SELECTION OF DROUGHT TOLERANT PARENTAL LINES OF WHEAT THROUGH MORPHO-MOLECULAR DIVERSITY AND MULTI-TRAIT GENOTYPE-IDEOTYPE DISTANCE INDEX

A THESIS

BY

NUSHRAT ZEMIN

STUDENT NO. 1701254

SEMESTER: July-December, 2023

SESSION: 2022-23

MASTER OF SCIENCE (MS)

IN

GENETICS AND PLANT BREEDING

DEPARTMENT OF GENETICS AND PLANT BREEDING

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY

DINAJPUR-5200

DECEMBER 2023

SELECTION OF DROUGHT TOLERANT PARENTAL LINES OF WHEAT THROUGH MORPHO-MOLECULAR DIVERSITY AND MULTI-TRAIT GENOTYPE-IDEOTYPE DISTANCE INDEX

A THESIS

BY

NUSHRAT ZEMIN

STUDENT NO. 1701254

SEMESTER: July-December, 2023

SESSION: 2022-23

Submitted to the Department of Genetics and Plant Breeding

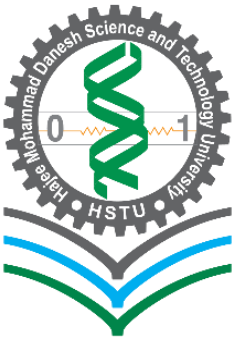
Hajee Mohammad Danesh Science and Technology University, Dinajpur

In partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE (MS)

IN

GENETICS AND PLANT BREEDING



DEPARTMENT OF GENETICS AND PLANT BREEDING

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY

DINAJPUR-5200

DECEMBER 2023

SELECTION OF DROUGHT TOLERANT PARENTAL LINES OF WHEAT THROUGH MORPHO-MOLECULAR DIVERSITY AND MULTI-TRAIT GENOTYPE-IDEOTYPE DISTANCE INDEX

A THESIS

BY

NUSHRAT ZEMIN

STUDENT NO. 1701254

SEMESTER: July-December, 2023

SESSION: 2022-23

Approved as to style and content by

_____	_____
(Professor Dr. Md. Arifuzzaman)	**(Dr. Md. Zaherul Islam)**
Supervisor	**Senior Scientific Officer, BWMRI**
	Co-Supervisor

_____

(Professor Dr. Md. Hasanuzzaman)
Chairman

DEPARTMENT OF GENETICS AND PLANT BREEDING

HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY

DINAJPUR-5200

DECEMBER 2023

Professor Dr. Md. Arifuzzaman
Department of Genetics and Plant Breeding
Hajee Mohammad Danesh Science & Technology University
Dinajpur-5200, Bangladesh
Phone No. : +8801718192717

Certification

This is to certify that the thesis entitled **"SELECTION OF DROUGHT TOLERANT PARENTAL LINES OF WHEAT THROUGH MORPHO-MOLECULAR DIVERSITY AND MULTI-TRAIT GENOTYPE-IDEOTYPE DISTANCE INDEX"** is a study, prepared by the examinee, bearing Registration No.: 1701254 of the Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The authoress has submitted this thesis to the Department as a partial fulfillment for the requirements of the degree "Master of Science in Genetics and Plant Breeding", is a record of original research work carried out by her under my supervision. The work is an original, unique one and to the best of my knowledge, no part of the thesis has been produced elsewhere for any other degree or diploma.

……..……..

(Professor Dr. Md. Arifuzzaman)

Supervisor

In the Name of Allah

&

My Beloved Parents and

Honorable Teachers

ACKNOWLEDGEMENTS

All gratitude is due to Allah Almighty, the omniscient, omnipresent, and omnipotent creator of all that exists, who enabled me to pursue an education in Agriculture and to accomplish and submit the thesis for the Master of Science (MS) degree in Genetics and Plant Breeding.

***Professor Dr. Md. Arifuzzaman**, Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur, has my heartfelt gratitude and profound respect for his helpful advice, scholastic guidance during planning and execution of the research, valuable suggestions, continuous support, and all sorts of support and help throughout the period of research work and preparation. His insightful comments and precise edits of this dissertation likewise merit sincere appreciation. **Dr. Md. Zaherul Islam**, Senior Scientific Officer, Bangladesh Wheat and Maize Research Institute, Dinajpur, has provided valuable suggestions, sincere assistance, scholarly instructions, and careful corrections to the dissertation's manuscript, for which I am profoundly grateful.*

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

I want to take this opportunity to thank the personnel of the Department of Genetics and Plant Breeding at the Hajee Mohammad Danesh Science and Technology University in Dinajpur for their cooperation and support throughout the study.

I would like to say thanks, to my classmates, friends and junior students of Genetics and Plant Breeding department for their active encouragement and inspiration.

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

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

The Author *December 2023*

SELECTION OF DROUGHT TOLERANT PARENTAL LINES OF WHEAT THROUGH MORPHO-MOLECULAR DIVERSITY AND MULTI-TRAIT GENOTYPE-IDEOTYPE DISTANCE INDEX

ABSTRACT

Drought is a major abiotic stress that significantly reduces wheat yield in the country. For this, the major aim of the study was to identify drought tolerant wheat genotypes from 100 diverse wheat genotypes. During the Rabi season of 2022–2023, the investigation was conducted at the experimental farm of Bangladesh Wheat and Maize Research Institute (BWMRI), Debiganj, Panchagarh and in the laboratory of the Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur and BWMRI, Dinajpur, Bangladesh. This study used a split plot design with two replications to examine twelve morpho-physiological traits: plant height, number of tillers per plant, proline content, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, root volume, root length, root shoot ratio, relative water content, and excise leaf water loss. The analysis of variances revealed significant high heritability was recorded for proline content, shoot dry weight and root dry weight. A significant positive correlation between root dry weight and shoot dry weight was observed in both control and drought conditions. Based on the morphological diversity 1HZWYT-425, 1HZWYT-430 RAJ, 11SATYN-9425, Jamal-10032, 1HZWYT-422 RAJ, BAW-1397, Jamal-9046, SABGPYT-4104, Jamal-9006 were identified in cluster I and cluster III as similar genotypes at drought condition. Jamal-9046, SABGPYT-4104, 1HZWYT-425, SABGPYT-4110, 1HZWYT-430 RAJ, 11SATYN-9425, Jamal-10032, Jamal-9006, 1HZWYT-422 RAJ and BAW-1397 were identified as drought tolerant genotypes among 100 genotypes through assessment of multi-trait genotype ideotype index (MGIDI). Based on the molecular diversity five SSR markers viz. WMS0691, GWM513, GWM495, Barc20 and Tagwm1037 were evaluated in 96 wheat cultivars for molecular characterizations. All of the SSRs were polymorphic across the 96 genotypes. In total, 24 alleles were detected and the number of alleles per locus ranged from 2 to 7 with an average of 4.8 alleles per locus. The most polymorphic alleles (seven) were produced by the marker GWM495, followed by Tagwm1037 (6), GWM513 (5), and WMS0691 (4). In contrast, Barc20 markers yielded the fewest polymorphic bands per locus of two (2). The highest PIC value (0.84) was recorded for GWM495, and GWM513 recorded second highest (0.78) and lowest by Barc20 (0.46). The genotypes 1HZWYT-425, 1HZWYT-430 RAJ, 11SATYN-9425, Jamal-10032, 1HZWYT-422 RAJ, BAW-1397, Jamal-9046, SABGPYT-4104, and Jamal-9006 were found to be comparable in cluster III, V, and VI based on molecular diversity.

Results obtained from the above two study suggested that the genotypes 1HZWYT-425, 1HZWYT-430 RAJ, Jamal-9046, 1HZWYT-422 RAJ and BAW-1397 should be important genetic materials for taking any future drought stress breeding programs in wheat.

CONTENTS

CHAPTER	TITLE		PAGE
	ACKNOWLEDGEMENT		**i**
	ABSTRACT		**ii**
	CONTENTS		**iii-vi**
	LIST OF TABLES		**vii-viii**
	LIST OF FIGURES		**ix**
	LIST OF APPENDICES		**x**
	SYMBOLS AND ABBREVIATION		**xi-xii**
I	**INTRODUCTION**		**1-4**
II	**REVIEW OF LITERATURE**		**5-13**
	2.1.	Occurrence of drought and its effects	5
	2.2.	Mechanism of drought tolerance	6
	2.3.	Genetic parameter studies in wheat	7
	2.4.	Correlation and regression analysis in bread wheat germpalsms	8
	2.5	Multivariate cluster analysis and principal component analysis	9
	2.6	Multi-trait Genotype Ideotype Distance Index (MGIDI)	10
	2.7	SSR Marker based DNA fingerprinting	11
	2.8	SSR based genetic diversity in Wheat	13
III	**MATERIALS AND METHODS**		**14-35**
	3.1	Experimental site and period	14
	3.2	Climate	14
	3.3	Germplasm	14
	3. A) Morpho-physiological characterizations of wheat genotypes at seedling stage under drought conditions		16
	3.4	Experimental design and set up	16
	3.5	Soil	16
	3.6	Collection of sand	17
	3.7	Preparation of sand	17

CONTENTS(Contd.)

CHAPTER	TITLE			PAGE
	3.8	Preparation of plastic pot		18
	3.9	Sowing of seeds		18
	3.10	Intercultural operation		19
		3.10.1	Irrigation	19
		3.10.2	Weeding	19
		3.10.3	Thinning	19
	3.11	General observation of the experimental pot		21
	3.12	Data recording		22
	3.13	Statistical analysis		24
		3.13.1	Mixed model analysis	25
		3.13.2	Estimation of genotypic and phenotypic Variances	26
		3.13.3	Estimation of heritability	27
		3.13.4	Descriptive analysis	28
		3.13.5	Partial correlation analysis	29
		3.13.6	Multivariate analysis	29
		3.13.7	Factor analysis	30
		3.13.8	Principal component analysis	31
		3.13.9	Multi-trait Genotype -Ideotype Distance Index	31
		3.13.10	Strengths and weaknesses	32
	3. B) Molecular characterization and diversity analysis in wheat using SSR markers			
	3.14	Genomic DNA Isolation		33
	3.15	DNA Quantification		33
	3.16	PCR Amplification and Electrophoresis Separation		34
	3.17	Microsatellite/Simple Sequences Repeat (SSR) Markers		34
	3.18	Molecular Statistical Analysis		35

CONTENTS(Contd.)

CHAPTER	TITLE			PAGE
IV	**RESULTS AND DISCUSSION**			**36-84**
	4. (A) Morpho-physiological characterizations of wheat genotypes at seedling stage under drought conditions			
	4.1	Mixed-model analysis		36
		4.1.1	Likelihood ratio test and variance Components	**37**
		4.1.2	Genetic parameter studies under control, drought and META Estimation of correlation coefficient of twelve characters	**39**
		4.1.3	Overall Performance of 100 Wheat Genotypes at 12 Morpho-Physiological Traits	**41**
		4.1.4	BLUPg (Best Linear Unbiased Prediction for Genotype Effect)	**43**
	4.2	Estimation of correlation coefficient of twelve characters		**45**
	4.3	Multivariate Analysis		**47**
		4.3.1	Cluster Analysis under drought condition	**49**
	4.4	Multi-trait genotype–idiotype distance index (MGIDI), genotype selection and trait adaptation		**51**
		4.4.1	Principle Component Analysis (PCA)	**52**
		4.4.2	Factor Analysis	**53**
		4.4.3	Selected genotypes and their strength and weaknesses	**54**
	4. (B) Assessment of genetic diversity in 100 wheat genotypes through molecular study			**57**
	4.5	Analysis of DNA fingerprinting based on SSR markers		**58**

CONTENTS(Contd.)

CHAPTER	TITLE	PAGE
	4.5 Analysis of DNA fingerprinting based on SSR markers	58
	4.6 Assessment of polymorphism from SSR Profiles	59
	4.7 UPGMA dendrogram	81
	4.8 Principal component analysis	83
	4.9 Interrelationship of phenotypic and molecular outcome	84
V	**SUMMARY AND CONCLUSION**	**125-129**
	REFERENCES	**130-144**
	APPENDICES	**145-148**

LISTS OF TABLES

TABLE	TITLE	PAGE
1	Plant materials used in the experiment	33-34
2	Doses and method of application of fertilizers for wheat	47
3	SSR Markers used for diversity analysis of wheat germplasms	50
4A	LRT for the genotypic effect showing the significance of traits in single treatment analysis	51
4B	Likelihood ratio test (LRT) for the genotypic effect showing the significance of traits in the genotype-environment interaction for multi-environmental trial analysis	52
5.1	Genetic parameter for control and drought condition	59
5.2	Genetic parameter for META	65
6	The statistical measures for the traits observed in control and drought condition: mean (x), confidence interval of mean (CI), and average deviation (AD)	92
7	Performance of the 100 wheat genotypes under control conditions	92
8	Performance of the 100 wheat genotypes under drought conditions	96
9	Blupg (Best unbiased linear predicted genotype) value observed by 100 genotypes through 12 morphological traits	
10	Distribution of 100 Wheat in six different clusters in drought condition	96
11	Cluster means for 12 different characters of 100 genotypes at drought condition	102
12	Intra (bold) and inter-cluster distance of 6 clusters of 100 genotypes	
13	Eigenvalue estimates by principal components analysis and the proportion of variance explained by them	102

LISTS OF TABLES (Contd.)

TABLE	TITLE	PAGE
14.1	Explained variance, factorial loadings after varimax rotation, and communalities obtained in the factor analysis considering control condition	103
14.2	Predicted genetic gains of the selected genotypes through multi-trait genotype-ideotype distance index considering control condition	103
15.1	Explained variance, factorial loadings after varimax rotation, and communalities obtained in the factor analysis considering drought condition	108-109
15.2	Predicted genetic gains of the selected genotypes through multi-trait genotype-ideotype distance index considering drought condition	115
16	Allele number, size range and polymorphism information content (PIC) found among 100 wheat genotypes for 5 microsatellite markers	119
17	Distribution of 100 Wheat in six different clusters by Molecular analysis	

LISTS OF FIGURES

FIGURE	TITLE	PAGE
1	Measuring of root length, root dry weight & root volume	32
2	Estimation of proline content in laboratory	38
3	DNA extraction, quantification and amplification of polymerase chain reaction estimation of proline content in laboratory	
4	Histogram of observed value different morphological traits among genotypes in control condition	
5	Histogram of observed value different morphological traits among genotypes in drought condition	39
6	Correlations among 12 different characters of wheat under control conditions and drought conditions	54
7	Dendrogram from UPGMA clustering for 100 wheat genotypes using Euclidean genetic distance based on twelve morphological characters under drought condition	84
8	The strength and weakness view among selected genotypes control and drought condition.	88
9	Multi-trait genotype ideotype distance index considering two treatments: (a) control; (b) drought.	89
10	Gel picture of microsatellite markers obtained from polyacrylamide gel electrophoresis using 100 bp DNA ladder.	93
11	An UPGMA cluster dendrogram showing the genetic relationships between 96 wheat genotypes based on the alleles detected by 5 microsatellite markers.	97
12	Multivariate heatmap illustrating the genetic diversity of 96 wheat genotypes based on the 5 SSR markers.	101
13	Two-dimensional principal component analysis (PCA) based on SSR polymorphisms in the 96 wheat genotypes.	106
14	The scree plot displays the principal coordinate analysis of the top 5 PCs for 96 wheat genotypes based on SSR marker data.	111-113

LISTS OF APPENDICES

APPENDIX	TITLE	PAGE
I	Map of Dinajpur Sadar Upazila showing the experimental area	145
II	Weather data of the experimental site during the period from December, 2022 to February, 2023	146
III	The soil nutrient composition of Genetics and Plant breeding Research plot of HSTU, Dinajpur	146
IV	Some photographs of research work	147-148

SYMBOLS AND ABBREVIATION

AEZ	:	Agro Ecological Zone
ANOVA	:	Analysis of Variance
BBS	:	Bangladesh Bureau of Statistics
BLUPg	:	Best Linear Unbiased Prediction for Genotype Effect
cm	:	Centimeter
CRD	:	Completely Randomized Design
CV	:	Coefficient of Variance
DAS	:	Days After Sowing
DAP	:	Di-Ammonium Phosphate
d.f.	:	Degree of Freedom
e.g.	:	For Example
etc.	:	And other similar things
et al.	:	And others people
FAOSTAT	:	Food and Agriculture Organization of the United Nations
FAO	:	Food and Agriculture Organization
g	:	Gram
GCV	:	Genotypic Coefficient of Variance
PCV	:	Phenotypic Coefficient of Variance
GE	:	Genotype by Environment interaction
GGE	:	Genotype and Genotype by Environment interaction
i.e.	:	That is
K	:	Potassium
kg	:	Kilogram
MGIDI	:	Multi-trait Genotype Ideotype Index
MoP	:	Muriate of potash
MS	:	Mean Sum of Square
MSe	:	Error Mean Square
MSg	:	Genotype Mean square
N	:	Nitrogen
OM	:	Organic Matter
P	:	Phosphorus
PAB	:	Pure and Applied Biology

PBI	:	Plant Breeding Institute
PCA	:	Principal Component Analysis
P^H	:	Potential for Hydrogen
rg	:	Genotypic correlation
RSS	:	Residual sum of squares
S	:	Sulfur
SE	:	Standard Error
SPAD	:	Soil Plant Analyses Development
SNP	:	Single-nucleotide polymorphism
Std. dev.	:	Standard deviation
STAR	:	Statistical Tool for Agricultural Research
USDA	:	United States Department of Agriculture
Zn	:	Zinc
°C	:	Degree Celsius

CHAPTER I

INTRODUCTION

The second most important commercially produced grain crop in the world is bread wheat (*Triticum aestivum L*., 2n=6x=42, AABBDD). It belongs to the genus *Triticum* and family 'Gramineae (Poaceae)'. Cultivated bread wheat is an allohexaploid, composed of three distinct genomes, A, B and D. Bread wheat genome is broad and narrow simultaneously. It is due to low frequency of hybridization between male *Aegilops tauschii* (DD) and female cultivated emmer *Triticum dicoccum* (AABB) naturally.

Wheat is a staple food for millions of people, being the second-most globally produced cereal after maize. Wheat provides 70% carbohydrate, 12% protein, 22% crude fiber, 2% fat, and essential micronutrients such as zinc and iron (Lemmens *et al.,* 2018; Alomari *et al.,* 2019). By 2050, global wheat production must be increased by 60%, from 3.5 tons per hectare, to nourish about 9 billion people (Borisjuk *et al.,* 2019; Langridge *et al.,* 2013). In the year 2023/2024, the global production volume of wheat amounted to almost 785 million metric tons (Bheel, 2024).

In Bangladesh, Bangladesh's wheat production for the 2020-21 marketing year remains stable at 1.25 million tons. The United States Department of Agriculture (USDA) forecasted that Bangladesh to import 5.12 million tons of wheat in the marketing year 2022-23 ($USDA_2$, 2024). The production of the 2023 winter wheat crop, harvested in the month of early to mid-April is officially estimated at 1.1 million tons, on the point of the 5-year average (FAO_2, 2023).

Recent work delineates the intensive impacts of global climate change on crop production not simply unanimously declared its negative impact on worldwide food production however conjointly expected the additional severe influence (Asseng *et al.,* 2019; Pravalie *et al.,* 2020, Khan *et al.,* 2020). Wheat can be produced in a varied range of agro-climatic environments; nevertheless, most of these environments have drought stress as one of the major constraints to their production and yield. The predicted global warming and climatic fluctuations will increase the frequency of drought, therefore leading to losses in wheat yield (Rijal *et al.,* 2021). However, wheat production is severely affecting and reducing by 29% due to various abiotic stresses, especially drought stress (Daryanto *et al.,* 2016 and Manickavelu *et al.,*

2012). Water shortage caused Around 17 -70% yield losses in cereals (Ahmed *et al.,* 2019a, 2019b, 2019c).

Drought is one of the most limiting factors especially in warm dry areas yielding crops (Qadir, 2018) that affect the growth and development of plants. Drought could be a terribly complicated natural hazard and encompasses a negative impact on the global ecosystem as a whole. Recently, over the past few decades, Bangladesh has been suffering from significant crop production losses due to high levels of climate variability (Prodhan *et al.,* 2020). North western region, namely, the Barind tract, is one of the largest drought-affected areas of Bangladesh (Islam *et al.,* 2019).

Wheat is grown in Bangladesh under irrigated & rainfed conditions during the winter seasons of November to March. This is typically not the period when there is a lot of precipitation. The groundwater level in Bangladesh is therefore rapidly decreasing every day as a result of the excessive usage of subsurface water for irrigation. Consequently, later phases of drought stress on wheat result in a sharp reduction in grain production (Jahan *et al.*, 2017).

Drought stress leads to inhibition of chemical activities that are related to decreasing in pigment content, plasma membrane stability, inflicting loss of membrane porosity and harm to the varied physiological and organic chemistry functions that eventually have an effect on the expansion of plant (Ma *et al.*, 2017, Ahmed *et al.,* 2019a, 2019b, 2019c).

Developmental response of plants to drought stress is manifested through enhanced root growth and suppressed shoot growth resulting in increased root shoot ratio (Sharp *et al.,* 2004, Yamaguchi and sharp, 2010 and Xu *et al.,* 2013). Considering the organs that could be most important in drought, roots are usually the earliest organ to perceive the drought stress and then communicate this to shoots and leaves (Bianco *et al.*, 2018). A combination of 20% faster root descent and more efficient roots can result in more effective water extraction from sub-soil (roots below 60cm) and provide yield benefits of 0.32-0.44 t ha^{-1} in wheat (Lilley and Kirkegaard,2011). Drought stress usually inhibits shoot growth but stimulates root growth to accelerate the remobilization of photo-assimilates from shoots to roots to cope with drought stress (Yamaguchi and Sharp, 2010). So, it is considered that water stress is usually less detrimental to grain yield when occurring early in the crop cycle (Blum, 1996). To survive under abiotic stresses plants have developed various adaptive strategies that manifest

in them morphological, physiological, developmental and molecular changes (Bohnert *et al.*, 1995 and Bray, 1997).

Drought-tolerance of wheat genotypes is a complex trait, being affected by several factors, including growth conditions, physiology, genotype, developmental stage, severity, and duration of drought, which implies diverse gene expression patterns and complex signaling pathways (Budak *et al*. 2015). Understanding the genetic, physiological, and biochemical mechanisms involved in the response of wheat genotypes to drought is useful to design breeding strategies for the selection of superior and drought-resilient genotypes (Nardino *etal*. 2022). However, understanding such mechanisms often requires genome wide association studies (Ballesta *et al*. 2020), in addition to physiological assessments and biochemical analyses (Abid *et al.,* 2018), which are often difficult and time-consuming. Alternatively, the response of wheat genotypes to drought can be easily assessed under field conditions by observing the impacts of the stress on agronomic traits (Sallam *et al*. 2018)

The selection of superior wheat genotypes through multivariate strategies can be designed using the multi-trait genotype ideotype distance index (MGIDI) (Olivoto and Nardino 2020), since this index is accurate, easy to interpret, and free of weighting coefficients and multi-collinearity problems. The single-environment multi-trait genotype–idiotype distance index (MGIDI) for simultaneous selection, considering mean performance and stability in the analysis of multi-environments using both fixed and mixed-effect models proposed by researchers (Olivoto, Lúcio, da Silva, Marchioro *et al.,* 2019; Olivoto, Lúcio, da Silva, Sari *et al.,* 2019). Moreover, most existing studies use phenotypic data to make inferences about the genotypes, which may not represent the true genetic value of individuals. Alternatively, the estimation of variance components by restricted maximum likelihood (REML) and prediction of genetic values by the best linear unbiased prediction (BLUP) allow the inference about best genotypes efficiently and accurately (C. Machado e Silva *et al*.2023). Factor analysis (FA) by giving a factorial score, idiotype design based on desirable and undesirable factors, and estimation of spatial probability based on genotype–idiotype distance enables researchers to measure genotype ranking (Rocha *et al.*, 2018). Due to variations in the environmental conditions, the genotype performance may vary from strength to weakness and vice versa (Al-Ashkar *et.al*. 2023).

The selection of parental genotypes for hybridization using effective molecular markers is a useful strategy. It permits speedy identification and choice of genetically unrelated oldsters

for strategic crosses. Hence practical genes may be recombined in dominant yield and yield-related traits, and quality traits for choice (Jernigan *et al.*, 2018; Sonrnezoglu and Terzi, 2018). Molecular markers provide a direct measure of genetic diversity and circumvent the environmental influences, providing complementary data through an efficient assessment of genetic diversity in crop genetic resources (Verma *et al.*, 2019). Several molecular markers have been used in the genetic analysis of bread wheat (Kumar *et al.*, 2019). SSR markers are extensively used in genetic studies because they are chromosome-specific, contain high polymorphic information, multi-allelic, and are omnipresent across the genome. Simple sequence repeats (SSR) markers are widely effective in drought tolerance genetic characterization in bread wheat (Sonmezoglu and Terzi, 2018) and have great sources of use for genetic diversity analysis because of their high level of polymorphism (Tamar *et al.*, 2016; Abbasabad *et al.*, 2017). Very few studies have investigated genetic diversity and molecular characterization in wheat genotypes in Bangladesh.

Considering the above facts, the research hypothesis might be the identification of potential drought tolerance genotypes. This might be achieved through suitable phenotypic selection methods like MGIDI and molecular characterization of the genotype Therefore, the research was undertaken to find out the drought-tolerant and diverse parents of wheat for future drought tolerance breeding using different wheat genotypes together with Bangladesh Wheat and Maize Research Institute (BWMRl) released varieties through the phenotypic and molecular genotypic data.

Therefore, this study was conducted to achieve the following specific objectives:

-To study the genetic variability and heritability of different morphological traits under controlled and drought-stressed conditions.

-To study cluster analysis and principal component analysis using morphological traits under controlled and drought-stressed conditions.

-To select the best plant genetic materials based on multi-trait assessment through the MGIDI index in early growth.

-To identify diverse parents for drought tolerance breeding in wheat using SSR markers.

CHAPTER II

REVIEW OF LITERATURE

Wheat is the world most important cereal crop in terms of production and area. It has been grown in a wide range of arid and semi-arid areas, where drought occurs frequently because of rainfall fluctuations in rain-fed regions, and water scarcity in irrigated regions. Drought stress tolerance is a complex trait that is obstructed by low heritability and deficiency of successful selection approaches. Therefore, selection of wheat genotypes should be adapted to drought stress. In addition, drought tolerance mechanism should be identified during the development of new cultivars in order to increase the productivity.

For the purpose of improving this crop, many researchers from around the world have worked on various aspects. The following beads contain a brief summary of the literature that is relevant to the goals of the current study.

2.1 Occurrence of drought and its effects

Global warming is a threat to world food security and lack of rain as a result of it has severely affected food security. The temperature increase has a direct impact on water resources and agricultural activities, leading to more severe drought. Agricultural production is threatened by a spring agricultural drought (between February and April) between 2050 and 2100 under the RCP4.5 scenario, which can have serious consequences on agricultural income as well as food security (Senna *et al.*, 2022). Plant productivity is declining because of various climatic events that have increased or changed, and they threaten global food security (Mickelbart*et al.*, 2015).

When plants are exposed to abiotic stress conditions such as drought, salinity, excessive rainfall and high temperature, this affects the development and growth of the plant negatively and it causes metabolic and physiological changes in the plant. Changing climate events are predicted to cause an increase in the frequency of floods, drought and high temperatures (Bita and Gerats 2013). In these events, drought is the major abiotic stress factor that adversely affects crop production and quality especially wheat. The wheat being affected negatively from drought and climate changes makes the situation worse (Huseynova and Rustamova, 2010).

Water deficit or drought is one of the many factors to which plant's metabolic machinery responds (Thomason *et al.,* 2018). Photosynthesis, stomatal activity, enzymes, adenosine triphosphate (ATP) synthesis, respiration, and many other important phenomena taking place in plants arc adversely affected by physiological stress, and if the stress is prolonged, plant growth and productivity arc severely affected. Among different biochemical responses, osmolyte biosynthesis and Function, water flux control, and membrane transport of ions for maintenance and reestablishment of homeostasis arc considered to be affected in particular (Hasegawa *et al.,* 2000).

Drought stress not only affects plant growth and development but ultimately productivity in almost all the cereals, thus it is one of the most serious threats to world agriculture (Hamayun*et al.,* 2010 and Subhani*et al.,* 2011). The situation demands crop breeding for drought stressed areas utilizing using traditional along with modern molecular techniques. The genes for drought tolerance arc regulated at once under drought conditions and produced the respected products that response to signal transduction, stress response and help the plant to withstand under drought stress (Zhou *et al.,* 2010).

Wheat production may decline substantially in China, India and Russia due to climate variability. Yield losses due to drought depend on the growth stage and severity of stress (Daryanto *et al.* 2016). To cope with the changing climatic conditions, breeding for drought tolerance using novel genetic resources is the most important strategy (Mwadzingeni*et al.* 2016). However, due to limited availability of resistance resources, it is not satisfactory for progress in breeding drought tolerant cultivars.

2.2 Mechanism of drought tolerance

Avoiding drought is an extremely important adaptation for survival in a water limiting environment. However, plant physiologists are generally more interested in plants that are able to tolerate drought (i.e., plants that have evolved n number of anatomical, developmental. biochemical, physiological and molecular adaptations to limit the drying out of vegetative tissues). The mechanisms for coping with drought in plants can be divided into three categories: I) escape, 2) avoidance, and 3) tolerance. Cultivars that have the ability to escape water stress are able to complete their life cycles before the water deficits can have an extreme effect on performance. Ideally, these cultivars exhibit high rates of growth and gas exchange using the available moisture to successfully reproduce before the time when water is limited.

Associated with successful escape of water stress are increased stem and root carbohydrate storage, and the ability to mobilize reserves during increasing drought (Kapoor *et al.,* 2020).

Plants may be able to tolerate moisture stress by avoiding tissue dehydration. This is done by maintaining tissue water potential as high as possible. A number of mechanisms exist by which plants can avoid dehydration by minimizing water loss. These include closing of stomata, reducing light absorbance (i.e., curling of leaves), possessing dense trachoma, which increase light reflectance, exhibiting a steep leaf angle, or by decreasing canopy leaf area by developing smaller leaves or shedding older leaves. Other ways to avoid dehydration is to maximize water uptake by increasing investment in root growth to provide the plant water from greater depth. Lastly, tolerance of water deficiency is associated with plants that. at the cellular level, have the ability to make osmotic adjustments, have more rigid cell walls or have smaller cells. At the organ level, plants with smaller leaves have the ability to tolerate drought stress as they have a greater ability to dissipate extra solar energy as well as a greater efficacy in controlling water loss through stomata (Wang *et al.,* 2020).

Success of certain crop plants under drought environments merely depends on presence of an optimum combination of these three resistance mechanisms. Breeding for drought in past has been facilitated by conventional breeding approaches by concentrating on yield and it's relating components. Breeding for new and improved cultivars against abiotic stress needs a thorough understanding of the reactions of plant tissues and organs against the prevailing stress.

Drought tolerance in wheat is a complex intrinsic response that is regulated by an interlinked network of genes, which are synchronized by some key players. At the molecular level, it is mainly regulated at transcription that involves the expression of genes, followed by the regulation at post transcription, translation, post translation, and epigenetic levels (Mohammadi, 2018).

2.3 Genetic parameter studies in wheat

Heidari *et al.* (2020) used sixteen advanced durum wheat breeding lines were evaluated under rain-fed and supplementary irrigation conditions in a randomized complete block design (RCBD) with three replications on seventeen agro-morphological characters to examine genetic value of the traits. Most traits revealed the highest coefficients of variation (CV). Results showed that the maximum phenotypic variance (PCV) of traits were generally higher

than genotypic coefficients of variance (GCV) under both conditions. Obtained heritability for several traits indicates that most likely the heritability is due to additive gene effects and selection may be effective in early generations for these traits. These parameters were estimated for a number of traits in vitro cultures as well.

Arifuzzaman *et al.* (2020) carried out an investigation with 25 wheat genotypes composed of cultivars and advanced lines to screen drought-tolerant bread wheat genotypes using multivariate analysis. The experiment was conducted in a split plot design with three replications and two treatments control and drought. High heritability was observed for all the traits except fag leaf length, fag leaf breadth and number of tillers per plant.

Alemu *et al.,* (2020) evaluated 64 durum wheat landraces using an 8 × 8 simple lattice designs. Analysis of variance indicated the presence of highly significant ($p < 0.01$) variations among accessions for all traits. The observed wide range of differences among genotypes for these main traits may be due to genetic differences of genotypes and selection can be effective for breeding programs. The highest values for both heritability (85.5%) and genetic advance as percent mean (19.96) for plant height indicates better possibility and easiness for trait improvement through selection.

In Atinafu *et al.* (2020) study, high heritability was estimated for tillers/plant, plant height, above ground biomass at Fereziye and characters that showed high heritability at Kotergedira were also tillers/plant, plant height, above ground biomass. For all traits, phenotypic coefficient of variation was highly higher than genotypic coefficient of variation this indicating that there was environmental influence on these traits.

Rana (2019) used ten wheat genotypes in his experiment namely BARI Gom 25, BARI Gom 26, BARI Gom 27, BARI Gom 28, BARI Gom 29, BARI Gom 30, BARI Gom 31, BARI Gom 32 and two advanced line BAW 1203 and BAW 1194. The results of the experiment revealed that there were a genetic variability and significant variations between genotypes were observed for all the characters.

According to Ullah *et al.,* (2018), genetic variability, heritability and correlation were observed in 63 wheat advanced lines. Genotypic (GCV) and phenotypic coefficients of variation (PCV) ranged from 1.60 to 15.74% and 1.74 to 19.91% for tested traits.

2.4 Correlation analysis in bread wheat germplasms

Belay *et al.,* (2021) showed a significant and positive correlation was found between photosynthetic pigments in both growth conditions. Proline exhibited a negative correlation with most of the investigated traits except root to shoot length ratio and all photosynthetic pigments which showed a positive and non-significant association.

Ferede *et al.,* (2020) initiated a study to showed significant difference ($P < 0.05$) among bread wheat genotypes for all studied traits. In the genetic linear regression analysis over years, only thousand seed weight showed positive significant increment, whereas grain yield, biological yield, days to physiological maturity, plant height and test weight showed positive non-significant increment.

2.5 Multivariate cluster analysis and principal component analysis

Thungo *et al.* (2020) found Cluster analysis grouped the CIMMYT bread wheat genotypes into six main groups. This aided identification and selection of genetically unrelated genotypes such as LM02, LM13, LM23, LM41, LM44, LM71, LM73 and LM75 suitable for population development. In line with agronomic data, genotypes such as LM02. LM I 3, LM23 and LM75 were identified as high-yielding and recorded grain yields of > 2 tons/ha under drought-stressed condition and possessed suitable yield component traits such as higher thousand kernel weight.

Amin *et al.* (2020) studied genetic divergences of 50 wheat lines through Mahalanobis's D2 and principal component analysis for fourteen characters. Genotypes were grouped into four different clusters. Cluster II comprised maximum number of genotypes (twenty-one) followed by cluster IV. The inter-cluster distance was maximum between clusters r and III (12.29) indicating wide genetic diversity between these two clusters followed by the distance between cluster I and cluster II (8.28), and cluster Ill and cluster IV (7.97). Among the characters, heading days, maturity days, plant height (cm), canopy temperature at vegetative stage, canopy temperature. Cluster I had the highest. mean for chlorophyll content at anthesis, and plant height (93 cm).

Arifuzzaman *et al.* (2020) carried out an investigation with 25 wheat genotypes composed of cultivars and advanced lines to screen drought-tolerant bread wheal genotypes using multivariate analysis. The experiment was conducted in a split plot design with three replications and two treatments control and drought. Based on the genetic distance, all the 25

genotypes were grouped into three different clusters. In control conditions. cluster number I contained most tolerant genotypes and under drought conditions, cluster number Ill contained most drought-tolerant genotypes. In biplot, the genotypes SATYN- 24, BARI Gom-24 and SATYN-2 contributed positively and correlated with number of tillers per plant, shoot fresh weight, flag leaf breadth, proline content and plant height under drought conditions.

Khodadadi *et al.* (2011) conducted an experiment at the Agricultural Research Farm of Shahed University, Tehran, Iran as a randomized complete block design with three replications. Cluster analysis based on squared Euclidean distance and ward's method, categorized the cultivars into seven groups. The highest genetic distance was observed between Sardari and Spn/Mcd/Cama/3/Nzr/4/Passarinho (SP) genotypes.

2.6 Multi-trait Genotype Ideotype Distance Index (MGIDI)

Silva *et al.,* (2023) conducted a study to evaluate the use of drought-tolerance indices for the selection of wheat genotypes, to compare the genetic gains using different selection strategies by means of a multi-trait index, and to select superior drought-tolerant wheat genotypes. The total of 31 tropical wheat lines was evaluated in two experiments. Five agronomic traits were accessed. The data were subjected to mixed model analysis, and four selection scenarios were designed. There was a significant effect of genotype for all traits. The inclusion of drought-tolerance indices in the selection index provided superior genetic gains in drought condition. Seven lines were selected due the high frequency of favorable alleles for drought-tolerance and other important agronomic traits. Drought-tolerance indices are appropriate for characterizing the response of wheat genotypes to drought stress. The inclusion of drought-tolerance indices along with agronomic traits in multi-trait selection strategies provides for superior gains in grain yield compared to the non-inclusion of the indices.

Alireza *et al.,* (2021) studied the effect of water-deficit stress on a core collection of landraces and wild relatives of wheat (including 180 samples belonging to four *Triticum* and eight *Aegilops* species [*T. boeoticum Bioss., T. urartuGandilyan., T. durum Def., T. aestivum L., Ae. speltoides Tausch., Ae. tauschiiCoss., Ae. caudata L., Ae. umbellulata Zhuk., Ae. neglecta L., Ae. cylindrica Host., Ae. crassaBoiss., and Ae. triuncialis*]) in terms of several physiological traits, root and shoot biomasses, and features of root system architecture (RSA). All genetic materials were subjected to water-stress treatment using a pot experiment under greenhouse conditions. To screen the most tolerant accessions, three selection indices, such

as Smith and Hazel (SH), factor analysis and ideotype-design (FAI), and the multi-trait genotype-ideotype distance index (MGIDI) were computed.

According to Farhad *et al.,* (2021), A best linear unbiased prediction (BLUP)-based multi-environmental stability analysis was conducted on three sets of genotypes across three consecutive years (2017, 2018, and 2019) under early and timely planting dates to identify genotypes for further breeding. A significant genotypic effect was observed for all traits in the single environment analysis. A genotype–environment interaction (GEI) was observed in the mixed-effect model, except for FLGLFA in Season 2 and SpkLng in Season 3 Residual components of variation were found to increase under early planting for all studied traits due to exposure of genotypes to early heat and a prolonged growing period. A higher GEI was observed in dissected phenological events such as BTH and GFD. Among phonological traits, it was found that DTB, GFD, and DAYSMT were strongly supporting selection gain throughout all seasons under early planting.

Al-Ashkar *et al.,* (2023) used multi-trait genotype-ideotype distance index (MGIDI) to detect the ideotype. Six tolerance multi-indices were used to test twenty wheat genotypes grown under multiple abiotic stresses. The G01, G12, G16, and G02 were selected as the appropriate and stable genotypes using the MGIDI with the six tolerance multi-indices. The pooled analyses (MGIDI) showed genotype G01 as the most stable candidate. The genotype (G01) is considered a novel genetic resource for improving productivity and stabilizing wheat programs under multiple abiotic stresses.

2.7 SSR Marker based DNA Fingerprinting

Thungo *et al.* (2020) studied twenty-four agronomically selected wheat genotypes sourced from the International Maize and Wheat Improvement Centre (CIMMYT)'s heat and drought tolerance nursery and four local check varieties were genotyped using 12 selected polymorphic SSR markers. Expected heterozygosity mean value of 0.58 indicated moderate genetic diversity for breeding. The studied wheat genotypes were delineated into six genetic groups using cluster analysis. Significant genotypic differences were observed for agronomic traits and GPC under NS and OS conditions. Genetically unrelated breeding parents including LM02, LM13, LM23, LM41, LM44, LM71, LM73 and LM75 were selected for population development and breeding for enhanced grain yield and protein content under heat and drought-stressed environments.

A total of sixteen SSR primers were examined by Kara et al. (2020); only eleven of them—WMC 14, WMC 15, WMC 17, WMC 20, WMC 21, WMC 24, WMC 25, WMC 27, WMC 48, WMC 50, and WMC 283—produced polymorphic bands. For WMC 16, WMC 18, WMC 19, WMC 22, and WMC 23 primers, no amplified products were found. Between 0.14 (WMC 21) and 0.70 (WMC 50 and WMC 17), the Polymorphism Information Content (PIC) ranged, with an average of 0.48 and 0.49. This indicates that the markers were highly informative.

Verma *et al.* (2019) suggested that a combined use of phenotypic and molecular data was a powerful approach to identify divergent parents because of the complementary nature of the two marker systems.

Sonmczoglu and Terzi (2018) found the results of the molecular studies identified and detected 15 polymorphic SSR markers which gave the clearest PCR bands among the control genotypes. At the end of the research, bread wheat genotypes which were classified for tolerance or sensitivity to drought and the genetic similarity within control varieties were determined by molecular markers.

Iqbal *et al.* (2016) tested SSR primer pairs (45) for polymorphism among selected wheat genotypes. The dendrogram results have shown the wheat genotype association with the levels of praline during induced drought stress. The relationship between pattern of drought responsive biochemical attributes and DNA markers in the selected wheat genotypes was recognized to select drought tolerant genotypes for sowing in drought affected areas of the country.

Bousba*et al.,* (2012) conducted a study where A total of 136 fragments were obtained from the 26 SSR primers and all the bands were polymorphic across all the genotypes screened, most of them were polymorphic. The polymorphism information content (PIC) values ranged from 38 % to 94%, with an average of 74%. A total of 136 fragments were obtained from the 26 SSR primers and all the bands were polymorphic across all the genotypes screened, most of them were polymorphic. The polymorphism information content (PIC) values ranged from 38 % to 94%, with an average of 74%.

2.8 SSR Marker based genetic diversity in wheat

Kara *et al.*, (2020) studied a total of 16 SSR primers tested, only 11 showed polymorphic bands (WMC 14, WMC 15, WMC 17, WMC 20, WMC 21, WMC 24, WMC 25, WMC 27, WMC 48, WMC 50, and WMC 283). No amplified products were obtained with WMC 16,

WMC18, WMC 19, WMC 22 and WMC 23 primers. The Polymorphism Information Content (PIC) was varied from 0.14 (WMC 21) to 0.70 (WMC 50 and WMC 17) with an average of 0.48 and 0.49. This implies that the markers were highly informative.

In Nahas*et al.,* (2020) study, 9077 ESTs related to drought tolerance in hexaploid wheat were downloaded from NCBI and assembled into 12062 contains and 4141 singletons. 81% of SSR-containing uniqueness had one chromosome location and the highest number of loci was found in chromosomes 1B (69). The distribution of genic SSR loci among the 21 wheat chromosomes, the three sub-genomes and the seven homoeologous groups of wheat chromosomes was significant, with $P<0.01$ indicating a non-random distribution.

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

Thungo *et al.,* (2020) studied twenty-four agronomically selected wheat genotypes sourced from the International Maize and Wheat Improvement Centre (CIMMYT)'s heat and drought tolerance nursery and four local check varieties were genotyped using 12 selected polymorphic SSR markers. The test genotypes were phenotyped using yield and yield-component traits, and grain protein content (GPC) under non-stressed (NS) and drought-stressed (DS) conditions. Expected heterozygosity mean value of 0.58 indicated moderate genetic diversity for breeding. Significant genotypic differences were observed for agronomic traits and GPC under NS and DS conditions.

Verma*et al., (*2019) suggested that a combined use of phenotypic and molecular data was a powerful approach to identify divergent parents because of the complementary nature of the two marker systems.

Sonmezoglu and Terzi (2018) revealed that 10 bread wheat cultivars (*Triticum aestivum* L.) and 9 breeding lines examined by using SSR microsatellite markers. The genotypes were screened with molecular markers for the presence of QTLs mapping to different

chromosomes. The molecular studies results were identified and detected 15 polymorphic SSR markers which gave the clearest PCR bands among the control genotypes. At the end of the research, bread wheat genotypes which were classified as tolerant or sensitive to drought and the genetic similarity within control varieties were determined by molecular markers.

Gurcan *et al.,* (2017) conducted an experiment that 50 hulled wheat populations from Kastamonu, Konya and Kayseri provinces and 15 tir wheats from Kars's provinces of Turkey showing some quantitative and qualitative traits of each population were determined.

Wang *et al.,* (2017) constructed a UPGMA clustering which indicated that the

238 T. urartu accessions could be classified into two subpopulations. The wide range of genetic diversity along with the manageable number of accessions makes it one of the best collections for mining valuable genes based on marker-trait association. The data demonstrated that SSRs and HMW-GSs were useful markers for identification of beneficial genes controlling important traits in T. urartu, and subsequently for their conservation and future utilization, which may be useful for genetic improvement of the cultivated hexaploid wheat.

Iqbal *et al.,* (2016) tested SSR primer pairs (45) for polymorphism among selected wheat genotypes. The dendrogram results had shown the wheat genotype association with the levels of proline during induced drought stress. The relationship between pattern of drought responsive biochemical attributes and DNA markers in the selected wheat genotypes was recognized to select drought tolerant genotypes for sowing in drought affected areas of the country.

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

Sehgal*et al.,* (2012) found that the molecular characterization and genetic diversity of 20 wheat genotypes was observed using 34 polymorphic Simple Sequence Repeats (SSR) screened primers. Shalimar-86 and Chakwal-86 showed the highest genetic diversity with

SH-02 and Ufaq respectively, giving a 98.94% genetic similarity and between Chakwal-50 and Bhakar minimum genetic diversity was observed which indicated that they are 74% similar. The current research found that SSR makers could enable to distinguish and characterize all of the genotypes.

CHAPTER III

MATERIALS AND METHODS

The field experiment was conducted at Breeder Seed Production Station, Bangladesh Wheat and Maize Research Institute, Debiganj; Panchagarh and the molecular experiment was conducted in the Department of Genetics and plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh and Bangladesh Wheat and Maize Research Institute (BWMRI), Dinajpur, Bangladesh. The materials and methods of these experiments are described below.

3.1 Experimental site

The experimental site was located at 26.11° N latitude and 88.76° E longitudes at an altitude of 46 m above from the mean sea level. This site is belonging to the agro-ecological region of the AEZ-1 (Old Himalayan Piedmont Plain).

3.2 Climate

The field experiment was conducted at Breeder Seed Production Station, BWMRI, Debiganj, Panchagarh during December 2022 to April 2023. During the growth period of this crop, the average atmospheric temperature range was 9.53-28.23°C. The relative humidity was 96.9-100% and the amount of rainfall was zero.

3.3 Wheat genotypes

A set of 100 diverse bread wheat genotypes consisting of old and new Bangladeshi elite varieties, exotic lines, synthetic hexaploid, and derived lines were used in the study. These wheat genotypes were collected from the Wheat Breeding Division of Bangladesh Wheat and Maize Research Institute (BWMRI), Dinajpur, Bangladesh (Table 1).

Table 1. Plant materials used in the experiment (Source: Bangladesh Wheat & Maize Research Institute, Dinajpur)

SL.NO.	Genotype	SL.NO.	Genotype
1	11SATYN-9406	26	1HZWYT-448 RAJ
2	11SATYN-9412	27	1HZWYT-449 RAJ
3	11SATYN-9416	28	29SAWYT-11 RAJ
4	11SATYN-9417	29	29SAWYT-3 RAJ
5	11SATYN-9425	30	29SAWYT-305
6	11SATYN-9426	31	29SAWYT-312
7	11SATYN-9428	32	29SAWYT-313
8	11SATYN-9437	33	29SAWYT-319
9	1HZWYT-410	34	BARIGOM-25
10	1HZWYT-412	35	BARIGOM-27
11	1HZWYT-417	36	BARIGOM-28
12	1HZWYT-418	37	BARIGOM-30
13	1HZWYT-421 RAJ	38	BARIGOM-31
14	1HZWYT-422 RAJ	39	BARIGOM-32
15	1HZWYT-423	40	BARIGOM-33
16	1HZWYT-425	41	BAW-1243
17	1HZWYT-427	42	BAW-1286 CVD-4
18	1HZWYT-428	43	BAW-1322 CVD-6
19	1HZWYT-430 RAJ	44	BAW-1340 CVD-7
20	1HZWYT-433	45	BAW-1390
21	1HZWYT-434	46	BAW-1394 CVD-9
22	1HZWYT-437 RAJ	47	BAW-1397
23	1HZWYT-439 RAJ	48	BAW-1399
24	1HZWYT-444	49	BAW-1401 CVD-12
25	1HZWYT-446	50	BAW-1403

Table 1. Plant materials used in the experiment(contd.) (Source: Bangladesh Wheat & Maize Research Institute, Dinajpur)

SL.NO.	Genotype	SL.NO.	Genotype
51	BAW-1407 AYT-7	76	Jamal-9033
52	BAW-1408 AYT-8	77	Jamal-9046
53	BAW-1411 AYT-7	78	Jamal-9048
54	BAW-1422 AYT-10	79	SABGPYT-1041
55	BAW-1425 AYT-11	80	SABGPYT-4053
56	BAW-1426 PYT-4	81	SABGPYT-4055
57	BAW-1427 PYT-5	82	SABGPYT-4056
58	BAW-1429 PYT-7	83	SABGPYT-4057
59	BAW-1430 PYT-8	84	SABGPYT-4075
60	Jamal-10008	85	SABGPYT-4079
61	Jamal-10010	86	SABGPYT-4104
62	Jamal-10020	87	SABGPYT-4110
63	Jamal-10024	88	SABGPYT-5050
64	Jamal-10026	89	SABGPYT-5082
65	Jamal-10028	90	SABGPYT-5094
66	Jamal-10029	91	SABGPYT-6006
67	Jamal-10032	92	SABGPYT-6016
68	Jamal-10038	93	SABGPYT-7055
69	Jamal-10059	94	SABGPYT-7056
70	Jamal-10089	95	SABGPYT-8011
71	Jamal-10105	96	SABGPYT-8082
72	Jamal-9006	97	WMRIGOM-1
73	Jamal-9007	98	WMRIGOM-2
74	Jamal-9015	99	WMRIGOM-3
75	Jamal-9030	100	WMRIGOM-4

3. A) Morpho-physiological characterizations of wheat genotypes at seedling stage under drought conditions

3.4 Experimental design and set up

The experiment was conducted in a Split Plot Design with two replications. Here, two treatments viz. control and drought stress were assigned to the sub-plots in which genotypes were assigned randomly. For this, ten seeds of individual genotypes were sown in plastic pots (20 cm×20 cm×22 cm) containing a mixture of top soil, silica sand, milled lava and peat dust etc. The total no. of pots used in this study was 400 (2×2×100). Considerable spacing was maintained among the pots for convenience of management operations. Water supply was done with a drip irrigation system by watering pots three times per day. The drought stress treatment was carried out 21 days after sowing (DAS) by eliminating the water supply completely. The control block was kept under continuous supply of irrigation.

3.5 Soil

The experimental land was medium high with fine sandy loam to fine sand texture. The soil profile depth was 0-70 cm. The soil pH was ranges from 5.1-6.0.

3.6 Collection of sand

The sand was collected from nearby source. Stone pieces, roots of different crops, brick pieces, polythene sheets, clods etc. were removed with the help of colander. Then the sand was finely sieved. Required and equal amount of sand was then placed in each plastic pot with the help of balance.

3.7 Preparation of sand

The collected sand was mixed with required dose of manures and fertilizer shown in Table 2. Then it was ready to fill up the plastic pot.

Table2. Doses and method of application of fertilizers for wheat

Name of the fertilizer/ manure	Application rate (kg/ha)
Cow dung	10000
Urea	260
Triple Super Phosphate (TSP)	150
Murate of Potash (MoP)	140
Gypsum	125
Boric acid	7

Source: Bangladesh wheat and Maize Research Institute, Dinajpur

3.8 Preparation of plastic pot

The plastic pots (12 cm×7 cm) were used in conducting the experiment. Finely prepared soil was used as a matrix for seed emergence. Each pot was filled up with finely mixtured sand up to 4.5cm.

3.9 Sowing of seeds

Ten seeds were sown in each pot singly at about 2 inch depth according to the experimental design on 10th December 2022.

3.10 Intercultural operations

When the seedlings were started to grow in the pot, it was always kept under careful observation. In addition, various intercultural operations were accomplished for better growth and development of wheat plants.

3.10.1 Irrigation

Water supply was done with a drip irrigation system by watering pots three times per day. Volumetric Moisture Content (VMC) was measured digitally with the frequency domain technique. In drought block, the plants were kept under stress for 21 days till VMC will reach the maximum drought stress threshold near to wilting point (VMC near to 0%). The control block was kept under continuous supply of irrigation.

3.10.2 Weeding

Weeding was done to keep the pots free from weeds, easy aeration of soil and to conserve soil moisture, which ultimately ensured better growth and development.

3.10.3 Thinning

Thinning was done to reduce the crop competition and to ensure vigorous growth and development of wheat plant. Thinning after 21 DAS was done to increase plant growth.

3.11 General observation of the experimental pot

The pot was observed time to time to detect visual difference among the treatment and any kind of infestation by weeds, insects and diseases so that considerable losses by pest should be minimized. The pot looked nice with normal green color plants.

3.12 Data recording

Data on different morphological and biochemical characters were recorded on pot and basis as per experimental requirement. For plant basis, the data were recorded on selected plants from each unit pot in a replication and were tagged individual plant. In total fourteen characters were studied. The all-experimental data were collected after harvesting of seedling 21 days aged. The plants were collected from pots and the following measurements were done.

i. Plant height in cm (PH)
ii. Number of tillers per plant (NTPP)
iii. Chlorophyll content (CC)
iv. Canopy temperature at ◦C (CT)
v. Number of dead leaves per plant (NDLP)
vi. Root fresh weight in gm. (RFW)
vii. Root dry weight in gm. (RDW)
viii. Shoot fresh weight in gm. (SFW)
ix. Shoot dry weight in gm. (SDW)
x. Root length in cm (RL)
xi. Root volume (RV)
xii. Relative water content (RWC)
xiii. Excise leaf water loss (ELWL)
xiv. Proline content (PC)

The details of these parameters are described under the following sub-heads.

i) Plant height

Plant height was expressed in centimeters by measuring the plant stalk from the base of the stem (at the soil surface) to the top of the canopy.

ii) Number of Tiller per Plant

Total number of Tiller per Plant was counted at 21 DAS.

iii) Chlorophyll Content

Chlorophyll content was measured as SPAD unit from the electronic instrument or device called chlorophyll meter. The chlorophyll was measured in the drought and control (irrigated) condition by keeping the healthier leaf in the meter. The readings were taken at sunshine hours.

iv) Canopy Temperature

Canopy temperature was measured by the infrared thermometer (IRT) known as plant Canopy Analyzer.

v) Number of Dead Leaves per Plant

Total number of dead leaves per Plant was counted at 21 DAS.

vi) Root Fresh Weight

Root fresh weight was estimated by using an electrical balance in gram (g). For those three seedlings were randomly sampled from each pot. After that radicle fresh weight were taken from individual plant and averaged.

vii) Root Dry Weight

The roots which were taken for fresh weight were kept into paper bags and labeled. After taking fresh weight the root portions of samples were oven dried at 60◦C for 72 hours and weighted by using an electrical balance in gram (g).

viii) Shoot Fresh Weight

Shoot fresh weight was recorded at 21 DAS. For those three seedlings were randomly sampled from each pot. Then fresh weights of the shoot were taken by using an electrical balance in gram (g) and averaged.

ix) Shoot Dry Weight

The same method used for root drying was followed for dry weight of shoot (g). After complete dried in oven those were weighed by electric balance and averaged carefully.

x) Root Length

Root length was measured from the ground level to the tip. The length of the root was measured in centimeter (cm) with the help of a centimeter scale.

xi) Root Volume

Root Volume was measured as the volume of water raised by the root in a measuring cylinder. It is measured in cubic-centimeter (cm^3)

xii) Proline Content (mg/g)

Proline analysis was carried out at the laboratory of the department of Agricultural Chemistry of Hajee Mohammed Danesh Science and Technology University, Dinajpur. Samples of the second top leaves from the flag leaf were harvested from the drought and control plots. Proline extraction was done following the acid-ninhydrin method according to Bates *et al.,* (1973). This was followed by UV-visible spectrophotometer analysis of the absorbance of the proline extract in toluene at a wavelength of 520nm.

The proline content on fresh weight basis as-

µ moles per g tissue = µg proline/ml × ml toluene/115.5× 1/g sample

xiii) Relative Water Content

At the middle of plant canopy, five fully developed leaf samples were taken from each of the selected plants from each plot, when drought appeared. After excision each sample was carefully taken to laboratory in polythene bag and fresh weight was recorded immediately. The leaf samples were kept in water for overnight to record turgid leaf weight. On next day the samples were oven dried at 70o C for six hours. The relative water content was measured using the following formula:

RWC= Fresh weight – Dry weight /Turgid weight – Dry weight

xiv) Excise leaf water loss

Three fully developed leaves were excised from selected plants and carefully were packed in polythene bags. The samples were brought into laboratory avoiding any water loss. Immediately at laboratory fresh weight of leaves was recorded and samples were left on laboratory benches for six hours. After six hours the weight of wilted leaves was recorded and samples were then be dried in oven at 70o C.

The ELWL was calculated using the following formula:

ELWL= Fresh weight –Wilted weight/ Dry weight

Figure1.Measuring of root length, root dry weight& root volume

Figure 2. Estimation of proline content in laboratory

3.13 Statistical analysis

The data obtained for different characters was recorded first on an MS Excel sheet. Afterwards, the data were analyzed using the software package R, version 4.2.3 (R Core Team 2023). The functions gamem_met(), gamem(), corr_ci(), and mgidi() of the R package Multi-Environment Trial Analysis-Metan 1.11.0 (Olivoto *et al.,* 2020) were used to compute the analysis in sections A, B, C, and D, respectively. Other data visualizations and graphs were prepared using Tidyverse 1.3.0 (Wickham *et al.,* 2019).

3.13.1 Mixed model analysis

Three selection indices, including multi-trait genotype-ideotype distance index (MGIDI), Smith-Hazel (SH), and factor analysis and ideotype-design (FAI) were used to select the desirable accessions in terms of a complex root and physiological traits. All analyses were computed in R software using the 'metan' package.

3.13.2 Estimation of genotypic and phenotypic variances

Genotypicandphenotypicvariancesweresestimatedbasedonmethodwhetherthegenotype, location and environment factors are defined as random or fixed reported by Dagnelie (1975).

$$\text{Genotypic variance } (\sigma^2_g) = (MSg - MSe)/r$$

Where,

MSg = Mean sum of squares for genotypes;

MS_e = Mean sum of squares for error

r= Number of replications;

And $\text{Phenotypic variance } (\sigma^2_p) = \sigma^2_g + \sigma^2_e$

Where,

σ^2_g=Genotypic variance

σ^2_e=Environmental variance/Error mean square

3.13.3 Estimation of heritability

Heritability in broad sense (h^2_b) was estimated following the formula of Johnson *et al.* (1955).

h^2_b (%) $=\frac{\sigma^2 g}{\sigma^2 p}\times 100$

Where,

σ^2_g=Genotypic variance;

and σ^2_p=Phenotypic variance.

3.13.4. Descriptive analysis

Descriptive statistics was used to compute the most used measures of central tendency, position and description. It was calculated by following equation:

$$Yij = \mu + \alpha i + Tj + \varepsilon ij$$

Where yij is the value observed for the ith genotype in the jth replicate (i = 1, 2 … g; j = 1, 2... r); being g and r the number of genotypes and replicates, respectively; αi is the random effect of the ith genotype; Tj is the fixed effect of the jth replicate; and εij is the random error associated to yij.

The agronomic data were subjected to mixed model analysis for the estimation of genetic parameters by the REML method, and prediction of genotypic values by BLUP according to

Eq. 1: $y = Xb + Zu + e$

in which: y = a vector η[∑jr =1(gr)]×1 of observations, that is, the response of the ith genotype in the jth block (i = 1, 2, ..., g; j = 1, 2, ..., r; y = [y11, y12, ..., ygr]′); b= a vector 1 × r of block fixed effects, b [γ1, γ2, ..., γr]′; g = a vector m [1 × g] of the random effects of genotype, g [α1, α2, ..., αg]′, g ~ N(0; I σg2); e = a vector n × 1 of the random error effects, e = [y11, y12, ..., ygr]′, e ~ N(0; I σg2); X, and Z = the incidence matrices of the effects.

3.13.5 Partial correlation analysis

To consider the influence a set of traits on the relationship between two traits the partial correlation is used. From Pearson's simple correlation matrix, the partial correlation is calculated by the following equation:

$$r_{xy.m} = \frac{-a_{xy}}{\sqrt{a_{xx}\, a_{yy}}}$$

Where $r_{xy.m}$ is the partial correlation coefficient between the traits * x * and * excluding the effects of the m * remaining traits of the set; −aij is the inverse element of the correlation matrix corresponding to xy, aiiajj are the diagonal elements of the inverse matrix of correlation associated with trait x and y, respectively. The significance of this correlation is also tested by the test * t * according to the following expression:

$$t_{calc} = r_{xy.m} \sqrt{\frac{n-v}{1-r^2 xy.m}}$$

Where t_{calc} is the calculated Student * t * statistic; $ r {xy.m} $ is the partial correlation coefficient for the traits x and y excluding the effect of the other * m * traits; * n * is the number of observations; and * v * is the number of traits.

3.13.6 Multivariate Analysis

The utilization of biometrical procedures has enabled the quantification and selection of genetically diverse parents for a hybridization program (Rao 1952). Multivariate analysis techniques, such as cluster analysis and principal component analysis (PCA) are effective methods for assessing genetic diversity by quantifying differences among multiple quantitative traits. Here, pooled mean data were utilized for clustering using Python software (Pilgrim and Willison 2009). A hierarchical agglomerative method was utilized, employing the Euclidean distances in Ward's method. The clustering process is structured in a manner that within-group variance is minimized, and for this reason earning it the name Ward's minimum variance method (Ward 1963). The optimal number of clusters was determined based on the point where the total clusters within-cluster variance exhibited a significant reduction.

3.13.7 Factor analysis

The MGIDI was used to rank the treatments based on the desired values of the studied trait. First, factor analysis was computed with (rXij) to account for the correlation structure and dimensionally reduction of the data, as follows

$$X = \mu + Lf + \varepsilon$$

Where, X is a p×1 vector of rescaled observations; μ is a p×1 vector of standardization means; L is a p × f matrix of factorial loadings; f is a p×1 vector of residuals, being p and f the number of traits and common factors retained, respectively. The eigenvalues and eigenvectors are obtained from the correlation matrix of rXij. The initial loadings are obtained considering only factors with eigenvalues higher than one. Then, the varimax rotation criteria is used for the analytic rotation and estimation of final loadings. Finally, the scores are computed as follows:

$$F = Z(A^T R^{-1})^T$$

Where F is a g × f matrix with the factorial scores; Z is a g × p matrix with the (rescaled) standardized means; A is a p × f matrix of canonical loadings, and R is a p × p correlation matrix between the traits. g, f, and p represent the number of treatments, factors retained, and analyzed traits, respectively. The number of factors retained was based on the Guttman-Kaiser criterion following the eigenvalues-greater-than-one rule.

3.13.8 Principal component analysis

To visually understand the relationships between trait and their association with the treatments, we conducted a Principal Component Analysis (PCA) with Xij containing the treatments in rows and traits in columns. A biplot was produced with the function fviz_pca_biplot() from the R package factoextra.

Data manipulation and the index computation were performed in the R Software using the package metan and the ecosystem of packages Tidyverse.

3.13.9 Multi-trait Genotype -Ideotype Distance Index

After the factor analysis, the MGIDI is computed as the Euclidean distance between the scores of treatments and the ideal treatment was computed as follows:

$$\text{MGIDI} = \sum_{j-1}^{f}[(\gamma ij - \gamma j)^2]^{0.5}$$

Where MGIDI is the multi-trait genotype-ideotype distance index for the ith treatment; γij is the score of the ith treatment in the jth factor (i = 1, 2... t; j = 1, 2...f), being t and f the number of treatments and factors, respectively; and γj is the jth score of the ideal treatment. The treatment with the lowest MGIDI is then closer to the ideal treatment and therefore presents desired values for all the p traits.

3.13.10 Strengths and weaknesses

The proportion of the MGIDI of the ith treatment explained by the jth factor (ωij) was used to show the strengths and weaknesses of the treatments and was computed as:

$$\omega ij = \frac{\sqrt{D^2\,ij}}{\sum_{j=1}^{f}\sqrt{D^2\,ij}}$$

where Dij is the distance between the ith treatment and ideal treatment for the jth factor. Low contributions of a factor suggest that the traits within such a factor are close to the ideal treatment.

3. B) Molecular characterization and diversity analysis in wheat using SSR markers

3.14 Genomic DNA Isolation

2-4 pieces of young leaves were collected in eppendorf tube and were dried for 6-7 days in silica gel. The samples were grinded using mortar and pestle. 800 µl of warmed (65°C) CTAB buffer was added to each tube and was vortexed thoroughly. Samples were incubated in water bath at 65°C for 45 minutes and at every 10 minutes they were mixed gently by inversion (400 µl of 2% β- Mercaptoethanol was added to 200 ml of extraction buffer prior to warming). The tubes were taken out of the water bath and left at room temperature for 5 minutes.600 µl chloroform Isoamyl alcohol was added (24:1). The samples were mixed by gently inversion for about 2 minutes (100 times) until two layers' mix. Then the samples were centrifuged for 4000 rpm at room temperature for 20 mins. The aqua phase was removed with wide bore pipette. The aqua phase was transferred to clean 1.5 ml tube then 2/3 volume of Ethanol was added and mixed gently to precipitate the nucleic acids. At this stage the samples were stored in 4°C for overnight. After that the samples were centrifuged at 10000 rpm for 20 minutes and the supernatant was discarded. Then the DNA was dried so that there was no ethanol. 500 µl of washing buffer was added

in each tube and the DNA was washed gently inversion. Again, the samples were centrifuged at 10000 rpm for 20 mins, then the supernatant was removed and the tubes were left on bench for drying.100 µl double distilled water was added, the samples were left for dissolving. Finally, the samples were stored in 4°C for short term.3.15 DNA Quantification

The Quality of the extracted DNA samples were also checked prior to PCR amplification through Quantification using a Thermo Scientific Nano Drop^ TM1000 Spectrophotometer (Thermo Fisher Scientific, USA).

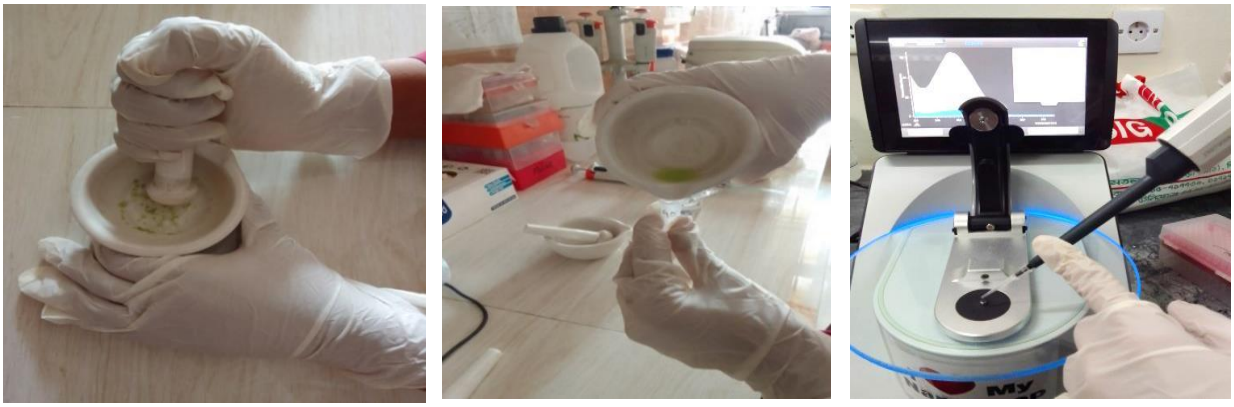


Figure 3. DNA extraction, quantification

3.16 PCR Amplification and Electrophoresis Separation

PCR amplifications were performed in 10 µL tube using a Veriti Thermal Cycler (Apliedbiosystems, USA). 2µL of template DNA, and 8 µL (0.5 of Forward primer, 0.5µL of Reverse primer, 2µL of nuclease free water and 5µL of G2 Green Master Mix) of reaction mixture was added in each tube. The PCR amplification was as follows: one cycle of 94°C for 5 min; 35 cycles of 95°C for 0.5 min, 53 to 58°C (depending on the specific primers) for 0.5 min and extension for 0.5 min; and a final extension at 72°C for 5 min. Reaction products were mixed with one fifth volume of loading buffer (100 mM/L EDTA pH 8.0,10 mM/L Tris-HCl pH 7.5, 5% Ficoll 400; 0.05% bromophenol, 0.05% xylenecyanol) and 2 µL were loaded vertically, for electrophoresis 8% denaturing polyacrylamide gels in 1 × TBE (90mM/LTris borate pH 8.3, 2 mM/L EDTA) at 50 mA

for 2 to 3 h (Wang *et al.*2007). Gels were then silver stained and photographed using x-ray viewer.

Figure 4. Amplification of polymerase chain reaction

3.17 Microsatellite/Simple Sequences Repeat (SSR) Markers

A total of 5 microsatellite (SSR) markers primer pairs (sigma Aldrich, Germany) covering all 5 chromosomes were selected for the genetic diversity analysis of the 96 genotypes of BWMRI as showed in the Table 3. These SSR primers with a distinct chromo some numbers were used for final Polymerase chain reaction (PCR) amplification. The original sources, repeat motifs, primer sequences, expected length and chromosomal position and other relevant information to these markers is published on the Grain Genes website (http://www.wheat.pw.usda.gov). The phenotypic characters, annealing temperature and primer sequences to SSR markers are shown in Table 3.

Table 3. SSR Markers used for diversity analysis of wheat germplasms

SSR Loci	Forward primer (5′–3′)	Reverse primer (5′–3′)	Annealing temperature (degree centigrade)	Phenotype
WMS0691	GGGAGGATATGAGGGCTCAC	GCACGTGATTGGTGAAAATG	56	Stay green
GWM513	ATCCGTAGCACCTACTGGTCA	GGTCTGTTCATGCCACATTG	56	Dwarf gene
Barc20	GCGATCCACACTTTGCCTCTTTACA	GCGATGTCGGTTTTCAGCCTTTT	58	Dwarf gene
Tagwm1037	CTTCATCTGCGACCTTCCAT	CTTTATTCCTGGTTATTGCC	54	Stay green
GWM495	GAGAGCCTCGCGAAATATAGG	TGCTTCTGGTGTTCCTTCG	56	Dwarf gene

3.18 Molecular Statistical Analysis

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

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

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

The Euclidean distance coefficients were estimated for all pairs of entries using the software 1 Euclidean distance matrix generated from seedling data was used as input data for cluster analysis based on un-weighted pair-group method of arithmetic average (UPGMA). A UPGMA dendrogram was created based on Euclidean genetic distances to estimate the level of relatedness among the varieties.

CHAPTER IV

RESULTS AND DISCUSSION

The experiment was conducted during the rabi season from November to February 2022-23. From this experiment, the outcome of the results and discussion of this research were obtained to analyze the variances, treatment association, variability, heritability, correlation of phenotypic and genotypic condition, multi-trait genotype ideotype (MGIDI) analysis, diversity analysis and principal component analysis morphologically and molecularly.

4.1 Mixed-model analysis

4.1.1 Likelihood ratio test and variance components

The statistical analysis using the likelihood ratio test demonstrated a significant impact of the genotype on all the trait categories in the single-environment analysis and multi-environment analysis. The statistical significance of the likelihood ratio for genotypes (LRTg) and the likelihood ratio for the genotype and environment interaction (LRTge) were observed in control and drought for all the morphological traits, except for the LRTg in the case of RL, SDW, RSR, SFW and RDW. All physiological traits except the PC was non-significant for the LRTg in multi-environment analysis (Table 4A & 4B).

Table 4A. LRT for the genotypic effect showing the significance of traits in single treatment analysis

VAR	CONTROL	DROUGHT
PH	119***	74.3***
NTPP	200***	96.4***
PC	777***	348***
RL	68***	74.4***
SDW	657***	513***
RSR	313***	149***
RWC	306***	239***
ELWL	141***	179***
SFW	311***	241***
RFW	175***	193***
RDW	141***	15***
RV	171***	186***

Here, *, ** and *** indicates significant at 5%, 1% and 0.1% level of probability, respectively, ELWL= excise leaf water loss, NTPP= no. of tiller per plant, PC= proline content, PH= plant height, RDW= relative water content, RFW= root fresh weight, RL= root length, RSR= root shoot ratio, RV= root volume, RWC=relative water content, SDW=shoot dry weight, SFW= shoot fresh weight

Table 4B. Likelihood ratio test (LRT) for the genotypic effect showing the significance of traits in the genotype-environment interaction for multi-environmental trial analysis

VAR	LRTg	LRTge
PH	17***	107***
NTPP	1.69*	247***
PC	0.955ns	826***
RL	9.09E-13ns	115***
SDW	2.36E-11ns	1.26e+3***
RSR	0.0955ns	344***
RWC	4.40*	466***
ELWL	6.51**	237***
SFW	6.82E-12ns	518***
RFW	26.1***	209***
RDW	-5.97E-13ns	187***
RV	39.4***	170***

Here, *, ** and *** indicates significant at 5%, 1% and 0.1% level of probability, respectively, ns= non-significant; ELWL= excise leaf water loss, NTPP= no. of tiller per plant, PC= proline content, PH= plant height, RDW= relative water content, RFW= root fresh weight, RL= root length, RSR= root shoot ratio, RV= root volume, RWC=relative water content, SDW=shoot dry weight, SFW=shoot fresh weight.

4.1.2 Genetic parameter studies under control, drought & META

Table 5.1 presents the mixed-model analyses for the morpho-physiological traits evaluated in the control and drought conditions. Mean heritability values ranged from 0.82 (RL) to 1.00 (PC and SDW) in the control condition, and from 0.54 (RDW) to 0.99 (PC) in the drought condition. Selective accuracies ranged from 0.90 (RL) to 1.00 (PC and SDW) in the control condition and from 0.73 (RDW) to 0.99 (DH) in the drought condition.

Table 5.2 presents the mixed-model analyses for the morpho-physiological traits evaluated in the META. Mean heritability values ranged from 0.03 (RL) to 0.72 (RV). Selective accuracies ranged from 0.03 (RL) to 0.85 (RV). Machado *et.al.* (2023) also found the same results.

Table 5.1 Genetic parameter for control and drought condition

Parameters	PH		NTPP		PC		RL	
	CON	DRT	CON	DRT	CON	DRT	CON	DRT
Gen_var	32	9.75	0.90	0.31	0.12	0.06	13.6	14.9
Gen (%)	83.6	72.6	93.1	78.9	100	98.5	70.5	72.7
Res_var	6.28	3.67	0.06	0.08	2.41E-5	1.01E-3	5.71	5.61
Res (%)	16.4	27.4	6.87	21.1	0.019	1.49	29.5	27.3
Phen_var	38.3	13.4	0.97	0.39	0.12	0.067	19.3	20.5
H^2	0.83	0.72	0.93	0.78	1.00	0.98	0.70	0.72
H^2mg	0.91	0.84	0.96	0.88	1.00	0.99	0.82	0.84
Accuracy	0.95	0.91	0.98	0.93	1.00	0.99	0.90	0.91
CVg (%)	8.38	8.51	22.8	18.8	59.2	10.4	12.8	14
CVr (%)	3.71	5.22	6.19	9.70	0.82	1.28	8.28	8.59
CV ratio	2.26	1.63	3.68	1.93	71.7	8.13	1.55	1.63

Here, PH= plant height, PC= proline content, RL=root length. CON= control, DRT=drought, Gen var = genotypic variance, Gen (%) =genotypic percentage, phenvar = phenotypic variance, H2=heritability, CVg =genotypic co-efficient covariance

Table 5.1 Genetic parameter for control and drought (contd.)

Parameters	SDW		RSR		RWC		ELWL	
	CON	DRT	CON	DRT	CON	DRT	CON	DRT
Gen_var	28.4	0.33	0.041	0.038	105	47.3	0.058	0.11
Gen (%)	99.9	99.7	97.9	88.2	97.7	95.4	87.2	91.4
Res_var	0.018	9.5E-5	8.95E-5	5.18E-3	2.49	2.27	8.66E-4	0.010
Res (%)	0.065	0.28	2.14	11.8	2.31	4.58	12.8	8.55
Phen_var	28.4	0.33	0.04	0.044	108	49.6	0.06	0.12
H^2	0.99	0.99	0.97	0.88	0.97	0.95	0.87	0.91
H^2mg	1.00	0.99	0.98	0.93	0.98	0.97	0.93	0.95
Accuracy	1.00	0.99	0.99	0.96	0.99	0.98	0.96	0.97
CVg (%)	40.7	37.7	63.6	48.9	14.8	18.7	4.68	10.8
CVr (%)	1.04	2.00	9.41	17.9	2.28	4.09	1.79	3.29

Here, ELWL= excise leaf water loss, RSR= root shoot ratio, RV= root volume, RWC=relative water content, SDW=shoot dry weight, CON= control, DRT=drought

Table 5.1 Genetic parameter for control and drought (contd.)

Parameters	SFW		RFW		RDW		RV	
	CON	DRT	CON	DRT	CON	DRT	CON	DRT
Gen_var	310	163	1.35	0.64	0.27	0.01	0.21	0.086
Gen (%)	97.8	95.5	91.1	92.6	87.2	37.5	90.7	92
Res_var	6.93	7.69	0.13	0.05	0.04	0.024	0.021	0.00751
Res (%)	2.19	4.50	8.91	7.41	12.8	62.5	9.35	7.96
Phen_var	317	171	1.48	0.69	0.31	0.039	0.23	0.094
H^2	0.97	0.95	0.91	0.92	0.87	0.37	0.90	0.92
H^2mg	0.98	0.97	0.95	0.96	0.93	0.54	0.95	0.95
Accuracy	0.99	0.98	0.97	0.98	0.96	0.73	0.97	0.97
CVg (%)	20.2	23.4	30.7	46.7	29.4	23.1	32.5	37.8
CVr (%)	3.01	5.09	9.61	13.2	11.3	29.9	10.4	11.1

Here, RDW=root dry weight, RFW= root fresh weight, RV= root volume, RWC=relative water content, SFW=shoot fresh weight., CON= control, DRT=drought

Table 5.2 Genetic parameter for META

Parameters	PH	NTPP	PC	RL	SDW	RSR	RWC	ELWL	SFW	RFW	RDW	RV
Phenotypic variance	25.9	0.68	0.096	19.9	14.4	0.042	78.7	0.094	244	1.09	0.17	0.16
Heritability	0.35	0.12	0.097	0.03	1.21E-14	0.03	0.20	0.24	0.04	0.46	7.48E-15	0.54
GEIr2	0.44	0.76	0.89	0.71	0.99	0.89	0.76	0.66	0.97	0.45	0.81	0.36
H^2mg	0.56	0.23	0.17	0.03	2.42E-14	0.06	0.34	0.40	0.04	0.65	1.65E-14	0.72
Accuracy	0.75	0.48	0.42	0.03	1.56E-7	0.24	0.58	0.63	0.04	0.80	1.28E-7	0.85
rge	0.7	0.87	0.99	0.71	0.99	0.92	0.96	0.86	0.97	0.84	0.81	0.802
CVg (%)	5.85	8.11	6.28	0.02	5.7E-7	9.95	7.58	3.63	0.04	25.8	3.15E-6	27.3
CVr (%)	4.28	7.67	1.48	8.43	1.35	15.3	2.91	2.36	3.81	11	15.6	11
CV ratio	1.37	1.06	4.26	0.00	4.21E-6	0.65	2.61	1.54	0.00	2.34	2.02E-7	2.47

Here, PH= plant height, NTPP=no. of tiller per plant, PC= proline content, RL=root length, SDW= shoot dry weight, RSR= root shoot ratio , RWC= relative water content , ELWL= excise leaf water loss ,SFW= shoot fresh weight , RFW= root fresh weight , RDW= root dry weight ,RV= root volume, CON= control, DRT=drought, Gen var = genotypic variance, Gen(%)=genotypic percentage, , h^2=heritability, CVg =genotypic co-efficient covariance

4.1.3 Overall Performance of 100 Wheat Genotypes at 12 Morpho-Physiological Traits

In the two environments, the average deviation for PH, NTPP, RFW, RL, RWC and RFW at drought condition was relatively low compared with control condition (Table 6). However, for other traits, average deviation appeared higher under drought condition, indicating a higher genotypic response under the drought condition; hence, the higher scope of genotype selection for those traits. The opposite was noted for other traits, suggesting that genotypic responses differed either due to genotypic effects or environmental variations. Variations in both the overall performance and variance components were detected among the different trait groups.

By analyzing the overall performance of 100 genotypes through 12 different morpho-physiological traits under two different environments or treatment, it was found variance among 100 genotypes (Table 7 & 8). The max mean value of Plant height (PH) had shown by GEN-1 (11SATYN-9406) for control condition and GEN-58 (BAW-1429 PYT-7) for drought condition. On the other hand, the min value of PH was found by GEN-78 for control which showed max mean value for No. of tiller per plant (NTPP) in drought condition. For control condition, GEN-58 (BAW-1429 PYT-7) was performed lower in root length (RL) but it was higher in plant height (PH) at drought condition. GEN-51 (BAW-1407 AYT-7) had shown maximum root length (RL) in control condition but lower in drought condition. GEN-90 (SABGPYT-5094) was lower in root shoot ratio (RSR) at control condition and root length (RL) at drought condition. The higher value of proline content (PC), Root length (RL), shoot dry weight (SDW), root shoot ratio (RSR), relative water content (RWC), shoot fresh weight (SFW), root fresh weight (RFW), root dry weight (RDW) and root volume (RV) was recorded by GEN-39 (BARIGOM-32), GEN-13 (1HZWYT-421 RAJ), GEN-85 (SABGPYT-4079), GEN-59 (BAW-1430 PYT-8), GEN-36 (BARIGOM-28), GEN-68 (Jamal-10038), GEN-71 (Jamal-10105), GEN-6 (11SATYN-9426) and GEN-63 (Jamal-10024) respectively at drought condition(Table 8).

Histogram showed the observed value of 12 morpho-physiological traits under both of control and drought condition (Figure 10& Figure 11). For plant height maximum genotypes had found under the range of 60 to 70cm in control condition (Figure10) and 32-40cm in drought condition (Figure 11). For no. of tiller per plant maximum genotypes were under the range of 3 to 5 in control but 2-3 in drought condition. Maximum genotypes for proline content were from 0.2 to 0.6 in control condition and from 2.0 to 2.5 in drought condition. For shoot dry weight (SDW), maximum genotypes were in 10-15gm at control condition and in 1-2gm at

drought condition. Again, for root length (RL) the range was 20-30cm at control and 25-35cm at drought condition. The range for root shoot ratio (RSR), relative water content (RWC), excise leaf water loss (ELWL), shoot fresh weight (SFW), root fresh weight (RFW), root dry weight (RDW) and root volume (RV) were 0.25-0.50, 60-80, 5.1-5.4, 70-90gm, 2.5-5.0gm, 1-2gm and 1-1.5 respectively at control condition and 0.1-0.5, 30-40, 2.5-3.5, 40-60gm, 1-2gm, 0.1-0.5gm and 0.5-1 respectively (Figure 10& Figure 11).

Table6. The statistical measures for the traits observed in control and drought condition: mean (x), confidence interval of mean (CI), and average deviation (AD)

Variable	AD		ci.t		$\bar{X}$	
	CON	DRT	CON	DRT	CON	DRT
ELWL	0.2	0.45	0.03	0.04	5.19	3.09
NTPP	0.76	0.21	0.13	0.08	4.18	2.98
PC	0.26	2.80	0.04	0.03	0.595	2.49
PH	5.06	0.12	0.86	0.51	67.5	36.7
RDW	0.40	0.55	0.07	0.02	1.77	0.524
RFW	0.93	3.53	0.16	0.11	3.78	1.72
RL	3.42	0.164	0.61	0.63	28.9	27.6
RSR	0.16	0.232	0.02	0.03	0.31	0.403
RV	0.34	5.26	0.06	0.04	1.41	0.78
RWC	8.39	0.45	1.44	0.98	69.3	36.8
SDW	4.07	10.5	0.74	0.08	13.1	1.54
SFW	13.6	0.282	2.48	1.82	87.4	54.5

Here, ELWL= excise leaf water loss, NTPP= no. of tiller per plant, PC= proline content, PH= plant height, RDW= relative water content, RFW= root fresh weight, RL= root length, RSR= root shoot ratio, RV= root volume, RWC=relative water content, SDW=shoot dry weight, SFW= shoot fresh weight., CON= control, DRT=drought.

Table 7. Performance of the 100 Wheat Genotypes under Control Conditions

Parameters	PH	NTPP	PC	RL	SDW	RSR	RWC	ELWL	SFW	RFW	RDW	RV
Ngen	100	100	100	100	100	100	100	100	100	100	100	100
OVmean	67.51	4.17	0.59	28.85	13.09	0.31	69.30	5.18	87.37	3.77	1.77	1.41
MinGEN	53 (G-78)	1.33(G-40)	0.05(G-74)	21.5 (G-58)	5.35 (G-20)	4e-04 (G-90)	46.84 (G-8)	4.48(G-4)	37.48 (G-37)	1.53(G-35)	0.94 (G-67)	0.75 (G-88)
MaxGEN	84.08 (G-1)	7.5 (G-80)	1.90(G-12)	44.5 (G-51)	28.46 (G-8)	0.92(G-9)	94.50 (G-51)	5.58(G-74)	129.03 (G-17)	8.42(G-69)	3.83 (G-64)	3.46 (G-64)

Here, ELWL= excise leaf water loss, NTPP= no. of tiller per plant , PC= proline content, PH= plant height, RDW= relative water content, RFW= root fresh weight , RL= root length, RSR= root shoot ratio, RV= root volume , RWC=relative water content , SDW=shoot dry weight , SFW= shoot fresh weight., Ngen= no. of total genotypes, OVmean= overall mean, MinGen= minimum genotype occupied by the particular trait, MaxGen= maximum genotype occupied by the particular trait.(G indicates genotypes mentioned at table 1.)

Table 8. Performance of the 100 Wheat Genotypes under Drought Conditions

Parameters	**PH**	**NTPP**	**PC**	**RL**	**SDW**	**RSR**	**RWC**	**ELWL**	**SFW**	**RFW**	**RDW**	**RV**
Ngen	100	100	100	100	100	100	100	100	100	100	100	100
OVmean	36.69	2.97	2.48	27.56	1.53	0.403	36.81	3.08	54.48	1.72	0.52	0.77
MinGEN	24.83 (G-90)	1.33 (G-76)	1.58 (G-68)	17.25 (G-51)	0.049 (G-90)	0.021(G-78)	24.03 (G-39)	2.29 (G-20)	31.05 (G-78)	0.55 (G-40)	0.23 (G-77)	0.24 (G-89)
MaxGEN	46 (G-58)	6.33 (G-78)	2.96 (G-39)	40.25 (G-13)	3.04 (G-85)	1.20(G-59)	70.81 (G-36)	3.86(G-5)	86.30 (G-68)	5.68(G-71)	1.316 (G-6)	2.05 (G-63)

Here, ELWL= excise leaf water loss, NTPP= no. of tiller per plant , PC= proline content, PH= plant height, RDW= relative water content, RFW= root fresh weight , RL= root length, RSR= root shoot ratio, RV= root volume , RWC=relative water content , SDW=shoot dry weight , SFW= shoot fresh weight., Ngen=no. of total genotypes, OVmean= overall mean, MinGen= minimum genotype occupied by the particular trait, MaxGen= maximum genotype occupied by the particular trait.(G indicates genotypes mentioned at table 1.)

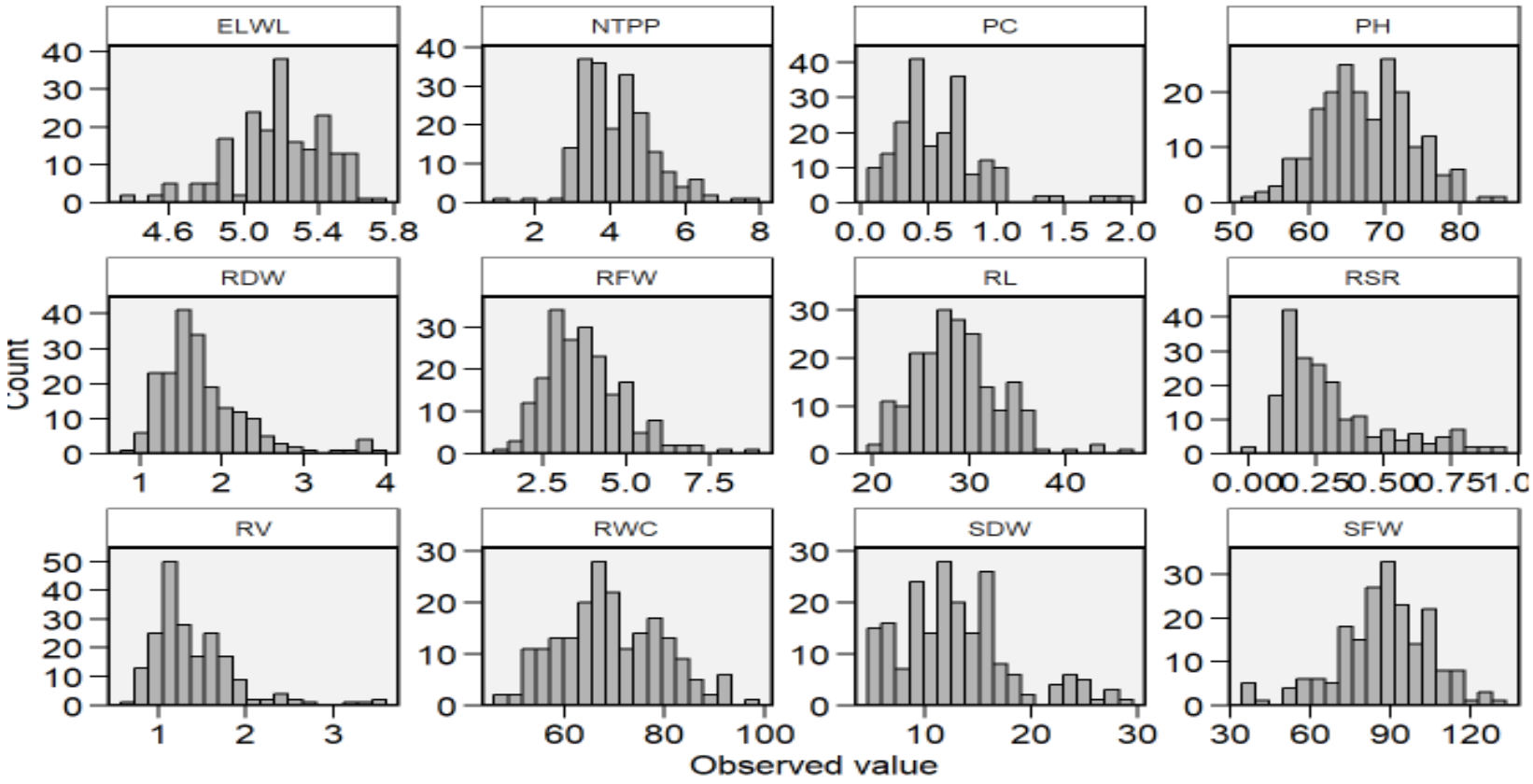


Figure4. Histogram of observed value different morphological traits among genotypes in control condition

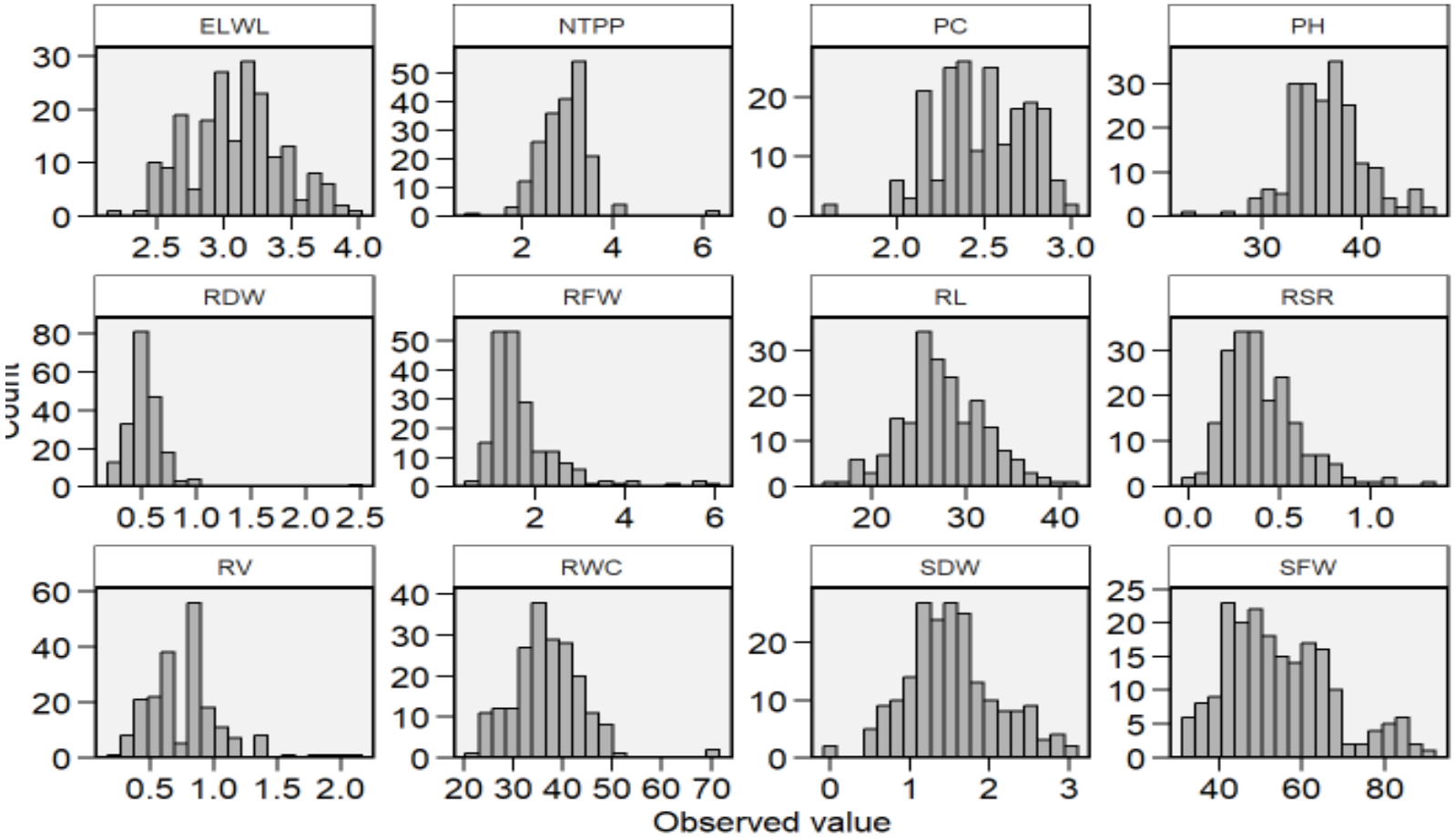


Figure 5. Histogram of observed value different morphological traits among genotypes in drought condition

4.1.4 BLUPg (Best Linear Unbiased Prediction for Genotype Effect)

Table shows best linear unbiased prediction for genotypic effect for 12 morphological traits among 100 wheat genotypes at control, drought and multi-environment trial analysis (META) respectively. The range of blupg value for plant height was 54.3-82.6 for control condition, 26.7-44.5 at drought condition and 46.9-59.2 at META. The range of blupg value for no. of tiller per plant was 1.44-7.38 at control condition, 1.53-5.93 at drought and 3.16-4.02 at META. Again, the blupg value range for proline content was 0.05-1.91 at control condition, 1.59-2.96 at drought condition and 1.44-1.69 at META respectively. For root length the range was 22.8-41.8 at control, 18.9-38.2s at drought and 22.5-28.2 at META. For shoot dry weight, the range was 5.35-28.5 at control, 0.05-3.04 at drought condition and 3.31-15.3 at META. For control condition, the range of blupg value for root shoot ratio, relative water content, excise leaf water loss, shoot fresh weight, root fresh weight, root dry weight and root volume were 0.003-0.092, 47.1-94.2, 4.53-5.56, 38-129, 1.64-8.20, 1.01-3.69 and 0.78-3.37 respectively. On the other hand, for drought condition the range of blupg value for root shoot ratio, relative water content, excise leaf water loss, shoot fresh weight, root fresh weight, root dry weight and root volume were 0.04-1.16, 24.3-70, 2.33-3.83, 31.6-85.6, 0.60-5.53, 0.36-0.96 and 0.26-2.00 respectively. And for META, the range of blupg value for root shoot ratio, relative water content, excise leaf water loss, shoot fresh weight, root fresh weight, root dry weight and root volume were 0.34-0.39, 48.1-62.1, 3.94-4.37, 39.3-70.5, 1.90-5.49, 0.74-1.15 and 0.70-2.26 respectively. The maximum blupg value at control condition were occupied by GEN-1 (for PH), GEN-80 (for NTPP), GEN-12 (for PC), GEN-51 (for RL), GEN-8 (for SDW), GEN-9 (for RSR), GEN-51 (for RWC), GEN-74 (for ELWL), GEN-17 (for SFW), GEN-69 (for RFW), GEN-64 (for RDW & RV) respectively. Again, the minimum blupg value at control condition were occupied by GEN-78 (for PH), GEN-40 (for NTPP), GEN-74 (for PC), GEN-58 (for RL), GEN-20 (for SDW), GEN-90 (for RSR), GEN-8 (for RWC), GEN-4 (for ELWL), GEN-70 (for SFW), GEN-35 (for RFW), GEN-67 (for RDW) & GEN-88 (RV) respectively. The maximum blupg value at drought condition were occupied by GEN-57 (for PH), GEN-77 (for NTPP), GEN-37 (for PC), GEN-12 (for RL), GEN-84 (for SDW), GEN-58 (for RSR), GEN-35 (for RWC), GEN-4 (for ELWL), GEN-67 (for SFW), GEN-70 (for RFW), GEN-5 (for RDW) & GEN-62 (for RV) respectively. Again, the minimum blupg value at drought condition were occupied by GEN-100 (for PH), GEN-100 (for NTPP), GEN-67 (for PC), GEN-58 (for RL), GEN-89 (for SDW), GEN-77 (for RSR), GEN-38 (for RWC), GEN-19 (for ELWL), GEN-77 (for SFW), GEN-39 (for RFW), GEN-76 (for RDW) & GEN-88 (RV) respectively. The maximum blupg value at

META were occupied by GEN-1 (for PH), GEN-80 (for NTPP), GEN-96 (for PC), GEN-12 (for RL), GEN-8 (for SDW), GEN-59 (for RSR), GEN-36 (for RWC), GEN-5 (for ELWL), GEN-67 (for SFW), GEN-69 (for RFW), GEN-63 (for RDW) & GEN-63 (for RV) respectively. Again, the minimum blupg value at META were occupied by GEN-90 (for PH), GEN-40 (for NTPP), GEN-68 (for PC), GEN-100 (for RL), GEN-20 (for SDW), GEN-24 (for RSR), GEN-40 (for RWC), GEN-4 (for ELWL), GEN-100 (for SFW), GEN-35 (for RFW), GEN-36 (for RDW) & GEN-89 (RV) respectively.

Table 9. Blupg(Best unbiased linear predicted genotype) value observed by 100 genotypes through 12 morphological traits

VALUE	TRT	PH	NTPP	PC	RL	SDW	RSR	RWC	ELWL	SFW	RFW	RDW	RV
MAX	CON	84.1	7.50	1.91	44.5	28.5	0.92	94.5	5.59	129	8.42	3.83	3.47
		G-1	G-80	G-12	G-51	G-8	G-9	G-51	G-4	G-17	G-69	G-64	G-64
	DRT	46	2.96	2.96	40.3	3.04	1.21	70.4	3.87	86.3	5.68	1.32	2.05
		G-58	G-39	G-39	G-13	G-85	G-59	G-36	G-5	G-68	G-71	G-6	G-63
	META	64.5	5.50	2.67	37.7	15.3	0.91	79.2	4.71	94.5	6.97	2.19	2.70
		G-1	G-80	G-12	G-16	G-8	G-59	G-36	G-5	G-74	G-69	G-63	G-63
MIN	CON	53	1.34	0.05	21.5	5.35	0.003	46.8	4.48	37.5	1.54	0.95	0.75
		G-78	G-40	G-74	G-58	G-20	G-90	G-8	G-5	G-38	G-35	G-67	G-88
	DRT	24.8	1.59	1.59	17.3	0.04	0.02	24	2.30	31.1	0.55	0.23	0.24
		G-90	G-68	G-68	G-59	G-90	G-78	G-39	G-20	G-78	G-40	G-77	G-89
	META	42.9	1.75	0.98	22.5	3.31	0.13	38.7	3.64	39.3	1.44	0.74	0.56
		G-90	G-40	G-68	G-76	G-20	G-78	G-40	G-100	G-98	G-35	G-36	G-89

Here, PH= plant height, NTPP=no. of tiller per plant, PC= proline content, RL=root length, SDW= shoot dry weight, RSR= root shoot ratio, RWC= relative water content, ELWL= excise leaf water loss ,SFW= shoot fresh weight , RFW= root fresh weight , RDW= root dry weight, and RV= root volume, CON= control, DRT=drought, META= Multi-Environmental Trial Analysis.

4.2 Estimation of correlation coefficient of twelve characters

The simple correlation coefficient among twelve important characters viz. plant height (cm)**,** no. of tiller per plant, proline content, root length(cm), shoot dry weight(g), root shoot ratio, relative water content, excise leaf water loss, shoot fresh weight(g) ,root fresh weight(g), root dry weight(g),root volume were analyzed for 100 wheat genotypes. The correlated characters and the values of the correlation coefficient on control condition and drought stress are presented in Figure 6.

In control condition, Plant height has a significant positive association with root shoot ratio and no. of tiller per plant, proline content, root length, excise leaf water loss, root fresh weight, root dry weight, root volume have a significant negative association. In drought conditions, root length, shoot dry weight has positive correlation with plant height and shoot fresh weight has a significant negative association. In case of no. of tiller per plant in control condition has significant positive association with root fresh weight and root dry weight. Proline content has a significant negative correlation with excise leaf water loss and root dry weight in control condition. Root length has a significant negative correlation with root volume in control condition. On the other hand, in drought condition, significant positive associations with shoot dry weight. In control and drought condition, root shoot ratio and relative water content has negative correlation with shoot dry weight and positive correlation with excise leaf water loss drought condition. Root shoot ratio has no significance in control and drought condition. Relative water content has significant negative association with root volume in control condition. Excise leaf water loss has a positive correlation with root volume in control condition and significant negative association with shoot fresh weight and root fresh weight in drought condition. In control and drought condition, root volume and root fresh weight have a significant positive correlation with shoot fresh weight. Root fresh weight has a significant positive correlation with root dry weight in control and significant negative correlation with root volume in drought condition. In control condition, root volume has a significant positive association with root dry weight. Ul-Allah *et al.* (2014) conducted an experiment and their findings related to current study in term of that shoot length is negatively correlated with root length.

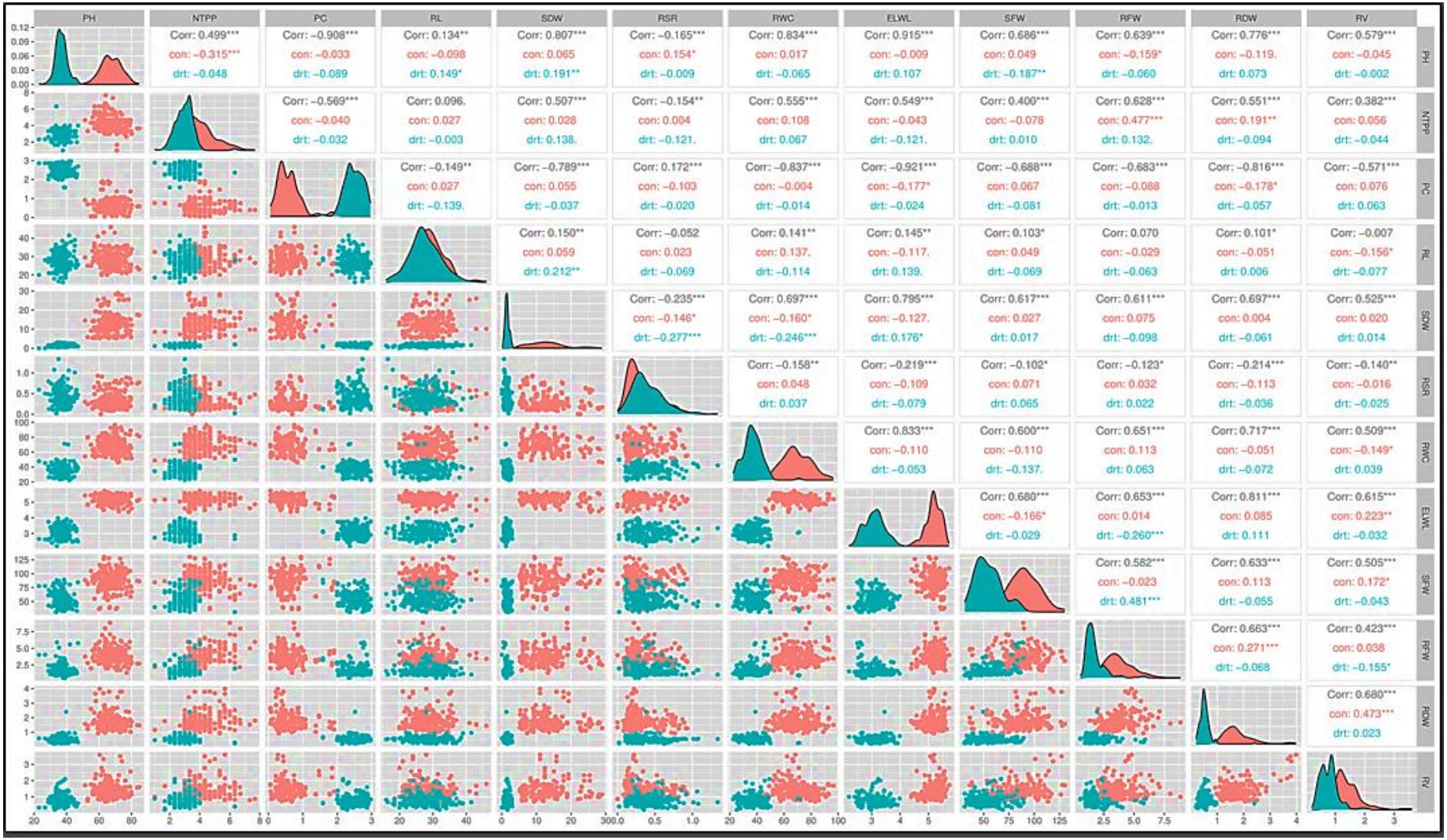


Figure 6: Correlations among 12 different characters of wheat under control condition and drought conditions

4.3Multivariate Analysis

Multivariate analysis or clustering refers is the task of grouping a set of objects in such a way that the objects in the same group (called cluster) are more similar (in some sense) to each other than to those in other groups (clusters).

4.3.1 Cluster Analysis under drought condition

Cluster analysis was performed with the relative mean values of each genotype. The standardized mean of 100 wheat genotypes were employed and an UPGMA dendrogram was constructed using these values (Figure 7). In this dendrogram, 100 wheat genotypes were grouped into six clusters. Maximum 34 genotypes were occupied in cluster VI followed by 20, 19, 13, 11 and 3 genotypes in cluster I, III, IV, V and II respectively (Table 10). The highest inter-cluster distance (7.58) was found among cluster II & IV which indicates higher diversity among them.

Among the six clusters, Cluster VI consisted of the largest number of genotypes. The cluster had a very high mean value only for shoot fresh weight. The cluster had the second largest (high) mean in plant height and relative water content. The cluster had moderate mean values for root length and shoot dry weight. The majority of the genotypes in this cluster showed lowest performance in most of the traits viz., no. of tiller of plant, proline content, root shoot ratio, excise leaf water loss, root fresh weight, root dry weight and root volume (Table 11).

Cluster I consisted of 20 genotypes. The cluster had the highest cluster mean plant height, second largest (high) mean value for relative water content, and root length. The moderate cluster mean values for shoot dry weight. Cluster I showed the lowest cluster mean values for most of the traits viz., no. of tiller of plant, proline content, root shoot ratio, excise leaf water loss, root fresh weight, root dry weight and root volume.

Cluster III showed the highest cluster means for shoot fresh weight. The cluster showed the second-largest mean values for plant height and relative water content. The cluster showed moderate cluster values for root length and shoot dry weight. The cluster showed the lowest cluster mean values for no. of tiller of plant, proline content, root shoot ratio, excise leaf water loss, root fresh weight, root dry weight and root volume.

Cluster number IV had a very high mean value for relative water content and shoots fresh weight. The cluster had the second largest (high) mean in plant height and root length. The cluster had moderate mean values for shoot dry weight. The majority of the genotypes in this cluster showed lowest performance in most of the traits viz., no. of tiller of plant, proline content, root shoot ratio, excise leaf water loss, root fresh weight, root dry weight and root volume.

Cluster number V had a very high mean value only for shoot fresh weight. The cluster had the second largest (high) mean in plant height and relative water content. The cluster had moderate mean values for root length and shoot dry weight. The majority of the genotypes in this cluster showed lowest performance in most of the traits viz., no. of tiller of plant, proline content, root shoot ratio, excise leaf water loss, root fresh weight, root dry weight and root volume.

Cluster number II includes only three genotypes. Cluster number II had a very high mean value only for shoot fresh weight. The cluster had the second largest (high) mean in plant height, relative water content. The cluster had moderate mean values for root length and shoot dry weight. The majority of the genotypes in this cluster showed lowest performance in most of the traits viz., no. of tiller of plant, proline content, root shoot ratio, excise leaf water loss, root fresh weight, root dry weight and root volume.

Dendrogram showed that within the cluster VI had the most diversity. Variation among them may be the result of the differences in their origin. Minimum genetic diversity is present between Cluster II, III, IV and Cluster V which had better characters contributing to drought tolerance.

Considering all the characters it appeared that the genotypes in the cluster III, IV and II had superior performance. The genotypes in this cluster had relatively higher plant height, higher no. of tiller per plant, higher shoot dry weight, root dry weight, relative water content, root length and root volume. These findings were in accordance with Asraf *et al.* (2022) and Javed *et al.* (2022).

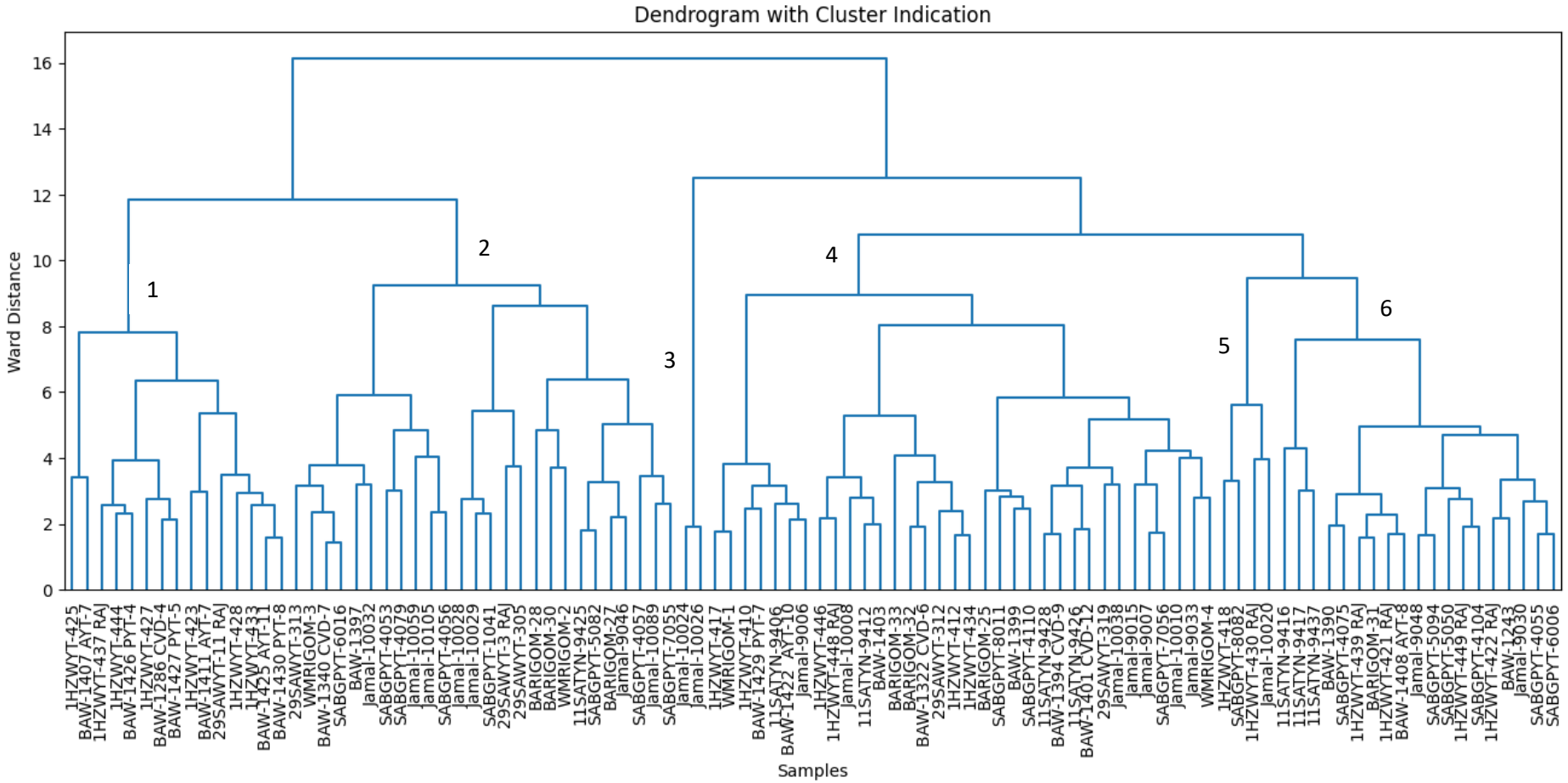


Figure7. Dendrogram from UPGMA clustering for 100wheat genotypes using Euclidean genetic distance based on twelve morphological characters

Table 10. Distribution of 100 Wheat in six different clusters in drought condition

Cluster	Number of genotypes	Genotypes in different clusters
I	20	11SATYN-9412, 11SATYN-9416, 11SATYN-9425, 11SATYN-9426, 11SATYN-9428, 1HZWYT-421 RAJ, 1HZWYT-422 RAJ, 1HZWYT-425, 1HZWYT-430 RAJ, 1HZWYT-439 RAJ, 1HZWYT-448 RAJ,1HZWYT-449 RAJ, 29SAWYT-305, 29SAWYT-313, BARIGOM-33, BAW-1243, BAW-1397, BAW-1401 CVD-12, BAW-1427 PYT-5, Jamal-10032
II	3	Jamal-10059, Jamal-10105, SABGPYT-5094
III	19	29SAWYT-3 RAJ, BAW-1403, Jamal-10010, Jamal-10028, Jamal-10029, Jamal-10038, Jamal-9006, Jamal-9015, Jamal-9030, Jamal-9046, SABGPYT-1041, SABGPYT-4053, SABGPYT-4057, SABGPYT-4075, SABGPYT-4079, SABGPYT-4104, SABGPYT-5082, SABGPYT-7056, SABGPYT-8011
IV	13	1HZWYT-427, 1HZWYT-433, 1HZWYT-434, BARIGOM-27, BARIGOM-28, BAW-1411 AYT-7, Jamal-10008, Jamal-10020, Jamal-10024, Jamal-10026, Jamal-9048, SABGPYT-8082, WMRIGOM-2
V	11	11SATYN-9406, 11SATYN-9417, 1HZWYT-446, BAW-1407 AYT-7, BAW-1425 AYT-11, BAW-1426 PYT-4, BAW-1429 PYT-7, BAW-1430 PYT-8, Jamal-9033, SABGPYT-5050, WMRIGOM-3
VI	34	11SATYN-9437, 1HZWYT-410, 1HZWYT-412, 1HZWYT-417, 1HZWYT-418, 1HZWYT-423, 1HZWYT-428, 1HZWYT-437 RAJ, 1HZWYT-444, 29SAWYT-11 RAJ, 29SAWYT-312, 29SAWYT-319, BARIGOM-25, BARIGOM-30, BARIGOM-31, BARIGOM-32, BAW-1286 CVD-4, BAW-1322 CVD-6, BAW-1340 CVD-7, BAW-1390, BAW-1394 CVD-9, BAW-1399, BAW-1408 AYT-8, BAW-1422 AYT-10, Jamal-10089, Jamal-9007, SABGPYT-4055, SABGPYT-4056, SABGPYT-4110, SABGPYT-6006, SABGPYT-6016, SABGPYT-7055, WMRIGOM-1, WMRIGOM-4

Table 11: Cluster means for 12 different characters of 100 genotypes at drought condition

Characters		Cluster mean				
	I	II	III	IV	V	V I
PH	38.06	34.66 (L)	35.01	36.59	38.79 (H)	36.36
NTPP	2.92	3.27	3.02	3.34 (H)	2.50 (L)	2.96
PC	2.36	2.79 (H)	2.33 (L)	2.50	2.39	2.64
RL	32.91 (H)	24.41	27.10	28.64	22.44 (L)	26.20
SDW	17.92 (H)	13.84	15.06	15.99	12.47 (L)	14.58
RSR	0.32 (L)	0.45	0.53 (H)	0.42	0.49	0.33
RWC	35.55	43.57	33.72 (L)	44.52 (H)	37.32	35.58
ELWL	3.31 (H)	2.58 (L)	2.91	2.86	2.89	3.24
SFW	15.8 (L)	82.07 (H)	65.91	44.74	48.60	53.21
RFW	0.59 (L)	5.08 (H)	2.15	1.43	1.51	1.44
RDW	0.67 (H)	0.46	0.45	0.44 (L)	0.55	0.54
RV	0.67	0.60 (L)	0.72	1.15 (H)	0.61	0.79

Table 12 Intra (bold) and inter-cluster distance of 6 clusters of 100 genotypes

Inter-cluster distance	1	2	3	4	5	6
1	**2.67**	7.71	5.00	5.39	5.52	4.42
2	7.71	**2.82**	6.23	7.58	7.17	6.84
3	5.00	6.23	**2.83**	5.37	4.86	4.38
4	5.39	7.58	5.37	**3.36**	5.67	4.88
5	5.52	7.17	4.86	5.67	**2.80**	4.44
6	4.42	6.84	4.38	4.88	4.44	**2.37**

4.4 Multi-trait Genotype–Idiotype Distance Index (MGIDI), genotype selection, and trait adaptation

4.4.1 Principal Component Analysis (PCA)

By analyzing the eigen values and cumulative frequency for the principal components obtained by the genetic correlation matrix, the first five main components presented eigenvalues with values > 1 (Table 16). Thus, according to Kaiser's criterion (Kaiser,1958), the data may be dimensionally reduced in five factors. The cumulative variance for the first five principal components accounted for ~61% of all genetic variability present in the dataset.

Table 13. Eigen value estimates by principal components analysis and the proportion of variance explained by them

PC	Eigen values	Variance (%)	Cum. variance (%)
PC1	1.99	16.6	16.6
PC2	1.64	13.7	30.2
PC3	1.36	11.4	41.6
PC4	1.24	10.3	51.9
PC5	1.07	8.89	60.8
PC6	0.98	8.19	69.0
PC7	0.9	7.48	76.5
PC8	0.78	6.52	83.0
PC9	0.67	5.6	88.6
PC10	0.57	4.77	93.4
PC11	0.48	3.96	97.3
PC12	0.32	2.67	100

Here, PC = Principal component

4.4.2 Factor Analysis

For control condition, the 12 traits were grouped into the five factors (FA) with the Comunalit Mean: 0.60 as follows (Table 13.1): The traits No. of tiller per plant (NTPP)&Root fresh weight (RFW) were constituted the first factor FA1 while the traits Shoot fresh weight (SFW), Root dry weight (RDW)&Root volume (RV) constituted the second factor FA2; In FA3, Root length (RL)&Excise leaf water loss (ELWL) were found. The Plant height (PH), Proline content (PC)&Root shoot ratio (RSR) were in FA4 while Shoot dry weight (SDW)&Relative water content (RWC) were constituted FA5. On the other hand, for drought condition, the 12 traits were grouped into the four factors (FA) with the Comunalit Mean: 0.49 as follows (Table 14.1): The traits Shoot dry weight (SDW), Root shoot ratio (RSR) & Relative water content (RWC) were constituted the first factor FA1 while the traits SFW & RFW constituted the second factor FA2; In FA3, No. of tiller per plant (NTPP), Excise leaf water loss (ELWL)&Root dry weight (RDW) were found. The Plant height (PH), Proline content (PC), Root length (RL)&Root volume (RV) were constituted in FA4.Loadings resulting from an orthogonal rotation range from − to + 1 and are the correlation coefficients between each trait and the factor. In most cases, closely related traits were grouped with in the same factor (Table 12.1 & 13.1). The average communalities obtained in the selection factors revealed that the factors explained a high proportion of the total variation observed.

The MGIDI provided desired selection differentials (SD) 12 studied traits (Table 13.2 & 14.2). The selection differentials ranged from -10.2% (RSR) to 54.0% (RDW) (Table 12.2). On the other hand, the selection differentials ranged from -27.0% (RV) to 26.9% (RFW) (Table 14.2). Table13.2 presents the predicted mean genotypic values and the gains from selection considering the control condition. ELWL, PH, PC, RSR and RWC showed negative gains, and NTPP, RFW, RDW, RL, RV, SFW and SDW showed positive gain. Table 14.2 presents the predicted mean genotypic values and the gains from selection considering the drought conditions, SFW, RDW, PC & RV showed negative gains, and SDW, RWC, RFW, NTPP, ELWL, PH & RL showed positive gain. Due to increasing mean value the positive gain achieved through the selected genotypes.

Table 14.1 Explained variance, factorial loadings after varimax rotation, and communalities obtained in the factor analysis considering control condition

VAR	FA1	FA2	FA3	FA4	FA5	Communality	Uniquenesses
PH	0.39	-0.06	0.08	-0.6	0.22	0.58	0.42
NTPP	-0.8	0.02	-0.08	0.15	-0.06	0.67	0.33
PC	0.29	0.12	-0.48	0.49	-0.01	0.57	0.43
RL	-0.08	-0.16	-0.47	0.07	-0.11	0.27	0.73
SDW	-0.14	-0.08	-0.17	0.04	0.87	0.8	0.2
RSR	-0.03	-0.17	-0.17	-0.73	-0.27	0.63	0.37
RWC	-0.23	-0.27	-0.23	-0.07	-0.54	0.47	0.53
ELWL	0.06	0.08	0.76	0.14	-0.12	0.62	0.38
SFW	0.15	0.61	-0.45	-0.2	0.07	0.64	0.36
RFW	-0.81	0.06	-0.01	-0.08	0.06	0.67	0.33
RDW	-0.42	0.65	0.22	0.03	0.03	0.65	0.35
RV	-0.02	0.81	0.23	0.12	0.01	0.72	0.28

*Comunalit Mean: 0.60. Here, PH= plant height, NTPP=no. of tiller per plant, PC= proline content, RL=root length, SDW= shoot dry weight, RSR= root shoot ratio, RWC= relative water content , ELWL= excise leaf water loss ,SFW= shoot fresh weight , RFW= root fresh weight , RDW= root dry weight ,RV= root volume,

Table 14.2 Predicted genetic gains of the selected genotypes through multi-trait genotype-ideotype distance index considering control condition

VAR	FACTOR	X0	Xs	SD	SDprc	h2	SG	SGprc
NTPP	FA1	4.18	4.95	0. 77	18.5	0.96	0. 74	17.8
RFW	FA1	3. 78	4.73	0.95	25.4	0.95	0.91	24.2
SFW	FA2	8.4	98. 7	11.3	12.9	0.98	11.2	12.8
RDW	FA2	1. 77	2. 73	0.95	54.0	0.93	0.89	50.3
RV	FA2	1. 41	2.08	0.66	47.4	0.95	0.63	45.1
RL	FA3	28.9	29.5	0.66	2.29	0.82	0.54	1.89
ELWL	FA3	5.19	5.08	-0.10	-1.95	0.93	-0.094	-1.81
PH	FA4	67.5	65.1	-2.46	-3.64	0.91	-2.24	-3.31
PC	FA4	0.59	0.54	-0.052	-8.76	1.0	-0.052	-8.76
RSR	FA4	0.31	0.28	-0.032	-10.2	0.98	-0.032	-10.1
SDW	FA5	13.1	15.0	1.91	14.6	1.00	1.91	14.6
RWC	FA5	69.3	69.2	-0.11	-0.17	0.98	-0.11	-0.17

Here, PH= plant height, NTPP=no. of tiller per plant, PC= proline content, RL=root length, SDW= shoot dry weight, RSR= root shoot ratio, RWC= relative water content , ELWL= excise leaf water loss ,SFW= shoot fresh weight , RFW= root fresh weight , RDW= root dry weight , RV= root volume. X0 = the original population mean, Xs = the mean of selected genotypes, SD= selection differential, SDperc= selection differential in percentage, SG= selection gain, SGperc= selection gain in percentage.

Table 15.1 Explained variance, factorial loadings after varimax rotation, and communalities obtained in the factor analysis considering drought condition

VAR	FA1	FA2	FA3	FA4	Communality	Uniquenesses
PH	0.19	-0.3	-0.14	0.44	0.34	0.66
NTPP	0.26	0.01	0. 71	0.09	0.59	0.41
PC	0.1	-0.05	0	-0.67	0.4	0.53
RL	0.34	-0.12	-0.1	0.55	0.44	0.56
SDW	0.81	-0.01	0.03	0.14	0.67	0.33
RSR	-0.61	0.11	-0.2	-0.12	0.44	0.56
RWC	-0.48	-0.33	0.47	0.04	0.57	0.43
ELWL	0.28	-0.2	-0.46	0.17	0.36	0.64
SFW	0.04	0.87	-0.05	-0.01	0. 75	0.25
RFW	-0.13	0. 73	0.33	0.12	0.67	0.33
RDW	-0.06	-0.09	-0.52	0.06	0.29	0. 71
RV	0.1	-0.23	-0.06	-0.5	0.31	0.69

*Comunalit Mean: 0.49. Here, PH= plant height, NTPP=no. of tiller per plant, PC= proline content, RL=root length, SDW= shoot dry weight, RSR= root shoot ratio, RWC= relative water content , ELWL= excise leaf water loss ,SFW= shoot fresh weight , RFW= root fresh weight , RDW= root dry weight ,RV= root volume,

Table 15.2 Predicted genetic gains of the selected genotypes through multi-trait genotype-ideotype distance index considering drought condition

VAR	FACTOR	X0	Xs	SD	SDprc	h2	SG	SGprc
SDW	FA1	1.54	1.70	0.16	10.8	0.99	0.16	10.8
RSR	FA1	0.40	0.37	-0.02	-6.53	0.93	-0.024	-6.12
RWC	FA1	36.8	37.9	1.03	2.81	0.97	1.01	2.74
SFW	FA2	54.5	53.5	-1.03	-1.89	0.97	-1.01	-1.85
RFW	FA2	1. 72	2.18	0.46	26.9	0.96	0.44	25.9
NTPP	FA3	2.98	3.20	0.22	7.58	0.88	0.19	6.69
ELWL	FA3	3.09	3.11	0.02	0. 70	0.95	0.020	0.67
RDW	FA3	0.52	0.47	-0.04	-8.80	0.54	-0.02	-4.80
PH	FA4	36. 7	36.8	0.06	0.18	0.84	0.05	0.15
PC	FA4	2.49	2.35	-0.13	-5.60	0.99	-0.13	-5.56
RL	FA4	27.6	30.8	3.23	11. 7	0.84	2. 72	9.86
RV	FA4	0. 78	0.56	-0.21	-27.0	0.95	-0.20	-25.9

Here, PH= plant height, NTPP=no. of tiller per plant, PC= proline content, RL=root length, SDW= shoot dry weight, RSR= root shoot ratio , RWC= relative water content , ELWL= excise leaf water loss ,SFW= shoot fresh weight , RFW= root fresh weight , RDW= root dry weight ,RV= root volume.X0 = the original population mean, Xs = the mean of selected genotypes, SD= selection differential, SDperc= selection differential in percentage, SG= selection gain, SGperc= selection gain in percentage.

4.4.3 Selected genotypes and their strength and weaknesses

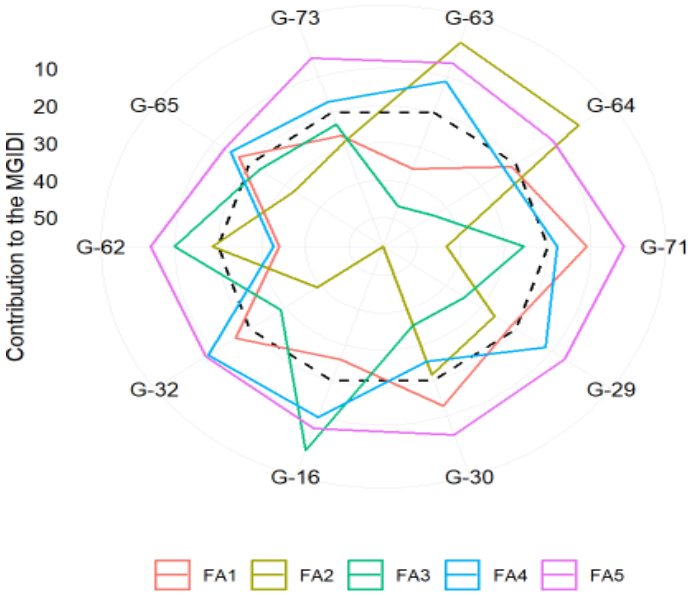
The strengths and weaknesses were studied to identify genotypes and traits to be selected for a much more efficient breeding program. The lower contribution of a factor in the genotype indicated that the genotype is highly selective for the trait in a particular factor. Figure 8 indicates the result of screening the investigated plant genetic accessions based on MGIDI index. In this figure, the red circle indicates the cut point according to the selection pressure (SI = 10%). The MGIDI index identified 10 as more desirable genotypes than others for each growth condition. Among these, G-16 (1HZWYT-425) was selected in both conditions, suggesting that it can maintain its ideal growth under both conditions.

In the control condition, the genotypes G-63 (Jamal-10024), G-64 (Jamal-10026), G-71 (Jamal-10105), G-29 (29SAWYT-3 RAJ), G-30 (29SAWYT-305), G-16 (1HZWYT-425), G-32 (29SAWYT-313), G-62 (Jamal-10020), G-65 (Jamal-10028) & G-73 (Jamal-9007) were selected (Figure 9a). The FA1 factor provided for the lowest contributions to genotype G-63 (Jamal-10024) & G-62(Jamal-10020) (Figure 8) thus G-63(Jamal-10024)& G-62(Jamal-10020) emphasizes the superiority of this genotype regarding no. of tiller per plant & root fresh weight. The smallest contributions provided for the factor FA2 were to genotypes G-16(1HZWYT-425) (Figure 8) suggested that they have higher shoot fresh weight, root dry weight and higher root volume. In the FA3, the lowest contributions to genotype G-63(Jamal-10024) & G-30(29SAWYT-305) provided that they have higher root length & excise leaf water loss. The lowest contributions provided for the factor FA4 were to genotypes G-62(Jamal-10020) revealed that it has higher plant height, proline content & root shoot ratio and the FA5 factor provided for the lowest contributions to genotypes G-64(Jamal-10026) as higher shoot dry weight and relative water content.

In the drought condition, the genotypes G-77 (Jamal-9046), G-86 (SABGPYT-4104), G-16(1HZWYT-425), G-87(SABGPYT-4110), G-19(1HZWYT-430 RAJ), G-5 (11SATYN-9425), G-67 (Jamal-10032) , G-72 (Jamal-9006),G-14 (1HZWYT-422 RAJ)& G-47 (BAW-1397) were selected (Figure 9b).In this strategy, the lowest observed contribution of the FA1 factor was for the genotypes G-77(Jamal-9046) & G-16(1HZWYT-425) (Figure 8) which revealed that they have higher shoot dry weight , root shoot ratio and relative water content. The FA2 factor provided for the lowest contributions to genotypes G-19(1HZWYT-430 RAJ) (Figure 8) as higher shoot fresh weight & root fresh weight. The lowest observed contribution of the FA3 factor was for the genotypes

G-14(1HZWYT-422 RAJ) as higher no. of tiller per plant, excise leaf water loss & root dry weight and The FA4 factor provided for the lowest contributions to genotypes G-72(Jamal-9006) & G-87(SABGPYT-4110) (Figure 8) which revealed that they have higher plant height, proline content, root length & root volume.

Control



Drought

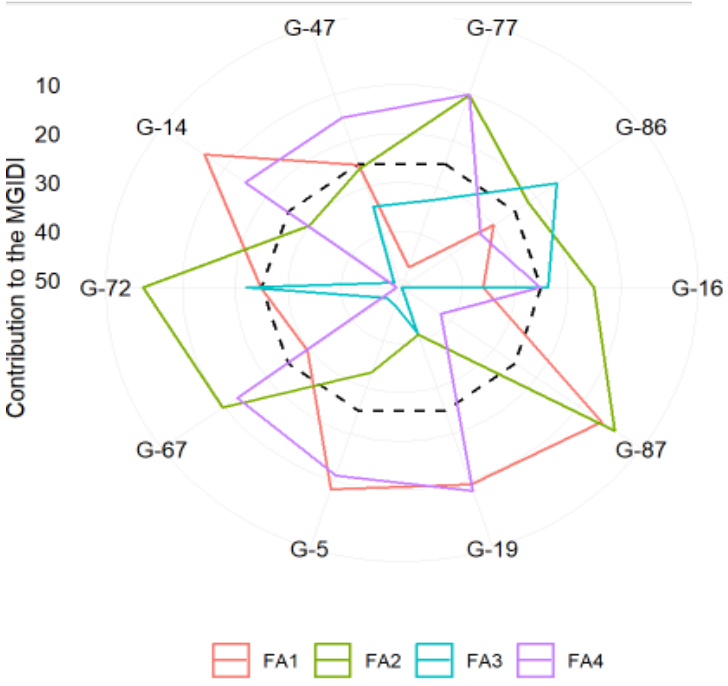


Figure8. The strength and weakness view among selected genotypes under control & drought condition. The dashed black line shows the theoretical value if all factors contributed equally.

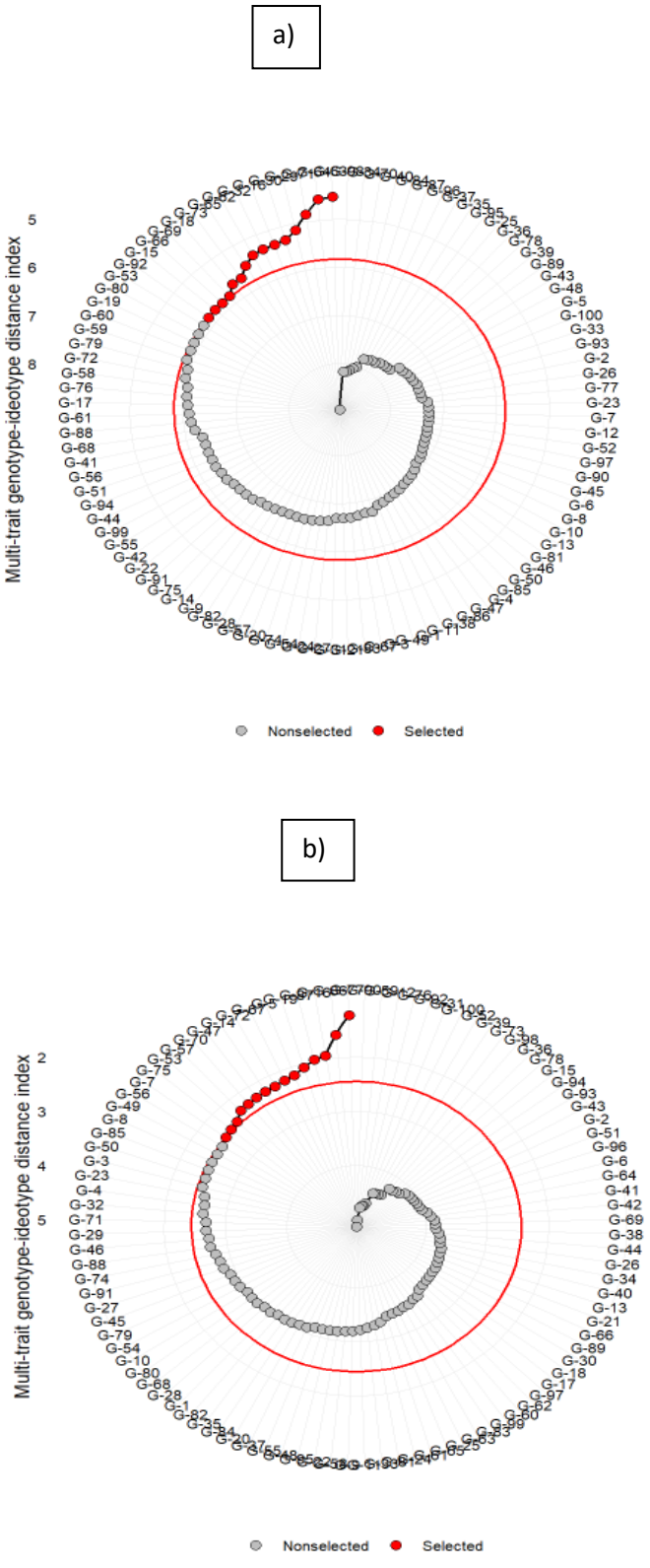


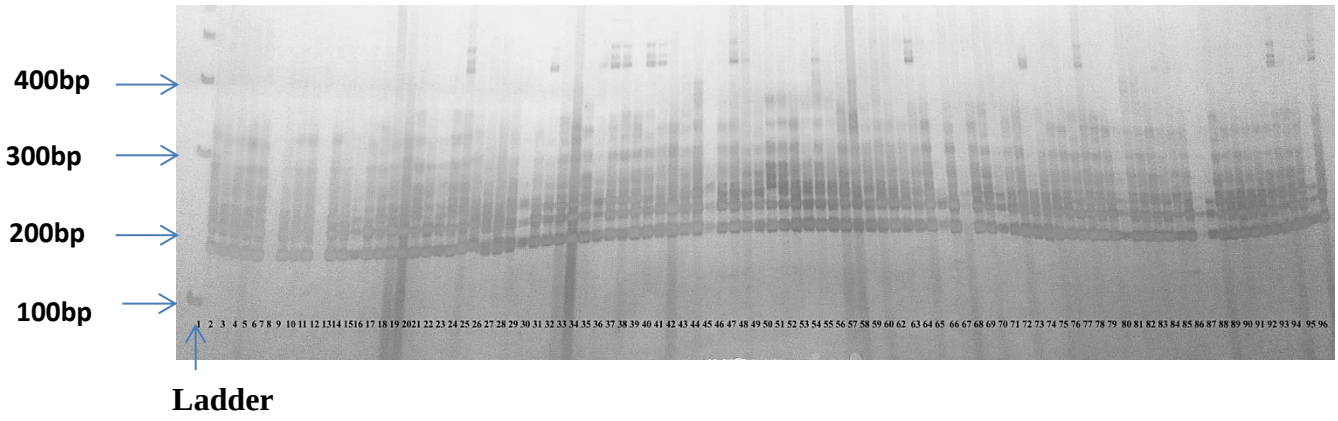
Figure 9. Genotype ranking and selected genotypes (in red) in ascending order for the multi-trait genotype ideotype distance index considering two treatments: (a) control; (b) drought. Red circle represents cut point according to the selection pressure.

4. (B) Assessment of genetic diversity in 100 wheat genotypes through molecular study

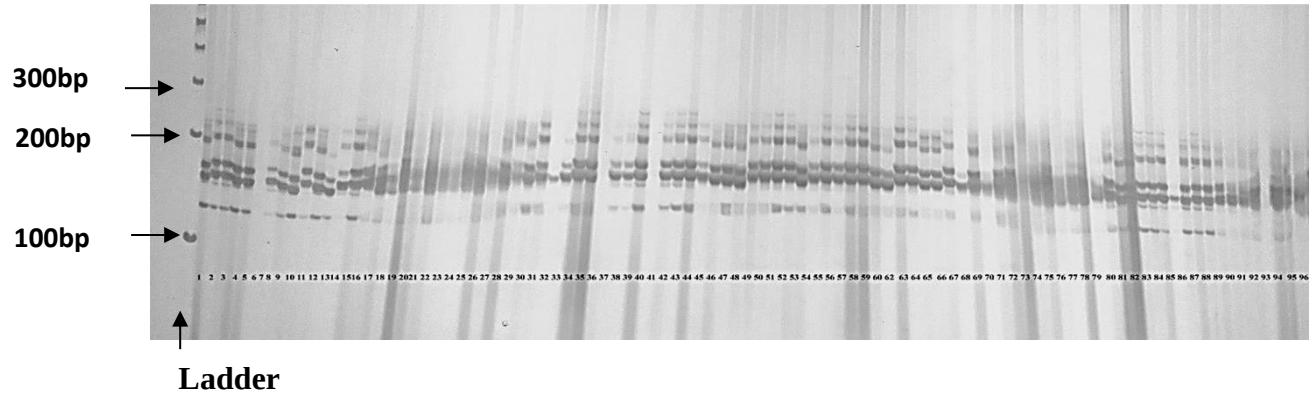
In this study, five (5) microsatellite markers were used. The molecular research was undertaken in the Molecular Breeding Laboratory of the Department of Genetics and Plant Breeding, HSTU and Bangladesh Wheat and maize Research Institute, Dinajpur. The experiment's outcomes are reported under the subsequent subheadings-

4.5 Analysis of DNA fingerprinting based on SSR markers

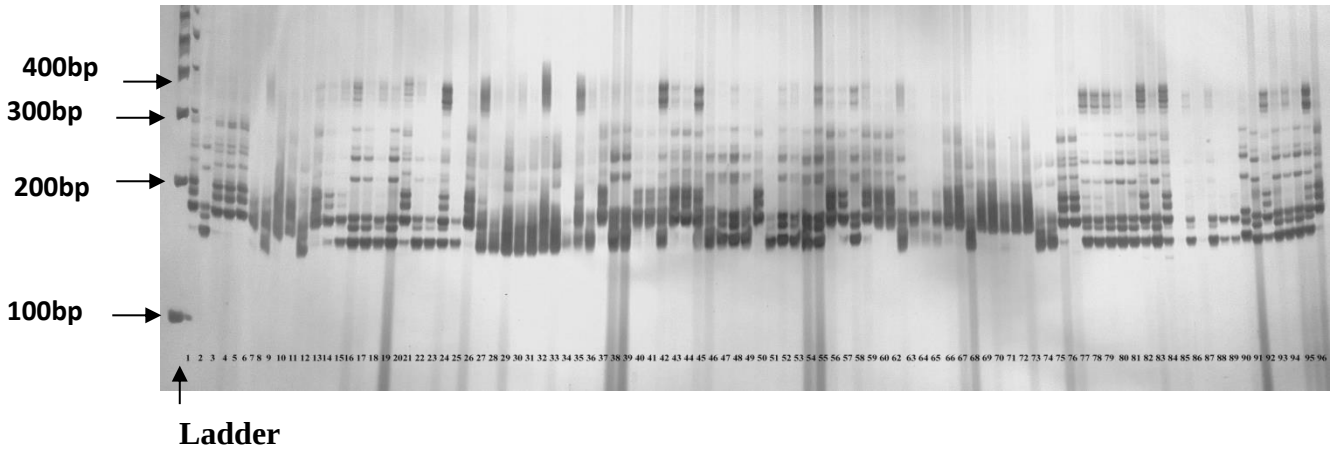
In the beginning, DNA was taken from young leaves of 3-week-old seedlings of 100 genotypes of wheat. DNA was extracted using modified CTAB method. Prior to PCR amplification, the quality of the extracted DNA samples was evaluated through quantification using a Thermo Scientific NanoDropTM1000 Spectrophotometer. One hundred different genotypes of wheat had DNA quantities ranging from 96 to 2600µl/mL. The 3 g/l. SSR markers revealed the polymorphic bands after polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis (PAGE) examination. Here, the distance between the DNA bands of the SSR/microsatellite markers was measured using a 100 bp DNA ladder. Figure 10displays a gel image of PCR-amplified fragments utilizing those SSR markers.



1. **WMS0691**

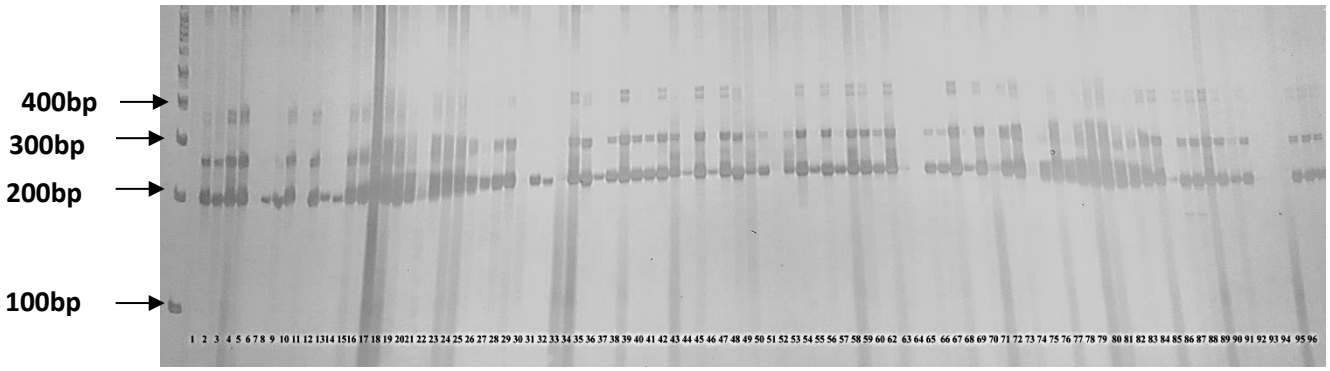


2. **GWM513**

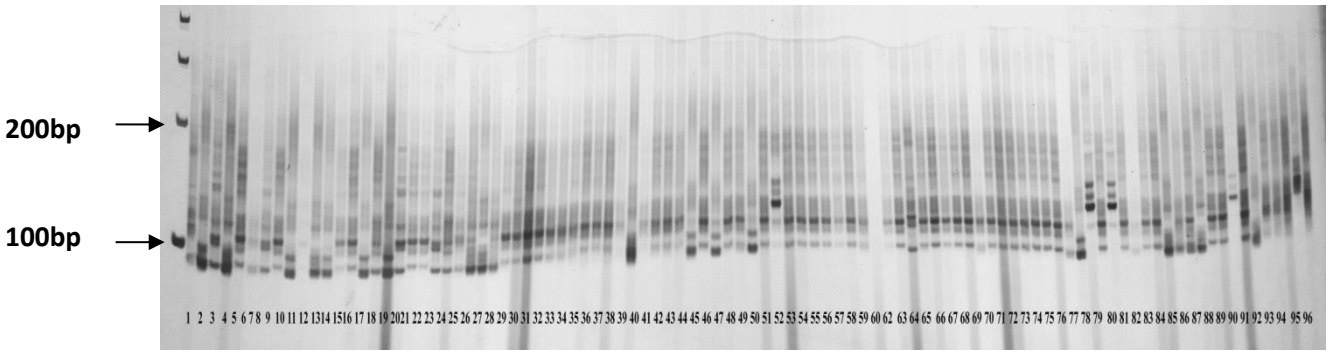


3. **GWM495**

Figure 10. Gel picture of microsatellite markers obtained from polyacrylamide gel electrophoresis using 100 bp DNA ladder. 1-96 indicates the genotype no. (According to Table.1)



Ladder **4. Barc20**



Ladder **5. Tagwm1037**

Figure 10. Gel picture of microsatellite markers obtained from polyacrylamide gel electrophoresis using 100 bp DNA ladder. 1-96 indicates the genotype no. (According to Table.1) (contd.)

4.6 Assessment of polymorphism from SSR Profiles

A total of 24 alleles were identified with 5 SSR markers over 96 wheat genotypes (Table 15). According to the Table 15, maximum range of band sizes was found by WMS0691 (146-400bp) which was followed by GWM495 (160-380bp) and Barc20 (200-360bp), respectively. The number of alleles per marker ranged from 2 to 7, with an average of 4.8 alleles across the 24 allele. The marker GWM495 produced the highest number of polymorphic alleles of seven (7) followed byTagwm1037 (6) and GWM513 with five (5), WMS0691 with four (4) respectively (Table 15). While Barc20 markers produced the least number of polymorphic bands per locus of two(2). The PIC (Polymorphism Information Content) values of SSRs ranged from 0.46 to 0.84 with an average of 0.41. The highest PIC value (0.84) was recorded for GWM495, and GWM513recorded second highest (0.78) and lowest by Barc20 (0.46). Dido *et al.* (2022) analyzed 49 SSR markers amplified a total of 478 alleles with an average of 9.755 alleles per marker were obtained of which 97.07% of the loci were observed to be polymorphic.

Table 16. Allele number, size range and polymorphism information content (PIC) found among 100 wheat genotypes for 5 microsatellite markers

Sl. No.	Markers	Allele No.	Size range (bp)	PIC
1	WMS0691	4	146-400	0.68
2	GWM513	5	120-210	0.78
3	GWM495	7	160-380	0.84
4	Barc20	2	200-360	0.46
5	Tagwm1037	6	100-160	0.62
Total		24		
Average		4.8		0.56

4.7 UPGMA dendrogram

The genetic distance-based results in the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis revealed six major clusters (Cluster I, Cluster II, Cluster III, Cluster IV, Cluster V and Cluster VI) in 100 wheat genotypes (Figure 11).

According to Table 16, Cluster I consisted of 16 genotypes namely- BARIGOM-25, BARIGOM-33, BARIGOM-31, 29SAWYT-319, BAW-1411 AYT-7, BAW-1401 CVD-12, BAW-1390, 29SAWYT-313, 1HZWYT-412, Jamal-10020, 29SAWYT-3 RAJ, BAW-1340 CVD-7, 1HZWYT-421 RAJ, BAW-1397, 29SAWYT-305, 29SAWYT-312. Again, cluster I was further divided into 2 sub-clusters (A and B). Among them the highest and lowest similarity found between sub-cluster A comprised viz, 29SAWYT-313, 1HZWYT-412, Jamal-10020, 29SAWYT-3 RAJ, BAW-1340 CVD-7, 1HZWYT-421 RAJ, BAW-1397, 29SAWYT-305, 29SAWYT-312. Sub cluster-B comprised of 7 genotypes viz. BARIGOM-25, BARIGOM-33, BARIGOM-31, 29SAWYT-319, BAW-1411 AYT-7, BAW-1401 CVD-12, BAW-1390.Cluster II consisted of 20 genotypes viz., BAW-1422 AYT-10, 1HZWYT-423, 1HZWYT-428,BARIGOM-28,BARIGOM-30, BAW-1394 CVD-9,BAW-1411 AYT-7,BAW-1425 AYT-11,1HZWYT-422 RAJ, SABGPYT-4055, SABGPYT-7055,SABGPYT-4053,SABGPYT-4057,SABGPYT-4079,BAW-1403,WMRIGOM-1, WMRIGOM-2,WMRIGOM-3,WMRIGOM-4,SABGPYT-8082.Again, cluster II was further divided into 2 sub-clusters (A and B). Sub cluster-A, included 15 genotypes viz. BAW-1422 AYT-10, 1HZWYT-423, 1HZWYT-428, BARIGOM-28, BARIGOM-30, BAW-1394 CVD-9, BAW-1411 AYT-7, BAW-1425 AYT-11,1HZWYT-422 RAJ, SABGPYT-4055, SABGPYT-7055, SABGPYT-4053, SABGPYT-4057,SABGPYT-4079,BAW-1403. Sub-cluster-B consisted of 5 genotypes namely- WMRIGOM-1, WMRIGOM-2, WMRIGOM-3, WMRIGOM-4, and SABGPYT-8082.Among them the highest and lowest similarity found. Cluster III consisted of 8 genotypes viz., 1HZWYT-410,1HZWYT-417,11SATYN-9412,11SATYN-9416,11SATYN-9406,11SATYN-9417,11SATYN-9425, and 11SATYN-9437. Again, cluster III was further divided into 2 sub-clusters (A and B). Sub cluster-A, included 3 genotypes viz.1HZWYT-410,1HZWYT-417 and 11SATYN-9412. Among them the highest and lowest similarity found. Sub-cluster-B consisted of 4 genotypes namely-11SATYN-9416, 11SATYN-9406,11SATYN-9417,11SATYN-9425, 11SATYN-9437 and the highest and lowest similarity found between them (Figure 11). Cluster IV consisted of 13 genotypes viz., Jamal-10032, Jamal-10089, Jamal-10008, Jamal-1010, Jamal-10059,

Jamal-1028,BARIGOM-27, BAW-1322 CVD-6,BAW-1286 CVD-4, BAW-1408 PYT-8, BAW-1427 PYT-5, BAW-1399, BAW-1243. Again, cluster IV was further divided into 2 sub-clusters (A and B). Sub cluster-A, included 6 genotypes viz. Jamal-10032, Jamal-10089, Jamal-10008, BARIGOM-27, Jamal-10059 and Jamal-10028. Sub-cluster-B consisted of 7 genotypes namely- Jamal-1010, BAW-1322 CVD-6,BAW-1286 CVD-4, BAW-1408 PYT-8, BAW-1427 PYT-5, BAW-1399 and BAW-1243. Cluster V consisted of 29 genotypes viz., Jamal-10024, BARIGOM-32, Jamal-10105, Jamal-9007,Jamal-9015, 1HZWYT-449 RAJ, 29SAWYT-11 RAJ,Jamal-10026,Jamal-10029, 29SAWYT-11 RAJ, 1HZWYT-449 RAJ, 11SATYN-9426, Jamal-9033,Jamal-9046,Jamal-9006,Jamal-9030,BAW-1426 PYT-4, 1HZWYT-437 RAJ, 1HZWYT-430 RAJ, 1HZWYT-439 RAJ, 1HZWYT-425, 1HZWYT-434,1HZWYT-433,1HZWYT-448 RAJ,1HZWYT-446, SABGPYT-6006, SABGPYT-6016,SABGPYT-5094,SABGPYT-5082,Jamal-10038,11SATYN-9428. Again, cluster IV was further divided into 2 sub-clusters (A and B). Sub cluster-A, included 15 genotypes viz. Jamal-10024, BARIGOM-32, Jamal-10105, Jamal-9007,Jamal-9015, 1HZWYT-449 RAJ, 29SAWYT-11 RAJ,Jamal-10026,Jamal-10029, 29SAWYT-11 RAJ, 1HZWYT-449 RAJ, 11SATYN-9426, Jamal-9033,Jamal-9046 andJamal-9006. Sub-cluster-B consisted of 14 genotypes namely- Jamal-9030,BAW-1426 PYT-4, 1HZWYT-437 RAJ, 1HZWYT-430 RAJ, 1HZWYT-439 RAJ, 1HZWYT-425, 1HZWYT-434,1HZWYT-433,1HZWYT-448 RAJ,1HZWYT-446, SABGPYT-6006, SABGPYT-6016,SABGPYT-5094,SABGPYT-5082,Jamal-10038 and 11SATYN-9428.Khalil *et al.*(2020) studied that cluster analysis and dendrogram showed the highest degree of genetic similarity between variety Arabiasuad and variety Arabiabiad (0.7619).

The heat map of population shows genetic diversity (Figure 12). The length of the amplified fragments at each locus is represented by a colored band and each locus has a unique contrast diagram of fragment size and color. Different colors represent different fragment sizes. Individuals of the same population are surrounded by black dotted frames.

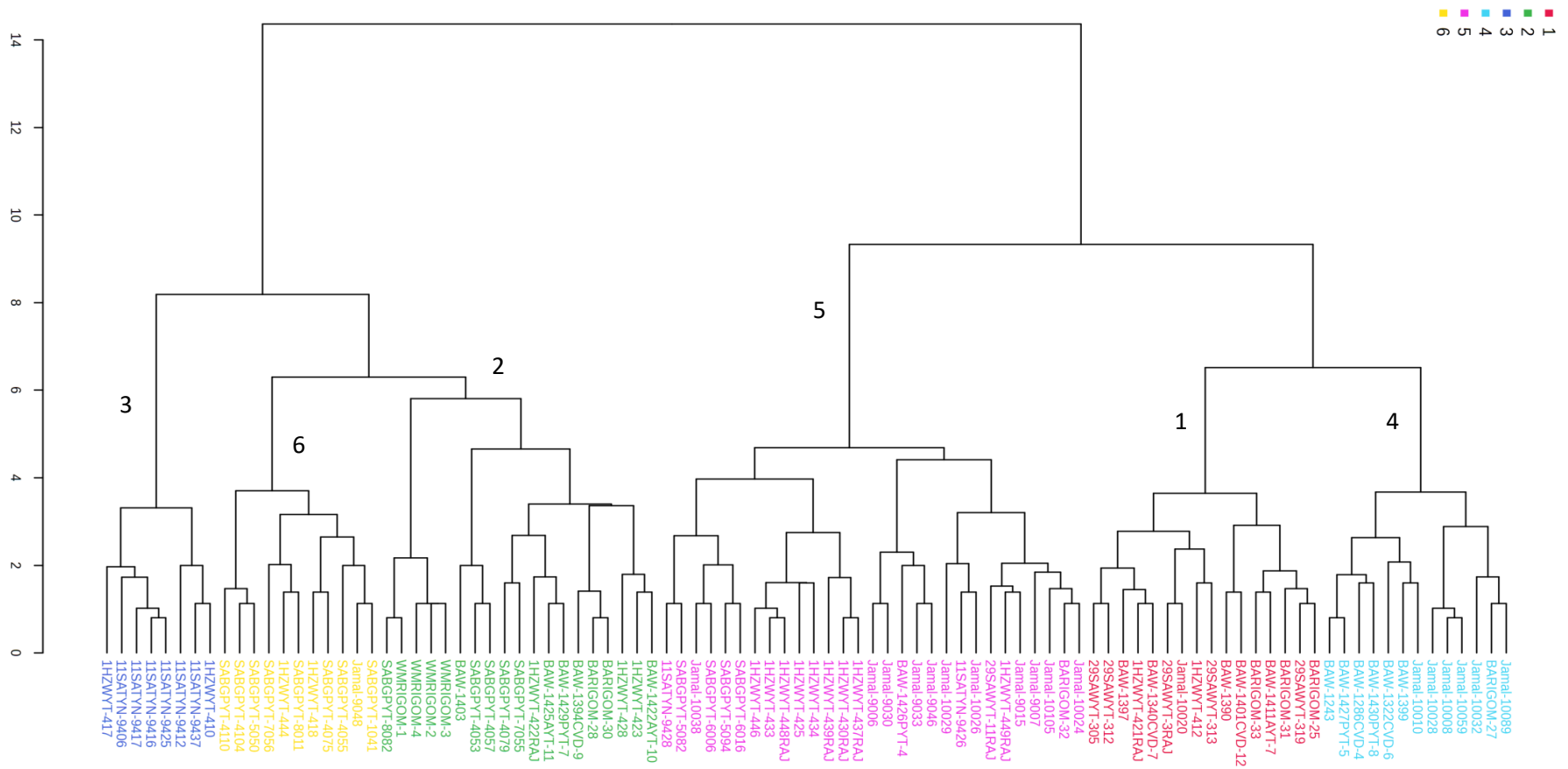


Figure 11: An UPGMA cluster dendrogram showing the genetic relationships between 96wheat genotypes based on the alleles detected by 5 microsatellite markers

Table 17 Distribution of 100 Wheat in six different clusters by Molecular analysis

Cluster	Number of genotypes	Genotypes in different clusters
I	16	BARIGOM-25, BARIGOM-33, BARIGOM-31, 29SAWYT-319, BAW-1411 AYT-7, BAW-1401 CVD-12, BAW-1390, 29SAWYT-313, 1HZWYT-412, Jamal-10020, 29SAWYT-3 RAJ, BAW-1340 CVD-7, 1HZWYT-421 RAJ, BAW-1397, 29SAWYT-305, 29SAWYT-312.
II	20	BAW-1422 AYT-10, 1HZWYT-423, 1HZWYT-428,BARIGOM-28,BARIGOM-30, BAW-1394 CVD-9,BAW-1411 AYT-7,BAW-1425 AYT-11,1HZWYT-422 RAJ, SABGPYT-4055, SABGPYT-7055,SABGPYT-4053,SABGPYT-4057,SABGPYT-4079,BAW-1403,WMRIGOM-1,WMRIGOM-2,WMRIGOM-3,WMRIGOM-4,SABGPYT-8082.
III	8	1HZWYT-410,1HZWYT-417,11SATYN-9412, 11SATYN-9416,11SATYN-9406, 11SATYN-9417,11SATYN-9425, 11SATYN-9437.
IV	13	Jamal-10032, Jamal-10089, Jamal-10008, Jamal-10010, Jamal-10059, Jamal-10028,BARIGOM-27, BAW-1322 CVD-6,BAW-1286 CVD-4, BAW-1408 PYT-8, BAW-1427 PYT-5, BAW-1399, BAW-1243.
V	29	Jamal-10024, BARIGOM-32, Jamal-10105, Jamal-9007,Jamal-9015, 1HZWYT-449 RAJ, 29SAWYT-11 RAJ,Jamal-10026,Jamal-10029, 29SAWYT-11 RAJ, 1HZWYT-449 RAJ, 11SATYN-9426, Jamal-9033,Jamal-9046,Jamal-9006,Jamal-9030,BAW-1426 PYT-4, 1HZWYT-437 RAJ, 1HZWYT-430 RAJ, 1HZWYT-439 RAJ, 1HZWYT-425, 1HZWYT-434,1HZWYT-433,1HZWYT-448 RAJ,1HZWYT-446, SABGPYT-6006, SABGPYT-6016,SABGPYT-5094,SABGPYT-5082,Jamal-10038,11SATYN-9428
VI	10	SABGPYT-1041,Jamal-9048,SABGPYT-4075, 1HZWYT-418,1HZWYT-444,SABGPYT-7056, SABGPYT-8011, SABGPYT-4110,SABGPYT-5050,SABGPYT-4104.

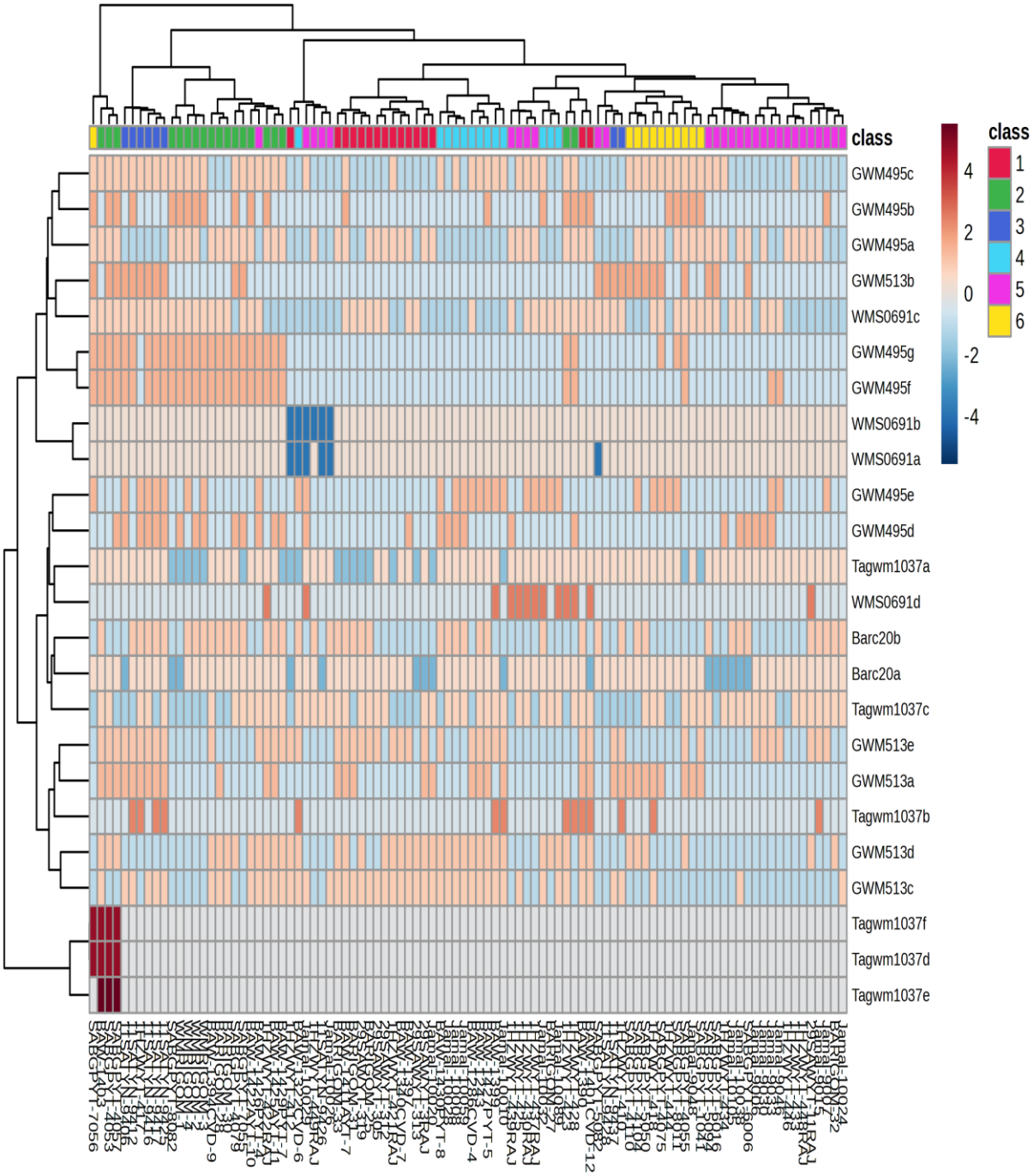


Figure 12. Multivariate heat map illustrating the genetic diversity of 96 wheat genotypes based on the 5 SSR markers

4.8 Principal component analysis

Principal Component Analysis Associations among 96 wheat genotypes were computed using PCA method. Here, first principal component (PC1), and the second principal component (PC2) explained 16.9% and 13.1 % of total variation, respectively and localization of genotypes in a 2D PCA plot indicates the genetic distances among the wheat genotypes (Figure 13). Here, Population I, Population II, Population III, Population IV, Population Vand Population VI were mixed with genotypes viz., In PCA scree plot, the green line on top showed the accumulated variance explained; the blue line underneath showed the variance explained by individual PC (Figure 14).

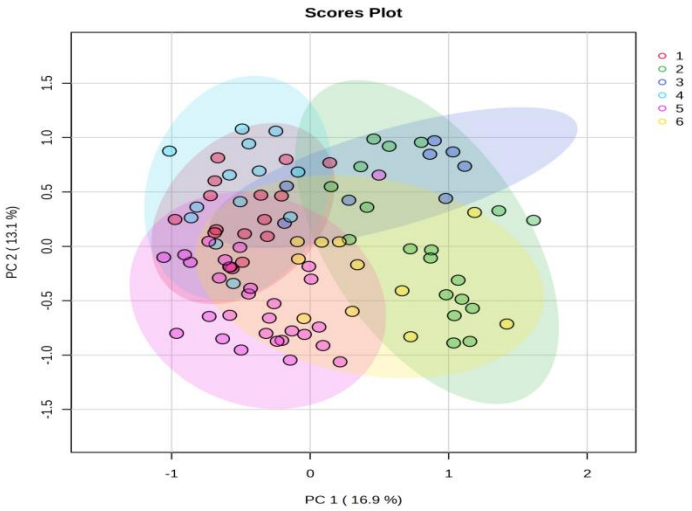


Figure 13: Two-dimensional principal component analysis (PCA) based on SSR polymorphisms in the 96 wheat genotypes.

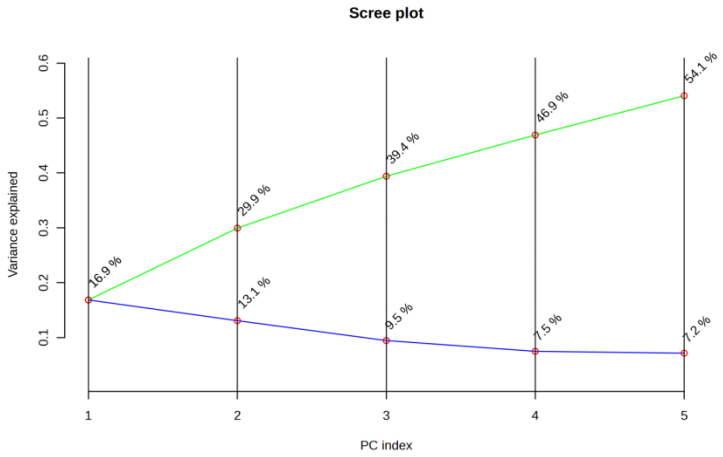


Figure 14:The scree plot displays the Principal coordinate analysis of the top 5 PCs for 96 wheat genotypes based on SSR marker data

4.9 Interrelationship of phenotypic and molecular outcome

Among 100 wheat genotypes, the genotypes 11SATYN-9425, 1HZWYT-422 RAJ, 1HZWYT-425, 1HZWYT-430 RAJ, BAW-1397, Jamal-10032, Jamal-9006, SABGPYT-4104 and SABGPYT-4110 are highly drought tolerant selected by multi-trait ideotype selection. Based on the morphological diversity 1HZWYT-425,1HZWYT-430 RAJ, 11SATYN-9425, Jamal-10032, 1HZWYT-422 RAJ, BAW-1397, Jamal-9046, SABGPYT-4104, Jamal-9006 were identified in cluster I and cluster III as similar genotypes.

Based on molecular study, the respected tolerant genotypes obtained from previous study i.e. 1HZWYT-425, 1HZWYT-430 RAJ and Jamal-9006 are located in same cluster as UPGMA cluster V.

CHAPTER V
SUMMARY AND CONCLUSION

The present investigation was conducted with one hundred wheat genotypes in CRD design with two replications at Breeder Seed Production Station, BWMRI, Debiganj, Panchagarh and Wheat Breeding laboratory, Bangladesh Wheat and Maize Research Institute, Dinajpur and Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh during Rabi season, 2022-2023. The objectives of the research work were to identify drought stress tolerance genotypes among the studied wheat germplasms using MGIDI and morpho-physiological as well as molecular diversity at seedling stage to assess the degree of divergence among the experimental wheat genotypes. In order to propose elite genotype with desired characters i.e. LRT, phenotypic and genotypic coefficient of variances, heritability,correlation,BLUPg, PCA, Multi-trial Genotype Ideotype Index (MGIDI), Factor analysis, Strength and Weakness were also studied. Apart from morphological analyses, molecular markers (SSRs) were also applied in order to molecular characterize these wheat germplasms at genetic level.

In morpho-physiological study, the observations were recorded on three selected plants per genotype at seedling stage in each replication. The statistical significance of the likelihood ratio for genotypes (LRTg) and the likelihood ratio for the genotype and environment interaction (LRTge) were observed in control and drought for all the morphological traits, except for the LRTg in the case of RL, SDW, RSR, SFW and RDW. All physiological traits except the PC was non-significant for the LRTg in multi-environment analysis.

From genetic parameter studies, Phenotypic variance was higher than genotypic variance at both control & drought condition. Mean heritability values ranged from 0.82(RL) to 1.00 (PC and SDW) in the control condition, and from 0.54 (RDW) to 0.99 (PC) in the drought condition. Selective accuracies ranged from 0.90(RL) to 1.00(PC and SDW) in the control condition and from 0.73 (RDW) to 0.99 (DH) in the drought condition, in the META. Mean heritability values ranged from 0.03(RL) to 0.72 (RV). Selective accuracies ranged from 0.03(RL) to 0.85(RV).

Descriptive analysis revealed that in the two environments, the average deviation for PH, NTPP, RFW, RL, RWC and RF at drought condition was relatively low compared with control condition. However, for other traits, average deviation appeared higher under

drought condition, indicating a higher genotypic response under the drought condition; hence, the higher scope of genotype selection for those traits.

By analyzing the overall performance of 12 different morphological traits under two different environment or treatment, it was found the max mean value of Plant height (PH), No. of tiller per plant (NTPP) , proline content (PC), Root length (RL), shoot dry weight (SDW), root shoot ratio (RSR), relative water content (RWC), shoot fresh weight (SFW), root fresh weight (RFW), root dry weight (RDW) and root volume (RV) was recorded by BAW-1429 PYT-7, Jamal-9048, BARIGOM-32, 1HZWYT-421 RAJ, SABGPYT-4079, BAW-1430 PYT-8, BARIGOM-28, Jamal-10038, Jamal-10105, 11SATYN-9426 and Jamal-10024 respectively at drought condition.

By considering blupg value, for control condition, the highest plant height was found in genotype 11SATYN-9406 (82.6cm), Genotype SABGPYT-4053 (7.38) the maximum number of tiller plant^{-1}. The highest proline content was exhibited in the genotype 1HZWYT-418 (1.91). The maximum root length (41.8) & relative water content (94.2) were revealed for the genotype BAW-1407 AYT-7. Genotype 11SATYN-9437 (28.5g) showed the highest shoot dry weight. The maximum amount of root shoot ratio was revealed for the genotype 1HZWYT-410 (0.91). The higher excise leaf water loss were revealed for the genotype Jamal-9015 (5.56). Genotype 1HZWYT-427(129g) showed highest shoot fresh weight. The maximum root fresh weight was found for the genotype Jamal-10059(8.20g). Maximum values for root dry weight (3.69) & root volume (3.37) were revealed for the genotype Jamal-10026.

In drought stressed condition, the highest plant height (82.6cm) was found in genotype BAW-1427 PYT-5. Genotype Jamal-9046 showed the maximum number of tiller plant^{-1}(7.38). The highest proline content (1.91mM) was exhibited in the genotype BARIGOM-30.The maximum root length was revealed for the genotype 1HZWYT-418 (41.8cm). Genotype SABGPYT-4075 (28.5g) showed the highest shoot dry weight. The maximum amount of root shoot ratio was revealed for the genotype BAW-1429 PYT-7 (0.91). The highest relative water content was revealed for the genotype BARIGOM-27 (94.2). The higher excise leaf water loss was revealed for the genotype 11SATYN-9417 (5.56). Genotype Jamal-10032 (129g) showed highest shoot fresh weight. The maximum root fresh weight was found for the genotype Jamal-10032 (8.20g). Maximum values for root

dry weight was revealed for the genotype 11SATYN-9425 (3.69g). The highest root volume was revealed for the genotype Jamal-10020 (3.37).

In META, the highest plant height was found in genotype 11SATYN-9406 (64.5cm). Genotype SABGPYT-4053 (5.50) showed the maximum number of tiller plant^{-1}. The highest proline content was exhibited in the genotype 1HZWYT-418 (2.67). The maximum root length was revealed for the genotype 1HZWYT-425 (37.7cm). Genotype 11SATYN-9437 (15.3g) showed the highest shoot dry weight. The maximum amount of root shoot ratio was revealed for the genotype BAW-1430 PYT-8 (0.91). The highest relative water content was revealed for the genotype BARIGOM-28 (79.2). The higher excise leaf water loss was revealed for the genotype 11SATYN-9425 (4.71). Genotype Jamal-9015 (94.5g) showed highest shoot fresh weight. The maximum root fresh weight was found for the genotype Jamal-10059 (6.97g). Maximum values for root dry weight (2.19g) & root volume (2.70) was revealed for the genotype Jamal-10024.

From the correlation coefficient study, Character association through correlation analysis studies revealed that in control and drought condition, root volume and root fresh weight have a significant positive correlation with shoot fresh weight. Root fresh weight has a significant positive correlation with root dry weight in control and significant negative correlation with root volume in drought condition. In control condition, root volume has a significant positive association with root dry weight. This indicated that simultaneous selection of these characters was important for drought tolerance. Hence, selection of genotypes for root association based on these traits could be useful for future breeding.

The multivariate analysis revealed that 100 wheat genotypes were distributed into six clusters. Among the six cluster, Considering all the characters it appeared that the genotypes in the cluster III (29SAWYT-3 RAJ, BAW-1403 etc.), IV (1HZWYT-427, 1HZWYT-433 etc.) and II (Jamal-10059, Jamal-10105, SABGPYT-5094)with the highest number of relative mean for plant height, no. of tiller per plant, shoot dry weight, root dry weight, relative water content, root length and root volume in drought condition. Those genotypes from these clusters having high mean values may be directly used for adaptation or may be used as parents in future hybridization programs.

By PCA analysis, it was founded the cumulative variance for the first five principal components accounted for ~61% of all genetic variability.

The MGIDI provided desired selection differentials (SD) 12 studied traits. The selection differentials ranged from − 0.032% (RSR) to 11.3% (SFW). ELWL, PH, PC, RSR and RWC showed negative gains, and NTPP, RFW, RDW, RL, RV, SFW and SDW showed positive gain at control condition.

MGIDI analysis revealed that, In the control condition, the genotypes Jamal-10024, Jamal-10026, Jamal-10105, 29SAWYT-3 RAJ, 29SAWYT-305, 1HZWYT-425, 29SAWYT-313, Jamal-10020, Jamal-10028&Jamal-9007 were selected where Jamal-10024& Jamal-10020 with higher no. of tiller per plant & root fresh weight, 1HZWYT-425 with higher shoot fresh weight, root dry weight & root volume, Jamal-10024&29SAWYT-305 with higher root length & excise leaf water loss,Jamal-10020 with higher plant height, proline content & root shoot ratio and Jamal-10026 with higher shoot dry weight & relative water content. In the drought condition, the genotypes Jamal-9046, SABGPYT-4104, 1HZWYT-425, SABGPYT-4110, 1HZWYT-430 RAJ, 11SATYN-9425, Jamal-10032, Jamal-9006, 1HZWYT-422 RAJ&BAW-1397 were selected. In this strategy, Jamal-9046&1HZWYT-425 were selected with higher shoot dry weight, root shoot ratio & relative water content, 1HZWYT-430 RAJ with higher shoot fresh weight & root fresh weight, 1HZWYT-422 RAJ with higher no. of tiller per plant, excise leaf water loss & root dry weight and Jamal-9006&SABGPYT-4110 with higher plant height, proline content, root length & root volume. The MGIDI index identified 20 as more desirable genotypes than others for each growth condition. Among these, 1HZWYT-425 was selected in both conditions, suggesting that it can maintain its ideal growth under both conditions.

In marker study (experiment 2), 5 SSR markers were evaluated in 100 wheat varieties for molecular characterization. All of the SSRs were polymorphic.

Total of 24 alleles were detected and the number of alleles per locus ranged from 2 to 7with an average of 4.8 alleles per locus. The marker GWM495 produced the highest number of polymorphic alleles of seven (7) followed by Tagwm1037 (6) and GWM513 with five (5), WMS0691 with four(4) respectively(Table 17). While Barc20 markers produced the least number of polymorphic bands per locus of two (2). The highest PIC value (0.84) was recorded for GWM495, and GWM513 recorded second highest (0.78) and lowest by Barc20 (0.46).The SSR markers in this study yielded reproducible polymorphic bands in ninety six genotypes of *Triticum aestivum*, providing a powerful and reliable molecular tool for analyzing genetic diversity and relationships in wheat.

Cluster analysis using UPGMA method delineated the 96 cultivars into six clusters comprising of 16, 20,8,13, 29 and 20 genotypes. The respected tolerant genotypes obtained from previous experiment i.e. 1HZWYT-425, 1HZWYT-430 RAJ and Jamal-9006 are located in same cluster as UPGMA cluster V.

Principal Component Analysis Associations among 96 wheat genotypes were computed using PCA method. Population I, Population II, Population III, Population IV, Population V and Population VI were mixed with genotypes .The graphical views of PCA showed the spatial distribution of the genotypes. The results indicated that the genotypes were placed far away from the centroid that means they were more genetically diverse. These genotypes are not sharing any genetic similarity between themselves and between the entire ninety six genotypes studied; they can be a potential parent in the future breeding programs.

As a result of genotype differences in reactions to water stress, moisture stress placed on all wheat genotypes indicates their capacity for drought tolerance. The most distant parental categories were clearly distinguished between genotypes using the current genetic diversity study. Due to their drought tolerance, this would facilitate hybridization as well as the identification of suitable donors in marker-assisted selection. High-yielding cultivars that are tolerant of drought could be created using efficient breeding method Bangladesh's disaster-prone regions.

Based on the findings of the present investigation following conclusions could be made.

- Jamal-9046, SABGPYT-4104, 1HZWYT-425, SABGPYT-4110, 1HZWYT-430 RAJ, 11SATYN-9425, Jamal-10032, Jamal-9006, 1HZWYT-422 RAJ&BAW-1397 were identified as drought tolerant genotypes among 100 genotypes through assessment of multi-trait genotype ideotype index (MGIDI).
- Based on the morphological diversity 1HZWYT-425,1HZWYT-430 RAJ, 11SATYN-9425, Jamal-10032, 1HZWYT-422 RAJ, BAW-1397, Jamal-9046, SABGPYT-4104, Jamal-9006 were identified in cluster I and cluster III as similar genotypes and might be useful for future hybridization program.
- Based on the molecular diversity genotypes were identified in 1HZWYT-425,1HZWYT-430 RAJ, 11SATYN-9425, Jamal-10032, 1HZWYT-422 RAJ, BAW-1397, Jamal-9046, SABGPYT-4104, Jamal-9006 cluster III, V and VI as

similar genotypes and might be useful for future hybridization program.

- Finally, considering the morphological and molecular study both the genotype 1HZWYT-425, 1HZWYT-430 RAJ, Jamal-9046, 1HZWYT-422 RAJ and BAW-1397 might be very effective in future drought tolerant breeding program.

REFERENCES

Abbas, M. W., Khan, M., Ahmad, F., Nawaz, H., Ahmad, J., Ayub, A., & Fahad, S. (2018). Germination and seedling growth of wheat as affected by seed priming and its duration. *Agricultural Research and Technology Journal*, *18*(3), 556062.

Abderrahmane, H., El Abidine, F. Z., Hamenna, B., & Ammar, B. (2013). Correlation, path analysis and stepwise regression in durum wheat (*Triticum durum* Desf.) under rainfed conditions. *Journal of Agriculture and Sustainability*, *3*(2), 122-131.

Abid M., Ali S., Qi L.K., Zahoor R., Tian Z., Jiang D., Snider J.L. & Dai T. (2018). Physiological and biochemical changes during drought and recovery periods at tillering and jointing stages in wheat (*Triticum aestivum* L.). *Scientific Reports*. 8: 4615.

Abou-Deif, M. H., Rashed, M. A., Sallam, M. A. A., Mostafa, E. A. H., & Ramadan, W. A. (2013). Characterization of twenty wheat varieties by ISSR markers. *Middle East Journal of Scientific Research*, *15*(2), 168-175. DOI: 10.5829/idosi.mejsr.2013.15.2.11044

Ahmad, S., Pasha, I., Saeed, M., & Shahid, M. (2017). Principal component analysis and correlation studies of spring wheats in relation to cookie making quality. *International Journal of Food Properties*, *20*(10), 2299-2313.

Ahmed, M., Qadir, G., Shaheen, F. A., & Aslam, M. A. (2017). Response of proline accumulation in bread wheat (*Triticum aestivum L.*) under rainfed conditions. *Journal of Agricultural Meteorology*, D-14.

Ahmad, M., Shabbir, G., Minhas, N. M., & Shah, M. K. N. (2013). Identification of drought tolerant wheat genotypes based on seedling traits. *Sarhad Journal of Agriculture*, *29*(1), 21-27.

Ahmed, H. G. M. D., Kashif, M., Rashid, M. A. R., Sajjad, M., & Zeng, Y. (2020). Genome wide diversity in bread wheat evaluated by SSR markers. *International Journal of Agriculture and Biology*, *24*(2), 263-272. DOI: 10.17957/IJAB/15.1433.

Ahmed, H. G. M. D., Khan, A. S., Khan, S. H., & Kashif, M. (2017). Genome wide allelic pattern and genetic diversity of spring wheat genotypes through SSR markers. *International Journal of Agriculture and Biology*, *19*, 1559-1565.

Ahomed, H. (2017). Screening for drought tolerance in wheat genotype by morphological and SSR markers. M.S. Thesis, Department of Biotechnology, Bangladesh Agricultural University, Mymensingh.

Aktaş, H. (2016). Drought tolerance indices of selected landraces and bread wheat (*Triticum aestivum* L.) genotypes derived from synthetic wheats. *Applied Ecology and Environmental Research*, *14*(4), 177-189. DOI: http://dx.doi.org/10.15666/aeer/1404_177189.

Al-Ashkar, I., Alderfasi, A., Ben Romdhane, W., Seleiman, M. F., El-Said, R. A., & Al-Doss, A. (2020). Morphological and genetic diversity within salt tolerance detection in eighteen wheat genotypes. *Plants*, *9*(3), 287.

Al-Ashkar, I.; Sallam, M.; Ibrahim, A.; Ghazy, A.; Al-Suhaibani, N.; Ben Romdhane, W.; Al-Doss, A.(2023). Identification of wheat ideotype under multiple abiotic stresses and complex environmental interplays by multivariate analysis techniques. Plants, 12, 3540.

Alemu, Y. A., Anley, A. M., & Abebe, T. D. (2020). Genetic variability and association of traits in Ethiopian durum wheat (*Triticum turgidium* L. var. *durum*) landraces at Dabat Research Station, North Gondar. *Cogent Food & Agriculture*, *6*(1), 1778604.

Al-Harbi, A. R., Wahb-Allah, M. A., & Abu-Muriefah, S. S. (2008). Salinity and nitrogen level affects germination, emergence, and seedling growth of tomato. *International Journal of Vegetable Science*, *14*(4), 380-392.

Ali M. B., & El-Sadek A. N. (2016). Evaluation of drought tolerance indices for wheat (*Triticum aestivum* L.) under irrigated and rainfed conditions. *Communications in Biometry and Crop Science* 11, 77–89.

Allard, R.W. (1960). Principles of plant Breeding. John willey and sons. Inc. New York. p. 36.

Amare, A., Mekbib, F., Tadesse, W., & Tesfaye, K. (2019). Screening of drought tolerant bread wheat (*Triticum aestivum* L.) genotypes using yield based drought tolerance indices. *Ethiopian Journal of Agricultural Sciences*, *29*(2), 1-16.

Anwar, J., Subhani, G. M., Hussain, M., Ahmad, J., Hussain, M., & Munir, M. (2011). Drought tolerance indices and their correlation with yield in exotic wheat genotypes. *Pakistan Journal of Botany*, *43*(3), 1527-1530.

Anwaar, H. A., Perveen, R., Mansha, M. Z., Abid, M., Sarwar, Z. M., Aatif, H. M. & Khan, K. A. (2020). Assessment of grain yield indices in response to drought stress in wheat (*Triticum aestivum* L.). *Saudi journal of biological sciences*, *27*(7), 1818-1823. DOI: https://doi.org/10.1016/j.sjbs.2019.12.009.

Arain, S. M., Sial, M. A., Jamali, K. D., & Laghari, K. A. (2018). Grain yield performance, correlation, and luster analysis in elite bread wheat (*Triticum aestivum* L.) lines. *Acta Agrobotanica*, *71*(4), 1-8. DOI: 10.5586/aa.1747.

Arifuzzaman, M., Barman, S., Hayder, S., Azad, M. A. K., Turin, M. T. S., Amzad, M. A., & Masuda, M. S. (2020). Screening of bread wheat (*Triticum aestivum* L.) genotypes under drought stress conditions using multivariate analysis. *Cereal Research Communications*, *48*(3), 301-308.

Ashraf, M., & Mehmood, S. (1990). Response of four Brassica species to drought stress. *Environmental and Experimental Botany*, *30*(1), 93-100.

Asmamaw, M., Keneni, G., & Tesfaye, K. (2019). Genetic diversity of Ethiopian durum wheat (*Triticum durum*Desf.) landrace collections as reveled by SSR markers. *Advances in Crop Science and Technology*, *7*(1), 413.

Atinafu, D. M., Alayachew, S. A., &Heterat, K.Z. (2020). Study of Genetic Variability in Some Bread Wheat Accessions (*Triticum aestivum* L.) in Gurage Zone, Ethiopia. *Asian Journal of Biological Sciences, 13: 309-317.* **DOI:** 10.3923/ajbs.2020.309.317.

Alireza Pour-Aboughadareh, Peter Poczai (2021). A dataset on multi-trait selection approaches for screening desirable wild relatives of wheat, Data in Brief, 39: 2352-3409. **DOI**:10.1016/j.dib.2021.107541.

Balota, M., Green, A. J., Griffey, C. A., Pitman, R., & Thomason, W. (2017). Genetic gains for physiological traits associated with yield in soft red winter wheat in the Eastern United States from 1919 to 2009. *European Journal of Agronomy*, *84*, 76-83.

Bano, S., Kaleri, A. A., Keerio, R., Memon, S., Kaleri, R. R., Akram, R., & Nazeer, S. (2017). Analysis of correlation and regression among M2 wheat mutant population for yield and its associated traits. *Journal of Basic & Applied Sciences*, *13*, 522-526.

Bates, L. S., Waldren, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water-stress studies. *Plant and soil*, *39*(1), 205-207.

Belay, G. A., Zhang, Z., & Xu, P. (2021). Physio-Morphological and biochemical trait-based evaluation of Ethiopian and Chinese wheat germplasm for drought tolerance at the seedling stage. *Sustainability*, *13*(9), 4605.

Belete, Y., Shimelis, H., Laing, M., & Mathew, I. (2021). Genetic diversity and population structure of bread wheat genotypes determined via phenotypic and SSR marker analyses under drought-stress conditions. *Journal of Crop Improvement*, *35*(3), 303-325.

Bhatta, M., Morgounov, A., Belamkar, V., Yorgancılar, A., & Baenziger, P. S. (2018). Genome-wide association study reveals favorable alleles associated with common bunt resistance in synthetic hexaploid wheat. *Euphytica*, *214*(11), 1-10.

Bheel, N., Kumar, S., Kirgiz, M. S., Ali, M., Almujibah, H. R., Ahmad, M., & Gonzalez-Lezcano, R. A. (2024). Effect of wheat straw ash as cementitious material on the mechanical characteristics and embodied carbon of concrete reinforced with coir fiber. Heliyon, 10(2).

Bhutto, A. H., Rajpar, A. A., Kalhoro, S. A., Ali, A., Kalhoro, F. A., Ahmed, M., Raza, S., & Kalhoro, N. A. (2016). Correlation and regression analysis for yield traits in wheat (*Triticum aestivum* L.) genotypes. *Natural Science*, *8*(03), 96-104. http://dx.doi.org/10.4236/ns.2016.83013.

Blum, A. (1996). Crop responses to drought and interpretation of adaptation. *Plant Growth Regulation*. 20, 135-148.

Blum, A., Munns, R., Passioura, J. B., Turner, N. C., Sharp, R. E., Boyer, J. S., & Hong, Z. (1996). Genetically engineered plants resistant to soil drying and salt stress: how to interpret osmotic relations?. *Plant Physiology*, *110*(4), 1051-1053.

Bohnert, H. J., Nelson, D. E., & Jensen, R. G. (1995). Adaptations to environmental stresses. *The plant cell*, *7*(7), 1099-1111.

Borisjuk, N., Kishchenko, O., Eliby, S., Schramm, C., Anderson, P., Jatayev, S., &Shavrukov, Y. (2019). Genetic modification for wheat improvement: from transgenesis to genome editing. *BioMed research international*, *2019*.

Bouabdelli, S., Zeroual, A., Meddi, M. *et al.* Impact of temperature on agricultural drought occurrence under the effects of climate change. *Theor Appl Climatol* **148**, 191–209 (2022). https://doi.org/10.1007/s00704-022-03935-7.

Bouslama, M., &Schapaugh Jr, W. T. (1984). Stress tolerance in soybeans. I. Evaluation of three screening techniques for heat and drought tolerance 1. *Crop science*, *24*(5), 933-937.

Bray, E. A. (1997). Plant responses to water deficit. *Trends in plant science*, *2*(2), 48-54.

Brunes, A. P., Araújo, Á. D. S., Dias, L. W., Villela, F. A., &Aumonde, T. Z. (2016). Seedling length in wheat determined by image processing using mathematical tools1. *Revista Ciência Agronômica*, *47*, 374-379.

Burton, G. M. (1952). Quantitative inheritance in grasses. Proc. 6 Tnt. Grassland cong. 1: 277-283.

Burton, G. W., &Devane, D. E. (1953). Estimating heritability in tall fescue (*Festuca arundinacea*) from replicated clonal material 1. *Agronomy journal*, *45*(10), 478-481.

Chachar, N. A., Chachar, M. H., Chachar, Q. I., Chachar, Z., Chachar, G. A., & Nadeem, F. (2014). Exploration of genetic diversity between six wheat genotypes for drought tolerance. *Climate Change outlook and adaptation*, *2*(1), 27-33.

Chairi, F., Vergara-Diaz, O., Vatter, T., Aparicio, N., Nieto-Taladriz, M. T., Kefauver, S. C., & Araus, J. L. (2018). Post-green revolution genetic advance in durum wheat: The case of Spain. *Field crops research*, *228*, 158-169.

Chaniago, I., Syarif, A., &Riviona, P. (2017). Sorghum seedling drought response: In search of tolerant genotypes. *International Journal on Advanced Science, Engineering and Information Technology*, *7*(3), 892-897. DOI: *https://doi.org/10.18517/ijaseit.7.3.1303.*

Chaudhary, R., Kumar, S., Singh, S., Prasad, J., Jeena, A. S., & Upreti, M. C. (2020). Study of genetic parameters and character association in wheat (*Triticum aestivum*L.). *International Journal of Chemical Studies*, *8*(3),2312-2315. DOI:https://doi.org/10.22271/chemi.2020.v8.i3ag.9555.

Chekole, N., Wassu, M., &Tebkew, D. (2016). Genetic variation, correlation and path coefficient analysis in Tef [Eragrostistef (Zucc.) Trotter] genotypes for yield, yield related traits at Maysiye, Northern Ethiopia. *American Journal of Research Communication*, 4(11), 73–102.

Chen, X., Min, D., Yasir, T. A., & Hu, Y. G. (2012). Genetic diversity, population structure and linkage disequilibrium in elite Chinese winter wheat investigated with SSR markers. *PLOS one*. 7(9): e44510. https://doi.org/10.1371/journal.pone.0044510.

Chipilski, R. R., Kocheva, K. V., Nenova, V. R., & Georgiev, G. I. (2012). Physiological responses of two wheat cultivars to soil drought. *Zeitschrift für Naturforschung C*, *67*(3-4), 181-186.

Chong, J., & Xia, J. (2018). MetaboAnalystR: an R package for flexible and reproducible analysis of metabolomics data. *Bioinformatics*, *34*(24), 4313-4314.

Chowdhury, A. (2014). Genetic analysis of morpho-physiological traits of spring wheat (*Triticum aestivum*L.) under drought stress, M.S. Thesis, Department of Genetics and Plant Breeding, Sher-e-Bangla Agricultural University, Dhaka.

Chutipaijit, S. (2016). Changes in physiological and antioxidant activity of indica rice seedlings in response to mannitol-induced osmotic stress. *Chilean Journal of Agricultural Research*, *76*(4), 455-462. http://dx.doi.org/10.4067/S0718-58392016000400009.

Cifci, E.A., &Yagdi, K. (2012). Study of genetic diversity in wheat (*Triticum aestivum L.*) varities using random amplified polymorphic DNA (RAPD) analysis. *Turkish Journal of Field Crops*. 17(1): 91-95.

Comstock, R. E., & Robinson, H. F. (1952). Genetic parameters, their estimation and significance. In *Proceedings of the 6th international Grassland congress*, 1, 248-291.

Dagnelie, P. (1975). *Theorie et methodesstatistiques, applications agronomiques: Vol. 2: Les methodes de l'inferencestatistique*. Les presses agronomiques.

Daryanto, S., Wang, L., & Jacinthe, P. A. (2016). Global synthesis of drought effects on maize and wheat production. *PloS one*, *11*(5), 1-15. e0156362. DOI: 10.1371/journal.pone.0156362.

Dhanda, S. S., & Sethi, G. S. (2002). Tolerance to drought stress among selected Indian wheat cultivars. *The Journal of Agricultural Science, 139*(3), 319-326.

Drikvand, R., Hossinpur, T., Ismaili, A., &Salahvarzi, E. (2012a). Assessment of drought tolerance indices for screening of rain fed wheat genotypes. *Journal of Food, Agriculture & Environment*, *10(1)*, 768-772.

Drikvand, R., Doosty, B., & Hosseinpour, T. (2012b). Response of rainfed wheat genotypes to drought stress using drought tolerance indices. *Journal of Agricultural Science (Toronto)*, *4*(7), 126-131.

Döring T.F., Annicchiarico, P., Clarke, S., Haigh, Z., Jones, H.E., Pearce, H., Snape, J., Zhan, J. and Wolfe, M.S. (2015). Comparative analysis of performance and stability among composite cross populations, variety mixtures and pure lines of winter wheat in organic and conventional cropping systems. *Field Crops Research*, 183: 235–245.

Edossa, D. C., Woyessa, Y. E., &Welderufael, W. A. (2016). Spatiotemporal analysis of droughts using self-calibrating Palmer's drought severity index in the central region of South Africa. *Theoretical and Applied Climatology*, *126*(3), 643-657.

Elshafei, A. A., Afiah, S. A. E. A., Al-Doss, A. A., & Ibrahim, E. I. (2019). Morphological variability and genetic diversity of wheat genotypes grown on saline soil and identification of new promising molecular markers associated with salinity tolerance. *Journal of Plant Interactions*, *14*(1), 564-571.

Eltaher, S., Sallam, A., Belamkar, V., Emara, H. A., Nower, A. A., Salem, K. F., Poland, J.& Baenziger, P. S. (2018). Genetic diversity and population structure of F3: 6 Nebraska winter wheat genotypes using genotyping-by-sequencing. *Frontiers in genetics*, *9*, 76.doi: 10.3389/fgene.2018.00076.

El-Bakatoushi, R. (2019). Genetic diversity of winter wheat (*Triticum aestivum* L.) growing near a high voltage transmission line. *Romanian Journal of Biology-Plant Biology*, 55(2): 71-87.

El-Rawy, M. A., & Hassan, M. I. (2014). Effectiveness of drought tolerance indices to identify tolerant genotypes in bread wheat (*Triticum aestivum* L.). *Journal of Crop Science and Biotechnology*, *17*(4), 255-266. DOI: https://doi.org/10.3389/fpls.2016.01276.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular ecology*, *14*(8), 2611-2620.

Hill, W. G., & Mackay, T. F. (2004). DS Falconer and Introduction to quantitative genetics. *Genetics*, *167*(4), 1529-1536.

FAO[1] World Food and Agriculture. Statistical Yearbook. Available online: http://www.fao.org/3/i3107e/i3107e.pdf (accessed on 20 May 2019).

FAO[2] (2017). Online sources GIEWS - Global Information and Early Warning System, Country Briefs, Bangladesh, Reference Date: 10-June-2020. http://www.fao.org/giews/countrybrief/country.jsp?code=BGD.

Farshadfar, E., Poursiahbidi, M. M., & Abooghadareh, A. P. (2012). Repeatability of drought tolerance indices in bread wheat genotypes. *International Journal of Agriculture and Crop Sciences*, *4*(13), 891-903.

Farshadfar, E., Romena, H., & Shabani, A. (2013). Evaluation of genetic parameters in agro-physiological traits of wheat (*Triticum aestivum* L.) under irrigated condition. *International journal of Advanced Biological and Biomedical Research*, *1*(4), 331-340.

Farhad, M., Tripathi, S. B., Singh, R. P., Joshi, A. K., Bhati, P. K., Vishwakarma, M. K., ... & Kumar, U. (2022). Multi-trait selection of bread wheat ideotypes for adaptation to early sown condition. *Crop Science*, *62*(1), 67-82.

Ferede, M., Worede, F., & Alemayehu, G. (2020). Phenotypic performance, genetic advance and regression analysis in bread wheat (*Triticum aestivum* L.) genotypes in Northwestern Ethiopia. *Cogent Food & Agriculture*, *6*(1), 1746227. https://doi.org/10.1080/23311932.2020.1746227.

Fernandez, G. C. (1992). Effective selection criteria for assessing plant stress tolerance. In *Proceeding of the International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress, Aug. 13-16, Shanhua, Taiwan, 1992* (pp. 257-270).

Fischer, R. A., & Maurer, R. (1978). Drought resistance in spring wheat cultivars. I. Grain yield responses. *Australian Journal of Agricultural Research*, *29*(5), 897-912.

Fleury, D., Jefferies, S., Kuchel, H., & Langridge, P. (2010). Genetic and genomic tools to improve drought tolerance in wheat. *Journal of experimental botany*, *61*(12), 3211-3222.

Gavuzzi, P., Rizza, F., Palumbo, M., Campanile, R. G., Ricciardi, G. L., & Borghi, B. (1997). Evaluation of field and laboratory predictors of drought and heat tolerance in winter cereals. *Canadian Journal of plant science*, *77*(4), 523-531.

Gerema, G., Lule, D., Lemessa, F., & Mekonnen, T. (2020). Morphological characterization and genetic analysis in bread wheat germplasm: A combined study of heritability, genetic variance, genetic divergence and association of characters. *Agricultural Science & Technology (1313-8820)*, *12*(4), 301-311. DOI: 10.15547/ast.2020.04.048.

Gezahegn, F., Sentayehu, A., & Zerihun, T. (2015). Genetic variability studies in bread wheat (*Triticum aestivum* L.) genotypes at Kulumsa agricultural research center, South East Ethiopia. *Journal of Biology, Agriculture and Healthcare*, 5(7), 89–98. https://www.researchgate.net/publication/308764540.

Gholipouri, A., Sedghi, M., Sharifi, R. S., & Nazari, N. M. (2009). Evaluation of drought tolerance indices and their relationship with grain yield in wheat cultivars. *Recent Research in Science and Technology*, *1*(4), 195-198.

Goldringer, I., van Frank, G., Bouvier d'Yvoire, C., Forst, E., Galic, N., Garnault, M., & Rivière, P. (2020). Agronomic evaluation of bread wheat varieties from participatory breeding: A combination of performance and robustness. *Sustainability*, *12*(1), 128.

Grzesiak, S., Hordyńska, N., Szczyrek, P., Grzesiak, M. T., Noga, A., &Szechyńska-Hebda, M. (2019). Variation among wheat (*Triticum easativum* L.) genotypes in response to the drought stress: I–selection approaches. *Journal of Plant Interactions*, *14*(1), 30-44. https://doi.org/10.1080/17429145.2018.1550817.

Guo, R., Yuan, G., & Wang, Q. (2011). Effect of sucrose and mannitol on the accumulation of health-promoting compounds and the activity of metabolic enzymes in broccoli sprouts. *Scientia Horticulturae*, *128*(3), 159-165.

Gurcan, K., Demirel, F., Tekin, M., Demirel, S., & Akar, T. (2017). Molecular and agro-morphological characterization of ancient wheat landraces of turkey. *BMC plant biology*, *17*(1), 1-10. DOI 10.1186/s12870-017-1133-0.

Hadi, B. H., Al-Maliky, R. J. M., Zaid, M. A., & Hassan, W. A. (2018). Estimation of some genetic parameters in bread wheat *Triticum aestivum* L. for WASIT and DIWANIYYA locations. *Euphrates Journal of Agriculture Science*, *9*(4), 1-12.

Hamayun, M., Khan, S. A., Khan, A. L., Shinwari, Z. K., Hussain, J., Sohn, E. Y., ... & Lee, I. J. (2010). Effect of salt stress on growth attributes and endogenous growth hormones of soybean cultivar Hwangkeumkong. *Pakistan Journal of Botany*, *42*(5), 3103-3112.

Haque, M. S., Saha, N. R., Islam, M. T., Islam, M. M., Kwon, S. J., Roy, S. K., & Woo, S. H. (2021). Screening for drought tolerance in wheat genotypes by morphological and SSR markers. *Journal of Crop Science and Biotechnology*, *24*(1), 27-39.

Hasegawa, P. M., Bressan, R. A., Zhu, J. K., & Bohnert, H. J. (2000). Plant cellular and molecular responses to high salinity. *Annual review of plant biology*, *51*(1), 463-499.

Heidari, S., Heidari, P., Azizinezhad, R., Etminan, A., & Khosroshahli, M. (2020). Assessment of genetic variability, heritability and genetic advance for agro-morphological and some in-vitro related traits in durum wheat. *Bulgarian Journal of Agricultural Science*, *26*(1), 120-127.

Hooshmandi, B. (2019). Evaluation of tolerance to drought stress in wheat genotypes. *Idesia*, *37*(2), 37-43.

Huseynova, I. M. (2012). Photosynthetic characteristics and enzymatic antioxidant capacity of leaves from wheat cultivars exposed to drought. *Biochim. Biophys. Acta Bioenerg.* 1817, 1516–1523. doi: 10.1016/j.bbabio.2012.02.037

Ilker, E., TATAR, Ö., Tonk, F. A., & Tosun, M. (2011). Determination of tolerance level of some wheat genotypes to post-anthesis drought. *Turkish Journal of Field Crops*, *16*(1), 59-63.

Iqbal, M. J., Maqsood, Y., Abdin, Z. U., Manzoor, A., Hassan, M., & Jamil, A. (2016). SSR markers associated with proline in drought tolerant wheat germplasm. *Applied biochemistry and biotechnology*, *178*(5), 1042-1052. DOI 10.1007/s12010-015-1927-1.

Isack, M. (2015). Combining ability, genetic advances and path coefficient analyses of maize hybrids developed from maize streak virus and downy mildew resistant recombinant inbred lines (M.Sc. Thesis). School of Graduate Studies of University of KwaZulu-Natal, South Africa, 17.

Islam, M. Z., Khalequzzaman, M., Prince, M. F. R. K., Siddique, M. A., Rashid, E. S. M. H., Ahmed, M. S. U., & Ali, M. P. (2018). Diversity and population structure of red rice germplasm in Bangladesh. *PLoS One*, *13*(5), e0196096.

Islam, M. S., Hossain, M. Z., & Sikder, M. B. (2019). Farmers' adaptation strategies to drought and their determinants in barind tract, Bangladesh. *SAARC Journal of Agriculture*, *17*(1), 161-174.

Islam, S., Haque, M. S., Emon, R. M., Islam, M. M., & Begum, S. N. (2012). Molecular characterization of wheat (*Triticum aestivum* L.) genotypes through SSR markers. *Bangladesh Journal of Agricultural Research*, *37*(3), 389-398. https://doi.org/10.20546/ijcmas.2019.802.095.

ISTA. 1999. International Rules for Seed Testing. Seed Science and Technology. International Seed Testing Association, Zurich, Switzerland. **27:** 155-199.

Iyem, E., Yildirim, M., & Kizilgeci, F. (2021). Germination, seedling growth and physio-biochemical indices of bread wheat (*Triticum aestivum* L.) genotypes under peg induced drought stress. *Agriculture and Forestry*, 67(1): 163-180. DOI: 10.17707/AgricultForest.67.1.14.

Izabela M., Ilona C.M., Edyta S., Maria F., Stanisław G. and Maciej T.G. (2013). Impact of osmotic Stress on physiological and biochemical characteristics in drought susceptible and drought-resistant wheat genotypes. *Acta physiologiae plantarum*, *35*(2), 451-461.

Jahan, A., & Ahmed, F. (2017). Effect of drought stress on growth and yield of wheat genotypes. *Bangladesh Agronomy Journal*, *20*(2), 97-105.

Johnson, H. W., Robinson, H. F., & Comstock, R. E. (1955). Estimates of genetic and environmental variability in soybeans. *Agronomy journal*, *47*(7), 314-318.

Kamrani, M., Ebadi, A., &Mehreban, A. (2016). Evaluation of Grain Yield-Based Drought Tolerance Indices for Screening Durum Wheat Genotypes. *Jordan Journal of Agricultural Sciences*, *12*(1), 649-665.

Kara, K., Rached-Kanouni, M.A.L.I.K.A., Mnasri, S., Khammar, H. & M'Barek, B.N. (2020). Genetic variability assessment in bread wheat (*Triticum aestivum*) grown in Algeria using microsatellites SSR markers, *Biodiversitas Journal of Biological Diversity*. 21(6): 2638-2644. DOI: 10.13057/biodiv/d210635.

Khan, M.A., Tahir, A., Khurshid, N., Ahmed, M., &Boughanmi, H. (2020). Economic effects of climate change-induced loss of agricultural production by 2050: a case study of Pakistan. *Sustainability*. 12(3): 1216. https://doi.org/10.3390/su12031216.

Kilic, H., & Yagbasanlar, T. (2010). The effect of drought stress on grain yield, yield components and some quality traits of durum wheat (*Triticum turgidum* ssp. *durum*) cultivars. *NotulaeBotanicae Horti Agrobotanici Cluj-Napoca*, *38*(1), 164-170.

Ktavii, M. N., Kiambi, D. K., Haussman, B., Semagn, K., Muluvi, G., Kairichi, M., &Machuka, J. (2014). Assessment of the genetic diversity and pattern of relationship of West African sorghum accessions using microsatellite markers. *African Journal of Biotechnology*, *13*(14).

Khammar, H., Kara, K., Rached-Kanouni, M.A.L.I.K.A., Mnasri, S., &M'Barek, B.N. (2020). Genetic variability assessment in bread wheat (*Triticum aestivum*) grown in Algeria using microsatellites SSR markers, *Biodiversitas Journal of Biological Diversity*. 21(6): 2638-2644. DOI: 10.13057/biodiv/d210635.

Khodadadi, M., Fotokian, M. H., &Miransari, M. (2011). Genetic diversity of wheat (*Triticum aestivum* L.) genotypes based on cluster and principal component analyses for breeding strategies. *Australian Journal of Crop Science*, *5*(1), 17-24.

Kulkarni, M., Soolanayakanahally, R., Ogawa, S., Uga, Y., Selvaraj, M. G., &Kagale, S. (2017). Drought response in wheat: key genes and regulatory mechanisms controlling root system architecture and transpiration efficiency. *Frontiers in chemistry*, *5*, 106.

Kumar, R., Bozdar, H. B., Jamali, K. D., & Sial, M. A. (2021). Evaluation of Yield and its Components in Bread Wheat (Triticum aestivum L.) Genotypes. *Journal of Applied Research in Plant Sciences (JOARPS)*, *2*(1), 76-82.

Kumar, A., Bharti, B., Kumar, J., Singh, G. P., Jaiswal, J. P., & Prasad, R. (2020). Evaluation of drought tolerance indices for identification of drought tolerant and susceptible genotypes in wheat (*Triticum aestivum* L.). *Electronic Journal of Plant Breeding*, *11*(3), 727-734.

Kumar, S., Kumari, J., Bansal, R., Kuri, B. R., Upadhyay, D., Srivastava, A., & Singh, R. (2018). Multi-environmental evaluation of wheat genotypes for drought tolerance. *Indian Journal of Genetics and Plant Breeding*, *78*(1), 26-35.DOI: 10.5958/0975-6906.2018.00004.4.

Kumar, P., Yadava, R. K., Kumar, S., & Kumar, P. (2016a). Molecular diversity analysis in Wheat genotypes using SSR markers. *Electronic Journal of Plant Breeding*, *7*(2), 464-468.

Kumar, V., Chattopadhyay, T., & De, N. (2016b). Molecular Characterization of a Few Wheat Genotypes Using Simple Sequence Repeat Markers.*International Journal of Science, Environment and Technology*, 5(4), 2459–2466.

Langridge, P. (2013). Wheat genomics and the ambitious targets for future wheat production. *Genome*, *56*(10), 545-547.

Lekshmi, S. S., & Ayona, J. (2018). Effect of drought stress (mannitol) on morphological physiological activity and anatomy of cow pea plant (Vigna unguiculata). *International Journal for Research in Applied Science and Engineering Technology*, *6*(7), 606-615.

Lemmen, D. S., & Warren, F. J. (2004). Climate change impacts and adaptation: a Canadian perspective.

Lilley, J. M., & Kirkegaard, J. A. (2011). Benefits of increased soil exploration by wheat roots. *Field Crops Research*, *122*(2), 118-130.

Liu, Y., Bowman, B. C., Hu, Y. G., Liang, X., Zhao, W., Wheeler, J., & Chen, J. (2017). Evaluation of agronomic traits and drought tolerance of winter wheat accessions from the USDA-ARS national small grains collection. *Agronomy*, *7*(3), 51.

Liu, J., Shi, S., Chang, E., Yang, W., & Jiang, Z. (2013). Genetic diversity of the critically endangered Thuja sutchuenensis revealed by ISSR markers and the implications for conservation. *International Journal of Molecular Sciences*, *14*(7), 14860-14871. DOI: 10.3390/ijms140714860

Longove, M. A., Farid, A., Shamsuddin, B., & Sher, A. (2014). Performance evaluation of different wheat varieties under agro-ecological conditions of Quetta (Balochistan). *Journal of Biology, Agriculture and Healthcare*, *4*(8), 39-43.

Loutfy, N., El-Tayeb, M. A., Hassanen, A. M., Moustafa, M. F., Sakuma, Y., &Inouhe, M. (2012). Changes in the water status and osmotic solute contents in response to drought and salicylic acid treatments in four different cultivars of wheat (*Triticum aestivum*). *Journal of Plant Research*, *125*(1), 173-184.

Machado e Silva, C., Mezzomo, H. C., Ribeiro, J. P. O., Freitas, D. S. and Nardino, M. (2023). Multi-trait selection of wheat lines under drought-stress condition. Bragantia, 82, e20220254. https://doi.org/10.1590/1678-4499.2022025.

Manickavelu, A., Kawaura, K., Oishi, K., Shin-I, T., Kohara, Y., Yahiaoui, N., &Ogihara, Y. (2012). Comprehensive functional analyses of expressed sequence tags in common wheat (*Triticum aestivum*). *DNA research*, *19*(2), 165-177.

Mardi, M., Naghavi, M. R., Pirseyedi, S. M., KAZEMI, A. M., RASHIDI, M. S., Ahkami, A. H., ... &Katsiotis, A. (2011). Comparative assessment of SSAP, AFLP and SSR markers for evaluation of genetic diversity of durum wheat (*Triticum turgidum* L. var. *durum*).

Marmar, A., Baenziger, S., Dweikat, I., & El Hussein, A. A. (2013). Preliminary screening for water stress tolerance and genetic diversity in wheat (*Triticum aestivum* L.) cultivars from Sudan. *Journal of Genetic Engineering and Biotechnology*, *11*(2), 87-94.

Meena, R. P., Tripathi, S. C., Chander, S., Chookar, R. S., Verma, M. A., & Sharma, R. K. (2015). Identifying drought tolerant wheat varieties using different indices. *SAARC Journal of Agriculture*, *13*(1), 148-161.

Mecha, B., Alamerew, S., Assefa, A., Dutamo, D., & Assefa, E. (2017). Correlation and path coefficient studies of yield and yield associated traits in bread wheat (*Triticum aestivum* L.) genotypes. *Advances in Plants and Agricultural Research*, *6*(5), 1-10.

Meng, Z., Duan, A., Dassanayake, K. B., Chen, D., Gao, Y., Wang, X., & Shen, X. (2016). Effects of regulated deficit irrigation on grain yield and quality traits in winter wheat. *Transactions of the ASABE*, *59*(3), 897-907.

Mdluli, S. Y., Shimelis, H., & Amelework, A. B. (2020). Genetic diversity and population structure of elite drought tolerant bread wheat (Triticum aestivum L.) genotypes. *Australian Journal of Crop Science*, *14*(9), 1362-1371.

Miller, T. E., Hutchinson, J., & Chapman, V. (1982). Investigation of a preferentially transmitted Aegilops sharonensis chromosome in wheat. *Theoretical and Applied Genetics*, *61*(1), 27-33.

Miri, A., Sabouri, H., Hossein Moghaddam, H., Soughi, H., Mollashahi, M., & Sajjadi, S. J. (2020). Genetic Structure of Wheat (Triticum aestivum L.) Grain Characteristics by Using Image Processing and Generation Mean Analysis Techniques. *Journal of Genetic Resources*, *6*(2), 131-141. *DOI: 10.22080/jgr.2020.18575.1180.*

Moayedi, A. A., Boyce, A. N., &Barakbah, S. S. (2010). The performance of durum and bread wheat genotypes associated with yield and yield component under different water deficit conditions. *Australian Journal of Basic and Applied Sciences*, *4*(1), 106-113.

Mondal, T., Banerjee A., Mondal, N.K.,& Datta, J.K. (2011). Assessment of drought tolerance of selected wheat cultivars under laboratory condition.*Journal of Agricultural Technology*, *7*(2), 383-393.

Moucheshi, A., Heidari, B., and Assad, M.T. (2012). Alleviation of drought stress effects on wheat using arbuscular mycorrhizal symbiosis. *International Journal of Agricultural Science* 291: 35-47.

Możdżeń, K., Bojarski, B., Rut, G., Migdałek, G., Repka, P., & Rzepka, A. (2021). Effect of drought stress induced by mannitol on physiological parameters of maize (*Zea mays L.*) seedlings and plants. *Journal of Microbiology, Biotechnology and Food Sciences, 2021*, 86-91. doi: 10.15414/jmbfs.2015.4.special2.86-91.

Mourad, A.M.I., Alomari, D.Z., Alqudah, A.M., Sallam, A., Salem, K.F.M. (2019). Recent Advances in Wheat (Triticum spp.) Breeding. In: Al-Khayri, J., Jain, S., Johnson, D. (eds) Advances in Plant Breeding Strategies: Cereals. Springer, Cham. https://doi.org/10.1007/978-3-030-23108-8_15

Mwadzingeni, L., Shimelis, H., &Tsilo, T. J. (2017). Variance components and heritability of yield and yield components of wheat under drought-stressed and non-stressed conditions. *Australian Journal of Crop Science*, *11*(11).

Mwadzingeni, L., Shimelis, H., Rees, D. J. G., &Tsilo, T. J. (2017). Genome-wide association analysis of agronomic traits in wheat under drought-stressed and non-stressed conditions. *PloS one*, *12*(2), e0171692.

Mwadzingeni, L., Shimelis, H., Tesfay, S., &Tsilo, T. J. (2016). Screening of bread wheat genotypes for drought tolerance using phenotypic and proline analyses. *Frontiers in Plant Science*, *7*, 1276.

Nahas, L. D., Alsamman, A. M., Hamwieh, A., Al-Husein, N., & Lababidi, G. (2020). Characterization of EST-SSR markers in bread wheat EST related to drought tolerance and functional analysis of SSR-containing unigenes. *Highlights in BioScience*, *3*, 1-12.DOI: 10.36462/H.BioSci.20203.

Naceur, A. B., Chaabane, R., El-Faleh, M., Abdelly, C., Ramla, D., Nada, A., & Sakr, M. (2012). Genetic diversity analysis of North Africa's barley using SSR markers. *Journal of Genetic Engineering and Biotechnology*, *10*(1), 13-21.

Narantsetseg, Ya., Sebastin, R., Bayarsukh, N., Myagmarsuren., Ya, Jung-Ro, L., Kyung-Jun, L., Myoung-Jae, S., Gyu-Taek, C., Kyung-Ho, M. & Gi-An, Lee. (2017). Genetic diversity and population structure of mongolian wheat based on SSR markers: implications for conservation and management. *Plant Breeding and Biotechnology*, *5*(3), 213-220.

Nehe, A. S., Foulkes, M. J., Ozturk, I., Rasheed, A., York, L., Kefauver, S. C., &Morgounov, A. (2021). Root and canopy traits and adaptability genes explain drought tolerance responses in winter wheat. *PloS one*, *16*(4), e0242472.

Nei, M., & Li, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences*, *76*(10), 5269-5273.

Nei, M., & Jin, L. (1989). Variances of the average numbers of nucleotide substitutions within and between populations. *Molecular Biology and Evolution*, *6*(3), 290-300.

Nikus O, M. A., Turk, A. M., & Al-Tawaha. (2004). Yield responses of sorghum (Sorghum bicolor, L) to manure supplemented with phosphate fertilizer under semi-arid Mediterranean conditions. *International Journal of Agriculture and Biology*, 6: 889-893

Nilanthi, D., Perera, P. C. D., & Gunarathna, P. G. T. M. (2015). Study the response of drought stress inducing by mannitol in germination to seedling stage of mung bean (Vigna Radiata L.) Variety MI5 and Variety Harsha. *International Journal of Scientific and Research Publications*, *5*(7), 1-4.

Nouraein, M., Mohammadi, S. A., Aharizad, S., Moghaddam, M., & Sadeghzadeh, B. (2013). Evaluation of drought tolerance indices in wheat recombinant inbred line population. *Annals of Biological Research*, *4*(3), 113-122.

Nowsherwan, I., Shabbir, G., Malik, S. I., Ilyas, M., Iqbal, M. S., & Musa, M. (2018). Effect of drought stress on different physiological traits in bread wheat. *SAARC Journal of Agriculture*, *16*(1), 1-6.

Odindo, A., Thungo, Z., Shimelis, H., Mashilo, J. &Shayanowako, A.(2020). Genetic relationship among selected heat and drought tolerant bread wheat genotypes using SSR markers, agronomic traits and grain protein content. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *70*(7), 594-604.

Ojha, R., Sarkar, A., Aryal, A., Rahul, K.C., Tiwari, S., Poudel, M., Pant, K.R. &Shrestha J. (2018). Correlation and path coefficient analysis of wheat (*Triticum aestivum* L.) genotypes. Farm and Management 3: 136-141.

Ojha, A., Pandey, M. P., Thapa, D. B., Ojha, B. R., & Kharel, R. (2018). Evaluation of early seedling, root and grain yield components of spring wheat genotypes in two sowing dates. *Asian Journal of Plant Sciences*, *17*(4), 191-197.

Ozturk, I., & Korkut, K. Z. (2018). Evaluation of drought tolerance indices and relation with yield in bread wheat genotypes under drought stress conditions. *Agriculture and Food*. 6(1), 359-367.

Parchin, R. A., Najaphy, A., Mohebodini, M. S. M., Vaseghi, A., Sohrabi-Babahadi, F., &Mostafaie, A. (2014). Comparing protein pattern and drought tolerant indicators as screening techniques for drought tolerance in common wheat genotypes. *International Journal of Plant, Animal and Environmental Sciences*, *4*(2), 251-258.

Pathak, G. N. (1940). Studies in the cytology of cereals. *Journal of Genetics*, *39*(3), 437-467.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945-959.

Poudel, M. R., Ghimire, S. K., Pandey, M. P., Dhakal, K. H., Thapa, D. B., & Khadka, D. K. (2019). Assessing genetic diversity for drought and heat stress tolerance of Nepalese wheat genotypes by SSR markers. *EurAsian Journal of BioSciences*, *13*(2), 941-941.

Prodhan, F. A., Zhang, J., Bai, Y., Sharma, T. P. P., & Koju, U. A. (2020). Monitoring of drought condition and risk in Bangladesh combined data from satellite and ground meteorological observations. *IEEE Access*, *8*, 93264-93282.

Qadir, S. A. (2018). Wheat Grains Germination and Seedling Growth Performance under Drought Condition. *Basrah Journal of Agricultural Sciences*, *31*(2), 44-52. DOI: https://doi.org/10.21276/basjas.

Ramakrishnan, M., Ceasar, S. A., Duraipandiyan, V., Al-Dhabi, N. A., &Ignacimuthu, S. (2016). Assessment of genetic diversity, population structure and relationships in Indian and non-Indian genotypes of finger millet (*Eleusine coracana*L.) Gaertn) using genomic SSR markers. *SpringerPlus*, *5*(1), 1-11.

Rana, S. (2019). Random Forest for Yield Prediction in Wheat (*Triticumaestivum* L.) M.S. Thesis, Department of Genetics and Plant Breeding,Hajee Mohammad Danesh Science and Technology University,DinajpurJune, 2019.

Ramirez-Vallejo, P., & Kelly, J. D. (1998). Traits related to drought resistance in common bean. *Euphytica*, *99*(2), 127-136.

Razmjoo, M., Mohammadi, R., &Shooshtari, L. (2015). In vitro evaluation of durum wheat genotypes for drought tolerance. *Journal on New Biological Reports*, *4*(1), 33-40.

Regmi, S., Poudel, B., Ojha, B. R., Kharel, R., Joshi, P., Khanal, S., & Kandel, B. P. (2021). Estimation of Genetic Parameters of Different Wheat Genotype Traits in Chitwan, Nepal. *International Journal of Agronomy*, *2021*. Article ID 6651325, 10 pages. https://doi.org/10.1155/2021/6651325.

Rijal, B., Baduwal, P., Chaudhary, M., Chapagain, S., Khanal, S., Khanal, S., & Poudel, P. B. (2021). Drought stress impacts on wheat and its resistance mechanisms. Malays. J. Sustain. Agric, 5, 67-76.

Robinson, H. F., Comstock, R. E., & Harvey, P. H. (1949). Estimates of heritability and the degree of dominance in corn. *Agronomy journal*.

Rodríguez-Ramilo, S. T., Toro, M. A., Wang, J., & Fernández, J. (2014). Improving the inference of population genetic structure in the presence of related individuals. *Genetics research*, *96*.

Rosielle, A. A., & Hamblin, J. (1981). Theoretical aspects of selection for yield in stress and non-stress environment 1. *Crop science*, *21*(6), 943-946.

Ruan, S., Xue, Q., &Tylkowska, K. (2002). Effects of priming on germination and health of rice (*Oryza sativa* L.) seeds. *Seed science and technology*, *30*(2), 451-458.

Saghafikhadem, A. (2012). The effect of drought on growth and yield of wheat. *American Journal of Scientific Research*. 44: 110-115.

Sajid, M., Ahamd, H., Jamal, Z., & Khan, W. (2016). Characterization of wheat germplasm for yield and root trait associated with drought tolerance. In conference proceedings: 14th national and 5th international conference of Botany, University of Karachi. Vol: AGR-O-35.

Salehi, M., Arzani, A., Talebi, M., &Rokhzadi, A. (2018). Genetic diversity of wheat wild relatives using SSR markers. *Genetika*, *50*(1), 131-141.

Salem, K. F., Röder, M. S., & Börner, A. (2015). Assessing genetic diversity of Egyptian hexaploid wheat (*Triticum aestivum* L.) using microsatellite markers. *Genetic resources and crop evolution*, *62*(3), 377-385.

Sanjar, P.E.A., and Yazdan, S.A. (2015). Evaluation of wheat (*Triticum aestivum* L.) genotypes under pre-and post-anthesis drought stress conditions.

Sassi, K., Abid, G., Jemni, L., Dridi-Al-Mohandes, B., & Boubaker, M. (2012). Comparative study of six varieties of durum wheat (*Triticum durum*Desf.) Vis-a-vis water stress. *Journal of Animal and Plant Sciences (JAPS)*, *15*(2), 2157-2170.

Schuster, I., Vieira, E. S. N., Silva, G. J. D., Franco, F. D. A., & Marchioro, V. S. (2009). Genetic variability in Brazilian wheat cultivars assessed by microsatellite markers. *Genetics and Molecular Biology*, *32*, 557-563.

Sehgal, S. A., Tahir, R. A., & Nawaz, M. (2012). Molecular characterization of wheat genotypes using SSR markers. *International Journal Bioautomation*, *16*(2), 119-128.

Seong, R. C., Chung, H. J., & Hong, E. H. (1988). Varietal responses of soybean germination and seedling elongation to temperature and polyethylene glycol solution. *Korean Journal of crop science*, *33*(1), 31-37.

Shamuyarira, K. W., Shimelis, H., Tapera, T., &Tsilo, T. J. (2019). Genetic advancement of newly developed wheat populations under drought-stressed and non-stressed conditions. *Journal of Crop Science and Biotechnology*, *22*(2), 169-176.https://doi.org/10.1007/s12892-018-0262-0.

Sharp, R. E., Poroyko, V., Hejlek, L. G., Spollen, W. G., Springer, G. K., Bohnert, H. J., & Nguyen, H. T. (2004). Root growth maintenance during water deficits: physiology to functional genomics. *Journal of experimental botany*, *55*(407), 2343-2351.

Sheibanirad, A., Farshadfar, E., & Najafi, A. (2018). Evaluation of drought tolerance in some bread wheat genotypes using drought resistance. *Journal of Plant Ecophysiology*, *9*(31), 1-14.

Shokat, S., Sehgal, D., Vikram, P., Liu, F., & Singh, S. (2020). Molecular markers associated with agro-physiological traits under terminal drought conditions in bread wheat. *International journal of molecular sciences*, *21*(9), 3156. doi:10.3390/ijms21093156.

Siddiquie, M. F. A., Bakhsh, M. Z. M., Ahmed, K., Sahar, A., & Aslam, H. A. (2020) Comparison between synthetic wheat derivatives and bread wheat (*Triticum aestivum*) at seedling stage under various levels of temperature and GA3 priming. *Journal of Bioscience and Agriculture Research, 23*(01), 1901-1910.

Singh, K., Punia, M., Singh, V., & Jagdale, V. (2017). Inter-generation correlation and regression analysis in F2 and F3 generations of wheat. *International Journal of Pure and Applied Bioscience.*, *5*, 809-816.

Singh, P., & Narayanan, S. S. (1993). Biometrical techniques in plant breeding. 1st EdnKalayani publishers. *New Delhi, India*.

Singh, R. K. (1985). Biometrical methods in Quantitative Genetic Analysis. Kalyani Pub. Ludhiana. *New Delhi, Revised Ed., 318*.

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

Soetaert, W., Vanhooren, P. T., & Vandamme, E. J. (1999). The production of mannitol by fermentation. In *Carbohydrate biotechnology protocols*. Humana Press. Pp. 261-275. http://DOI: 10.1007/978-1-59259-261-6_21.

Solangi, A. H., Solongi, N., Jatoi, W. A., Solangi, M. K., Solangi, S. K., Memom, S., Soomro, S. (2021). Drought Tolerance Indices of Wheat (*Triticum Aestivum* L.) Genotypes Under Water Deficit Conditions. *Plant Cell Biotechnology and Molecular Biology*,*22*, 1-19.

Soomro, A. A., Rashid, M. A. R., &Kaleri, F. N. (2015). Direct and indirect influence of yield components on grain yield of wheat. *American-Eurasian Journal of Agricultural & Environmental Sciences*, *15*(4), 603-606. DOI: 10.5829/idosi.aejaes.2015.15.4.12595.

Sönmezoğlu, Ö. A., & Terzi, B. (2018). Characterization of some bread wheat genotypes using molecular markers for drought tolerance. *Physiology and molecular biology of plants*, *24*(1), 159-166.doi: 10.1007/s12298-017-0492-1.

Subhani, G. M., Hussain, M., Ahmad, J., & Anwar, J. (2011). Response of exotic wheat genotypes to drought stress. *Journal of Agricultural Research*, *49*(3), 293-305.

Thomason, K., Babar, M. A., Erickson, J. E., Mulvaney, M., Beecher, C., & MacDonald, G. (2018). Comparative physiological and metabolomics analysis of wheat

(*Triticum aestivum* L.) following post-anthesis heat stress. *PLoS One*, *13*(6), e0197919.

Thungo, Z., Shimelis, H., Odindo, A., Mashilo, J., &Shayanowako, A. (2020). Genetic relationship among selected heat and drought tolerant bread wheat genotypes using SSR markers, agronomic traits and grain protein content. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *70*(7), 594-604.

Tomar, R. S. S., Tiwari, S., Naik, B. K., Chand, S., Deshmukh, R., Mallick, N.,& Tomar, S. M. S. (2016). Molecular and morpho-agronomical characterization of root architecture at seedling and reproductive stages for drought tolerance in wheat. *PloS one*, *11*(6), e0156528.

Torrion, J. A., & Stougaard, R. N. (2017). Impacts and limits of irrigation water management on wheat yield and quality. *Crop Science*, *57*(6), 3239-3251.

Ullah, I., Akhtar, N., Mehmood, N., Shah, I. A., & Noor, M. (2014). Effect of mannitol induced drought stress on seedling traits and protein profile of two wheat cultivars. *Journal of Animal and Plant Sciences*, *24*(4), 1246-1251.

Ullah, N., Ullah, H., Afridi, K., Mukhtar, A. L. A. M., Jadoon, S. A., Khan, W. U., & Uddin, H. (2018). Genetic variability, heritability and correlation analysis among morphological and yield traits in wheat advanced lines. *BiyolojikÇeşitlilikveKoruma*, *11*(1), 166-180.

United States Department of Agriculture (USDA[1]), World Wheat Production 2020/2021.online source: www.worldagriculturalproduction.com/crops/wheat.aspx.

United States Department of Agriculture (USDA[2]) (2020) Bangladesh grain production challenged with adverse weather, online sources, https://www.world-grain.com/articles/14491-bangladesh-grain-production-challenged-with-adverse-weather#:~:text=Bangladesh's%20wheat%20production%20for%20the,the%202020%2D21%20marketing%20year.

Vangelis, H., Tigkas, D., & Tsakiris, G. (2013). The effect of PET method on reconnaissance drought index (RDI) calculation. *Journal of Arid Environments*, *88*, 130-140.

Vaiman, D., Mercier, D., Moazami-Goudarzi, K., Eggen, A., Ciampolini, R., Lépingle, A., & Guérin, G. (1994). A set of 99 cattle microsatellites: characterization, synteny mapping, and polymorphism. *Mammalian Genome*, *5*(5), 288-297.

Verma, H., Borah, J. L., & Sarma, R. N. (2019). Variability assessment for root and drought tolerance traits and genetic diversity analysis of rice germplasm using SSR markers. *Scientific reports*, *9*(1), 1-19.

Verma, O.P., Rajpoot, P. & Rajbahadur. (2015). Genetic variability, correlation and path coefficient analysis for yield and it's contributing traits in wheat (*Triticum aestivum*). *International Journal of Science and Research.* 4(9): 1481-1484.

Wang, X., Luo, G., Yang, W., Li, Y., Sun, J., Zhan, K., & Zhang, A. (2017). Genetic diversity, population structure and marker-trait associations for agronomic and grain traits in wild diploid wheat *Triticum urartu*. *BMC plant biology*, *17*(1), 1-17.

Wang, X., Li, Q., Yang, M., Zhang, J., Huang, M., Cai, J., ... & Jiang, D. (2021). Crosstalk between hydrogen peroxide and nitric oxide mediates priming-induced drought tolerance in wheat. *Journal of Agronomy and Crop Science*, *207*(2), 224-235.

Wang, Y., Yang, C., Liu, G., Zhang, G., & Ban, Q. (2007). Microarray and suppression subtractive hybridization analyses of gene expression in Puccinelliatenuiflora after exposure to NaHCO3. *Plant Science*, *173*(3), 309-320.

Wei, T., Simko, V., Levy, M., Xie, Y., Jin, Y., & Zemla, J. (2017). Package 'corrplot'. *Statistician*, 56(316):e24.

Wu, J., Zhou, L., Liu, M., Zhang, J., Leng, S., & Diao, C. (2013). Establishing and assessing the Integrated Surface Drought Index (ISDI) for agricultural drought monitoring in mid-eastern China. *International Journal of Applied Earth Observation and Geoinformation*, *23*, 397-410.

Xu, W., Jia, L., Shi, W., Liang, J., Zhou, F., Li, Q., & Zhang, J. (2013). Abscisic acid accumulation modulates auxin transport in the root tip to enhance proton secretion for maintaining root growth under moderate water stress. *New Phytologist*, *197*(1), 139-150.

Ya, N., Raveendar, S., Bayarsukh, N., Ya, M., Lee, J. R., Lee, K. J., Myoung-Jae, S., Gyu-Taek, C., Kyung-Ho, M. & Lee, G. A. (2017). Genetic diversity and population structure of mongolian wheat based on SSR markers: implications for conservation and management. *Plant Breeding and Biotechnology*, *5*(3), 213-220.

Yadav, S., Vijapura, A., Dave, A., Shah, S., & Memon, Z. (2019). Genetic diversity analysis of different wheat (*Triticum aestivum* L.) varieties using SSR markers. *International Journal of Current Microbiology and Applied Science*, *8*(2), 839-846.

Yamaguchi, M., Valliyodan, B., Zhang, J., Lenoble, M. E., Yu, O., Rogers, E. E., ... & Sharp, R. E. (2010). Regulation of growth response to water stress in the soybean primary root. I. Proteomic analysis reveals region-specific regulation of phenylpropanoid metabolism and control of free iron in the elongation zone. *Plant, cell & environment*, *33*(2), 223-243.

Yao, J., Ma, H., Yang, X., uocai Yao, G., & Zhou, M. (2014). Inheritance of grain yield and its correlation with yield components in bread wheat (Triticum aestivum L.). *African Journal of Biotechnology*, *13*(12).

Zampieri, M., Ceglar, A., Dentener, F., &Toreti, A. (2017). Wheat yield loss attributable to heat waves, drought and water excess at the global, national and subnational scales. *Environmental Research Letters*, *12*(6), 064008. DOI: 10.1088/1748-9326/aa723b.

Zegeye, F., Alamirew, B., &Tolossa, D. (2020). Analysis of Wheat Yield Gap and Variability in Ethiopia. *Agricultural Economics*, *5*(4), 89-98.

Zerga, K., Mekbib, F., &Dessalegn, T. (2017). The Mean Performance of Different Bread Wheat (*Triticum Aestivum*. L) Genotypes in Gurage Zone, Ethiopia. *Landscape Architecture and Regional Planning*, *2*(1), 29-35.

Zhang, D., Bai, G., Zhu, C., Yu, J., & Carver, B. F. (2010). Genetic diversity, population structure, and linkage disequilibrium in US elite winter wheat. *The Plant Genome*, *3*(2), 117–127. doi: 10.3835/plantgenome2010.03.0004.

Zhao, T., & Dai, A. (2015). The magnitude and causes of global drought changes in the twenty-first century under a low–moderate emissions scenario. *Journal of climate*, *28*(11), 4490-4512.

Zhou, G. A., Chang, R. Z., & Qiu, L. J. (2010). Overexpression of soybean ubiquitin-conjugating enzyme gene GmUBC2 confers enhanced drought and salt tolerance through modulating abiotic stress-responsive gene expression in Arabidopsis. *Plant molecular biology*, *72*(4), 357-367.

Zorić, M., Dodig, D., Kobiljski, B., Quarrie, S., & Barnes, J. (2012). Population structure in a wheat core collection and genomic loci associated with yield under contrasting environments. *Genetica*, *140*(4), 259-275.

APPENDICES

Appendix I: Map of Debiganj Upazila showing the experimental area

Appendix II: Weather data of the experimental site during the period from December, 2022 to February, 2023

Months	Relative humidity (%)	Temperature		Total rainfall (mm)
		Minimum (°C)	Maximum (°C)	
December/ 2022	100	8.9	25.8	0.00
January/ 2023	98.8	9.4	30	0.00
February/ 2023	96.9	10.3	28.9	0.00

Source: Bangladesh Wheat and Maize Research Institute, Dinajpur (BWMRI).

Appendix III: Some photographs of research work