xwmc-112	9	210-390	0.82
3	Barc-20	7	195-390	0.84
4	Gwm-495	9	160-300	0.87
	Average	7.75		0.84

4.7.2 Population structure of wheat varieties

In the 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. 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 the number of subpopulations in the main population. According to Evanno *et al.,* (2005) and Rodríguez-Ramilo *et al.,* (2014) test, the highest log-likelihood was performed and yielded K=2 (Figure 22). 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 (27.27%) of total genotypes i.e. 3 genotypes (HSTUW 1, HSTUW 2, and HSTUW 3) were pure, where not found any admixture. In the case, Population II comprised (72.73%) of total genotypes i.e. 8 genotypes; 7 genotypes (HSTUW 4, HSTUW 5, HSTUW 6, HSTUW 7, HSTUW 8, HSTUW 10, and BARRI Gom 32) were found pure and 1 genotype (HSTUW 9) were found heterogeneous. Phylogenetic dendrogram based on genetic distance revealed a similar trend to the population structure analysis, revealing two possible subpopulations using model-based STRUCTURE (Figure 23). Results revealed there are two possible divergent groups in tested representative wheat germplasms.

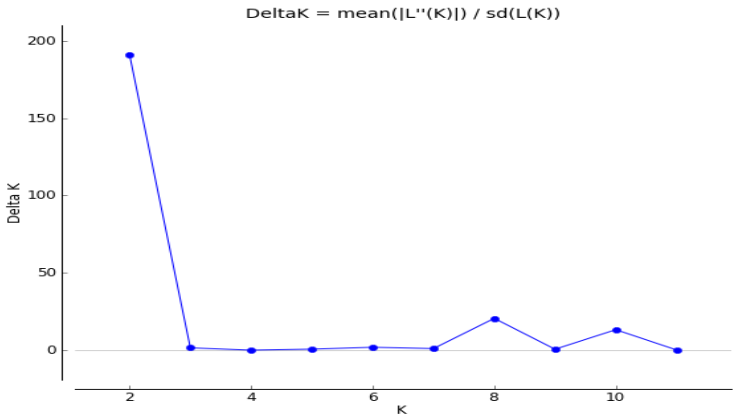

Figure 22: The best number of groups among locations estimated by Evanno test methods

(A) Sort by Q

(B) Plot in multiple lines

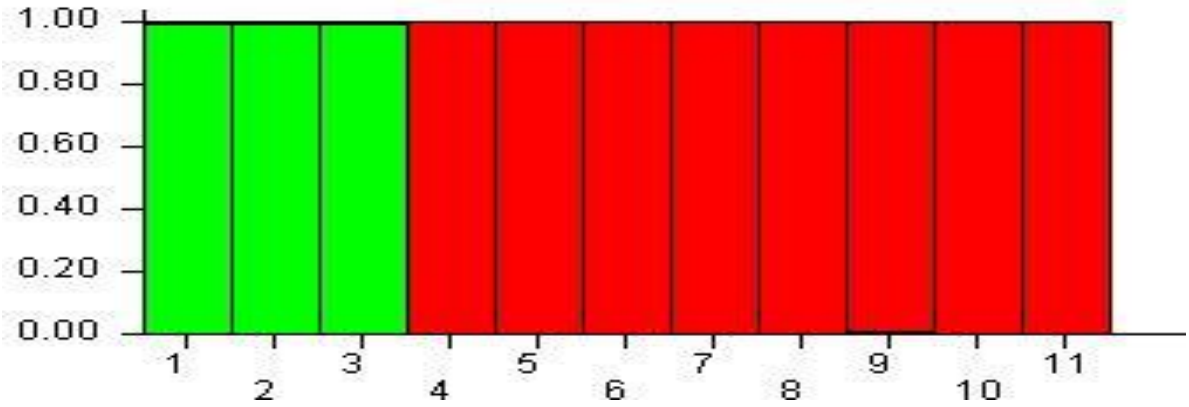

Figure 23: Model-based population structure plot for each isolate with K=2, using Structure with SSR markers data. Color codes are Population I green and Population II red (A). The code of each genotype (B) corresponds to the description in Table 1(1st table of all genotypes with sources)

4.7.3 Genetic diversity among the 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 24). These techniques have been applied in the wheat breeding scheme 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 2.5 and 4.5 indicating significant genetic variation among the wheat accessions studied. Both Groups could be further separated into three subgroups. Subgroup-1 comprised 4 genotypes, Subgroup-2 comprised 4 genotypes, and Subgroup-3 comprised 3 genotypes. The highest genetic similarity was observed between HSTUW 2 and HSTUW 3 (97%) followed by HSTUW 4, HSTUW 5, HSTUW 6 (100%), and HSTUW 7, HSTUW 8, and HSTUW 10 (85%) (Figure 24). So, HSTUW 2 and HSTUW 3 are genetically more similar while HSTUW 7 and HSTUW 8 HSTUW 10, and HSTUW 1 show a minimum of 85% divergent. In the case of heatmap clustering, the genotype and SSR microsatellite marker association show the different clustering groups based on their association (figure 25). Several studies using SSR have resulted in the successful clustering of wheat cultivars representing the diversity among their performance based on their molecular studies. This type of marker is very effective in delineating diversity based on parental source by grouping cultivars (Kitavi *et al.,* 2014) as well as groups 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.,* 2018) have been reported in genetic diversity studies.

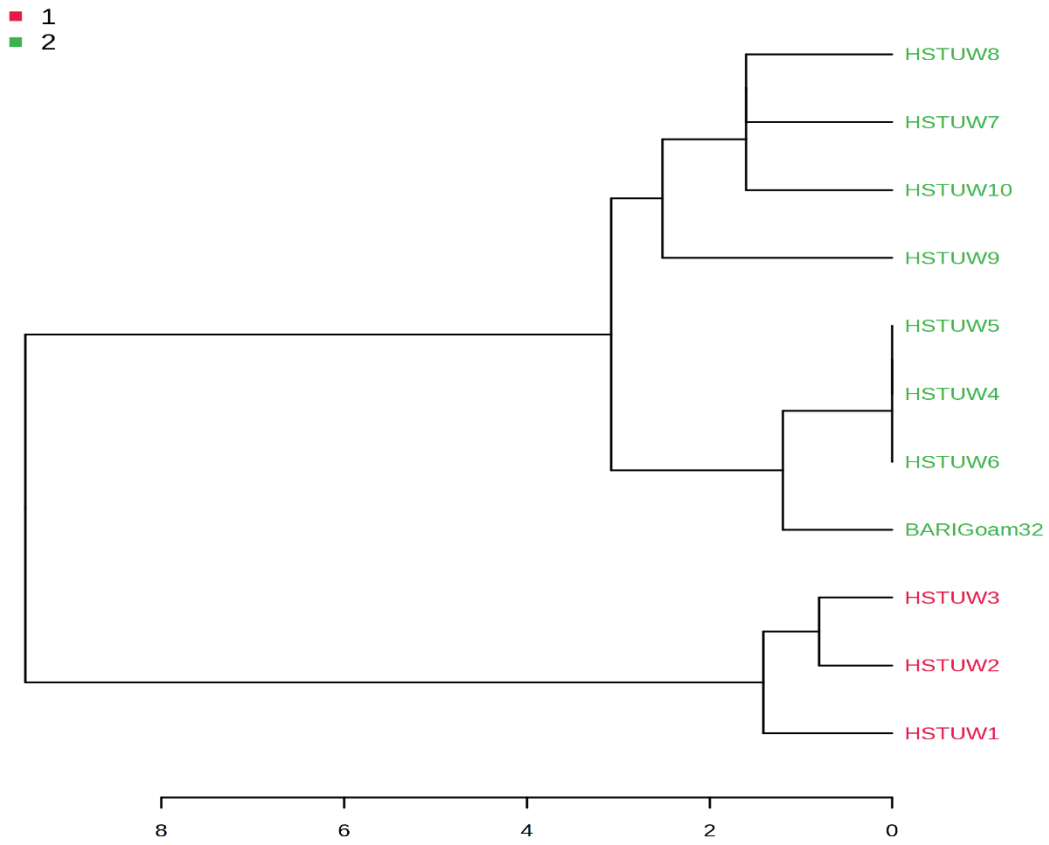

Figure 24: UPGMA tree displaying the distribution of the wheat genotypes in two groups, and presenting the genetic similarities and dissimilarities within and between the groups. Names of the genotypes are given on the termini of branches

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

Cluster	Size	Control	
		Genotypes	
I	3	HSTUW 1, HSTUW 2, and HSTUW 3	
II	8	Sub group I	HSTUW 4, HSTUW 5, HSTUW 6, and BARI Gom 32
		Sub group II	HSTUW 7, HSTUW 8, HSTUW 9, and HSTUW 10,

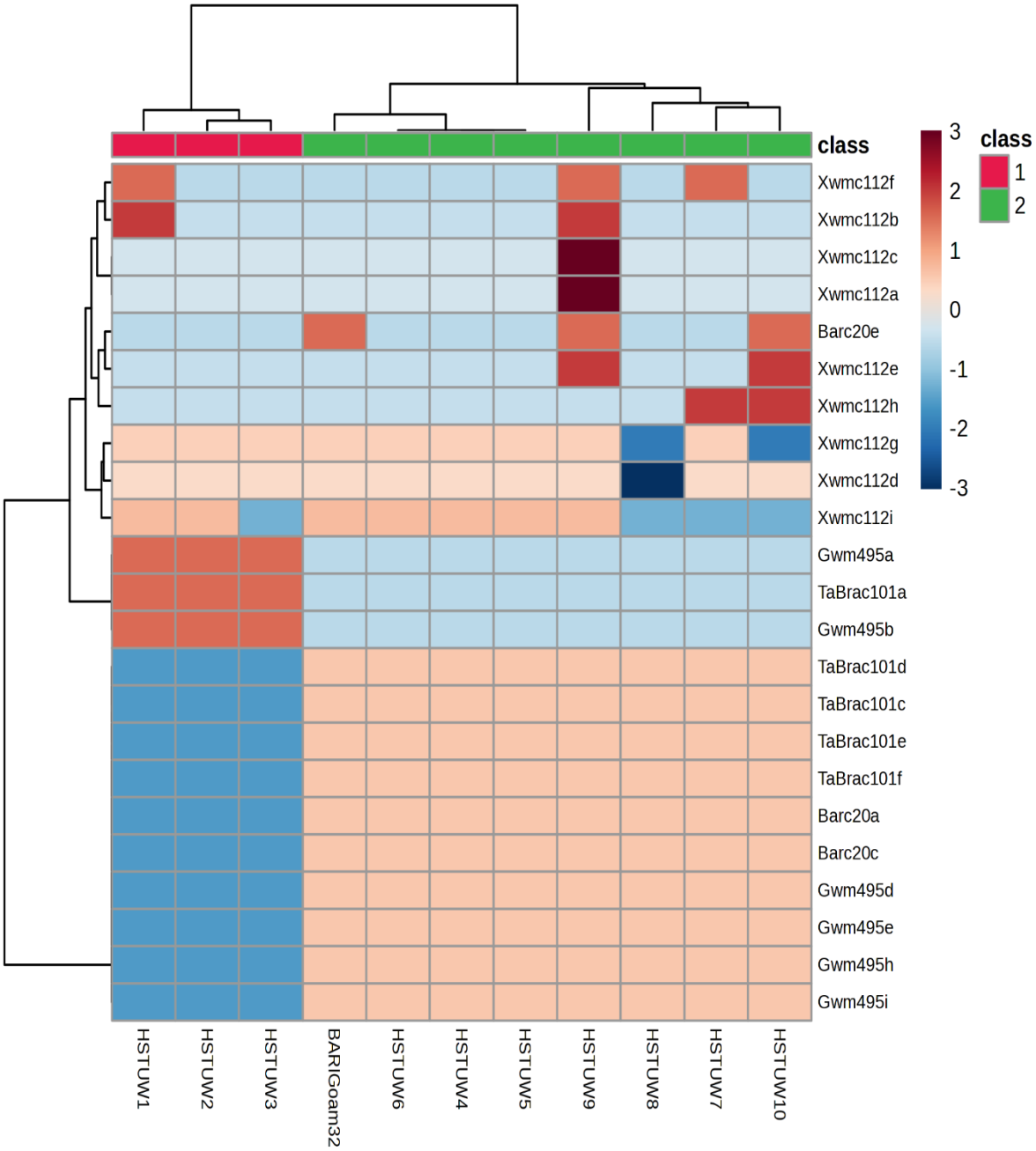

Figure 25: Heatmap cluster analysis based on genotypical traits

CHAPTER V

SUMMARY AND CONCLUSION

The present investigation was conducted with 11 wheat genotypes in RCBD design with three replications at Hajee Mohammad Danesh Science Technology University, Dinajpur, Bangladesh during the *Rabi* season, 2022-2023. The objectives of this research work were to study the mean performances of Heat tolerance advance wheat genotypes, to study the genetic variability and character associations, to estimate genetic diversity based on morpho-physiological traits, to characterize heat tolerance wheat genotypes based on SSR markers, and to identify superior line for multilocation yield trial based on both phenotypic and molecular genetic diversity outcomes.

The observations were recorded on ten randomly selected plants per genotype in each replication. Analysis of variance for yield and yield contributing characters revealed highly significant variations among the genotypes for all the traits, indicating the presence of an ample amount of variability which can be exploited for the selection of characters for crop improvement programs.

The mean squares against all the genotypes were found significant for all the characters studied. The mean performance of all traits, the grand mean, and the range value indicated that there was a wide range of variation among the studied genotypes for the quantitative traits. Mean performances of the 11 genotypes showed the highest yield per plot(g) recorded in HSTUW 1 (2812.73g) followed by HSTUW 8 (2671.95g), and HSTUW 4 (2665.91g) respectively. The genotypic coefficient of variation was 8.45 and the phenotypic coefficient of variation was 10.69 with a heritability of 63.73 and genetic advance in percentage of mean 10.04. In the case of 1000 grain weight, the average seed index was 41.89±1.73 g, and the range of 1000 grain weight was estimated at 31.99 to 47.23 consisting of the highest genotype of HSTU developed advanced line HSTUW 8, followed by BARI Gom 32, HSTUW 6, HSTUW 10 and HSTUW 4 respectively. The genotypic coefficient of variation was 11.65 and the phenotypic coefficient of variation was 13.68 with a heritability of 72.54 and genetic advance in percentage of mean 20.44. That result indicated that there was a high scope to isolate a good genotype for this character in the present materials. However, the number of spike per 1 square meter significantly differed from 351.67 - 505.67 with a mean value of 412.24±18.39 (Table 13). HSTUW 7 (351.67) produced the lowest number of spike per 1 square meter whereas HSTUW 1 (505.67) produced the highest number of spike per 1 square meter followed by BARI Gom

32 (483.33), HSTUW 2 (477.00), HSTUW 3 (435.33) and HSTUW 9 (385.00). The genotypic coefficient of variation was 12.44 and the phenotypic coefficient of variation was 14.65 with a heritability of 72.17 and genetic advance in percentage of mean 21.77.

The character average grain per spike ranged from (30.93 to 45.20) with a mean value of 40.08±1.49. The highest average grain per spike was found in HSTUW 9 (45.20), followed by HSTUW 5 (45.03), HSTUW 3 (44.50), HSTUW 7 (43.57), HSTUW 6 (41.63), and the lowest number of spike per 1 square meter was found in BARI Gom 32 (30.93). The genotypic coefficient of variation was 10.81 and the phenotypic coefficient of variation was 12.58 with a heritability of 73.87 and genetic advance in percentage of mean 19.14. whereas, the tallest genotype was HSTUW 4 (116.33cm). The genotypic coefficient of variation of the tallest genotypes was 7.76 and the phenotypic coefficient of variation was 8.04 with a heritability of 93.13 and genetic advance in percentage of mean 15.42. The lower days to heading and days to maturity were found at 51.67 days (HSTUW 1) and 102.33 days (HSTUW 1) Where the mean was 63.52±1.04 and 110.82±0.60 respectively. Additionally, The HSTUW 5 (10.73 cm) showed the largest spike length followed by HSTUW 9 (10.58 cm), HSTUW 4 (9.90 cm), HSTUW 7 (9.85 cm), HSTUW 6 (9.84 cm). The lowest average spike length HSTUW 1 was revealed at 8.58 cm. The average range of average spike length was measured from 8.58 cm to 11.94 cm with a mean value of 9.61±0.31. The genotypic coefficient of variation was 6.38 and the phenotypic coefficient of variation was 8.45 with a heritability of 57.10 and genetic advance in percentage of mean 9.94. Similarly, the character average grain weight per spike was HSTU-developed advanced line 1.888 g for HSTUW 5 with the Mean values of the parameter being 1.65 g with the SE value ±0.09. The genotypic coefficient of variation was 12.73 and the phenotypic coefficient of variation was 15.62 with a heritability of 66.47 and genetic advance in percentage of mean21.38. That result indicated that there was a high scope to isolate a good genotype for this character in the present materials.

The correlation coefficients of 11 wheat genotypes among a total of 9 yields and yield contributing characters showed that the yield per plot has a very strong positive significant correlation with 1000 grain weight (0.8292) at a 5% level of probability yield-contributing characters showed positive non-significant correlations with average plant height (0.4214), average grain weight per spike (0.3554), and negative non-significant correlations with days to heading (-0.0393), days to maturity (-0.1188), number of spike per 1 square meter (-0.1795), average spike length (-0.0584), and average grain per spike (-0.4879) at the genotypic level but at the phenotypic level, yield per plot has a significant correlation with 1000 grain weight

(0.5199) while the moderate positive significant association with average plant height (0.3459), But the character yield per plot exhibited a moderate negative significant association with average grain per spike (-0.4269), and had negative non-significant association with Days to maturity (-0.1123) and Days to heading (-0.0416) at phenotypic level.

All nine traits have at least one significant correlation with others at the genotypic and phenotypic level at phenotypic level indicating all the traits under study directly or indirectly have a relationship with yield per plot. The highest direct effect (3.573) was observed in Grain weight per spike at the genotypic level but at the phenotypic level, it was observed in 1000 grain weight (1.108). Grain weight per spike contributes indirectly through average spike length to yield per plot at the genotypic level. In the case of phenotypic level Grain weight per spike contributes indirectly through the number of spike per 1 square meter to yield per plot. Considering the situation, selection through 1000 grain weight, grain weight per spike, and average spike length may effectively increase yield per plot.

This present study was conducted with wheat genotypes. A total of four SSR/microsatellite markers were used to identify the variation among the genotypes. The SSR/microsatellite markers viz. Gwm495, Barc20, TaBarc101, and Xwmc112 were found polymorphic. A total of 31 alleles were detected and the number of alleles per locus ranged from 6 to 9 with an average of 7.75 alleles per locus

In the case of population genetic structure analysis, 11 wheat genotype data were generated by SSR marker data. 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. The highest log-likelihood was performed and yielded K=2. This means that all populations represent two distinct clusters. 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. The most polymorphic microsatellite markers were Xwmc-112 and Gwm-495 with 9 alleles followed by Barc-20 with 7 alleles and TaBarc-101 with 6 alleles respectively. The PIC values for the analyzed SSR markers ranged from 0.82 for TaBarc-101 to 0.87 for Gwm-495, with an average of 0.84 per marker (Table 18). The highest number of alleles per locus Gwm-495 and the highest PIC value 0.87 were estimated.

Here, population I consisted (27.27%) of total genotypes i.e. 3 genotypes (HSTUW 1, HSTUW 2, and HSTUW 3) were pure, where not found any admixture. In the case, Population II comprised (72.73%) of total genotypes i.e. 8 genotypes; 7 genotypes

(HSTUW 4, HSTUW 5, HSTUW 6, HSTUW 7, HSTUW 8, HSTUW 10, and BARRI Gom 32) were found pure and 1 genotype (HSTUW 9) were found heterogeneous. Phylogenetic dendrogram based on genetic distance revealed a similar trend to the population structure analysis, revealing two possible subpopulations using model-based STRUCTURE. The cluster analysis revealed two major clusters (Group I and II) with a similarity coefficient varying between 2.5 and 4.5 indicating significant genetic variation among the wheat accessions studied. Both Groups could be further separated into three subgroups. Subgroup-1 comprised 4 genotypes, Subgroup-2 comprised 4 genotypes, and Subgroup-3 comprised 3 genotypes. The highest genetic similarity was observed between HSTUW 2 and HSTUW 3 (97%) followed by HSTUW 4, HSTUW 5, HSTUW 6 (100%), and HSTUW 7, HSTUW 8, and HSTUW 10 (85%). So, HSTUW 2 and HSTUW 3 are genetically more divergent while HSTUW 7 and HSTUW 8 HSTUW 10, and HSTUW 1 show a minimum of 85% divergent.

In conclusion, variation is one of the important pre-conditions for effective selection. Significant variation was observed in the base population. There was variability among existing HSTU-developed lines and the trait found through path analysis has a direct effect on Average grain per spike, especially 1000 grain weight. If 1000 grain weight can be carefully selected then it will help to select good yielding wheat lines. Especially if 1000 grain weight plays a positive role, then if we select the lines that have bold grains and besides, if we consider Average grain per spike, then we can easily select advanced lines of wheat with high yield. Then, if we do further selection with these two characters' 1000-grain weight and Average grain per spike the selection will be very effective. And later, the genetic advance will be found in the selected lines. Concerning the above discussion, we have found that there was variability at the molecular level and in the lines by using different primers/markers. So, finally, as a part of our detailed research, the variability of HSTU-developed existing lines, 1000 grain weight, and Average grain per spike are two very good traits, based on which will help us to select the breed later.

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