IDENTIFICATION OF HIGH YIELDING AND STABLE BARLEY GENOTYPES THROUGH FIELD PHENOTYPING IN MULTI-LOCATION TRIALS AND MOLECULAR APPROACHES

A THESIS

BY

AFRIN AJAHAN PAPIA

STUDENT NO. 2205097

SEMESTER: July-December, 2023

SESSION: 2022-23

MASTER OF SCIENCE (MS)
IN
GENETICS AND PLANT BREEDING

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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

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Certification

This is to certify that the thesis entitled **"IDENTIFICATION OF HIGH YIELDING AND STABLE BARLEY GENOTYPES THROUGH FIELD PHENOTYPING IN MULTI-LOCATION TRIALS AND MOLECULAR APPROACHES"** is a study, prepared by the examinee, bearing Registration No.: 2205097 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. Farhad****, Senior Scientific Officer, BWMRI, Bangladesh, 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. Md. Hasanuzzaman, Professor Dr. Md. Abul Kalam Azad, 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, brother and other relatives whose sacrifices, inspiration and continuous blessings paved the way for my higher studies.

December 2023 *The Author*

IDENTIFICATION OF HIGH YIELDING AND STABLE BARLEY GENOTYPES THROUGH FIELD PHENOTYPING IN MULTI-LOCATION TRIALS AND MOLECULAR APPROACHES

ABSTRACT

Barley *(Hordeum vulgare)* is a nutrient rich cereal and there is a limited number of cultivars available in Bangladesh. The study was aimed to analyze high yielding stable genotypes by genotypes-environment interactions (GEI) and to locate the unique genotype by deploying molecular characterization. To get a reliable stable genotype, rather than relying on a single analysis, employing a combination of methods to measure the nature of the GEI in various dimensions is often more effective. We investigated GEI using parametric and non-parametric stability statistics including multi-trait genotype ideotype distance index (MGIDI), additive main effects and multiplicative interaction (AMMI), and GGE. Nine barley genotypes comprised of six exotic lines and three Bangladesh Agricultural Research Institute (BARI) released varieties were evaluated in a randomized complete block design across three locations (Dinajpur, Rangpur and Panchagarh) during the rabi season of 2022-2023. Various parametric and nonparametric stability statistics were calculated using a web-based STABILITYSOFT program. Based on the analysis, EEB_450, BARI Barley-7 and EEB_91 identified as superior and high yielding genotypes. The additive effects analysis of AMMI model revealed significant effects of genotype, environment, and GEI on number of grains per spike (NGPS), yield per plant (YPP) and yield per plot (YPPLOT). EEB_450 and EEB_91 for NGPS, EEB_18 and EEB_152 for YPP, and EEB_450 and EEB_91 for YPPLOT emerged as stable genotypes with optimal performance according to the AMMI model. The likelihood ratio test indicated significant effects of genotype and GEI all studied traits. Regarding NGPS, YPP and YPPLOT, EEB_450, BARI Barley-7 and BARI Barley-9 had high best linear unbiased prediction (BLUP) value and were identified as suitable genotypes. The GGE biplot method determined the most favorable location for specific genotypes. Among three experimental locations, Panchagarh was identified as the superior environment for barley cultivation with EEB_450 being the top performing genotype considering all traits. Using the multi-trait genotype ideotype distance index (MGIDI), EEB_450, BARI Barley-7 and BARI Barley-9 were deemed the most ideal genotypes. Analysis of genetic variation using SSR markers, the genotype EEB_450 revealed phenotypically stable and showed variations with the BARI released check varieties BARI Barley-7, BARI Barley-8 and BARI Barley-9. Therefore, EEB_450 is recommended as a superior performing genotype in three locations and can be released as a variety in Bangladesh. The combination of multi-location trials and molecular approaches facilitates the identification and selection of high-yielding, stable barley genotypes with desirable agronomic traits. This integrated approach enhances the efficiency and precision of barley breeding programs, ultimately contributing to sustainable agricultural productivity and food security.

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SYMBOLS AND ABBREVIATION

AEZ	:	Agro Ecological Zone
ANOVA	:	Analysis of Variance
BBS	:	Bangladesh Bureau of Statistics
cm	:	Centimeter
CRD	:	Completely Block Design
CV	:	Coefficient of Variation
DAS	:	Days After Sowing
DAP	:	Di-Ammonium Phosphate
d.f.	:	Degree of Freedom
e.g.	:	For Example
etc.	:	And the rest
et al.	:	And others people
EEB	:	Eastern European Barley
FAOSTAT	:	Food and Agriculture Organization of the United Nations
FAO	:	Food and Agriculture Organization
g	:	Gram
GA	:	Genetic Advance
GAM	:	Genetic Advance Over Mean
GCV	:	Genotypic Coefficient of Variation
PCV	:	Phenotypic Coefficient of Variation
GE	:	Genotype by Environment interaction
GGE	:	Genotype and Genotype by Environment interaction
i.e.	:	That is
K	:	Potassium
kg	:	Kilogram
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
ppm	:	Parts Per Million
RBD	:	Randomized Block Design
RCBD	:	Randomized Complete Block Design
rg	:	Genotypic correlation
RSS	:	Residual sum of squares
S	:	Sulphur
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
AMMI	:	Additive main effect and multiple interaction
GGE	:	Genotype plus genotype environmental interaction
LRT	:	Likelihood ratio test
BLUP	:	Best linear unbiased predection
MGIDI	:	Multi-trait genotype-ideotype distance index
ASV	:	AMMI stability value
WAASB	:	Weighted average of absolute scores of the *i*th genotype
MTSI	:	Multi-trait stability index

CHAPTER I

INTRODUCTION

Barley (*Hordeum* spp.) is a self-pollinating diploid species (2n=2x=14) with a genome size of 4.79 billion letters of genetic code, which is approximately twice as large as the genome of a human being (Mascher *et al.*, 2017). It is a member of the Poaceae family and comes in three different types: 2-row type barley (*H. disticum*), 4-row type barley (*H. vulgare*), and 6-row type barley (*H. hexasticum*). Among the cereals, barley is the firstborn domesticated food-cereal across the globe (Wang *et al*. 2015). It is the fourth most commonly used crop after wheat, rice and corn worldwide and this crop is more durable than other grains for stress conditions (drought, salinity, temperature stress), which is important in arid and semiarid regions based on rainfall grows (Vaezi *et al.*, 2017). Barley, a member of the grass family, is one of the eight founder crops and one of the oldest domesticated crops. It is a significant cereal worldwide, used for human food, animal feed, and malting substrates.

Barley, a cereal primarily found in temperate regions, is cultivated in Russia, Canada, and Germany, with mountain slope cultivation in Tibet, Nepal, Ethiopia, and the Andes. Modern plant breeding activities in the recent century resulted in the development of a large number of elite varieties with a higher yield, better quality, and resistance to stresses, but the narrow genetic background (Fischbeck *et al*., 2003). Replacement of highly adapted landraces by modern varieties performing under optimal conditions but failing under harsh environments (Yahiaoui *et al*., 2014). The disappearance of most landraces of crop plants from practical farming (Fischbeck *et al*., 2003 and Jones *et al*., 2011). Although in many countries, landraces were completely replaced by modern varieties starting from a long time back, in some marginal areas, to take advantage of the specific adaptations of landraces to the agro-ecosystem, their cultivation still continues (Jones *et al*., 2008).

Barley is a widely consumed food crop in Africa, the Middle East, Nepal, South America, and Asia. Its husk protects the coleoptile during germination, aids in filtration, and is preferred for malt recovery. Malt is used in brewing, distillation, baby foods, confectionaries, cocoa-malt drinks, and medicinal syrups. In barley, Grain protein content is closely related to feeding quality as well as malting and brewing processes. High protein concentration is attributed to feeding quality, while

low protein content is favorable for barley malt and beer production (See *et al*., 2002). Barley water, a decoction of barley in water, is used for medicinal purposes and inflammation treatment. Barley products like Sattu and Missi roti are traditionally used. It was once more important than wheat in agriculture. But now it stands fourth in world cereal production after wheat, rice, and maize, and is mainly used as animal feed (around 85% of global production) (Schulte *et al*., 2009). Approximately 65-70% of the produced barley in the world is used as animal feed, 33-35% as malt in beer, whiskey and biodiesel production and 2-3% as human food in food production.

Studying genetic variability in crops is important for improving the crops and enhancing the production. Genetic variability is the occurrence of differences among varieties due to differences in their genetic composition and/or the environment in which they are raised (Tehulie, 2022). Heritability is a measure of the value of selection for particular characters and an index of their transmissibility. It plays a predictive role in breeding, expressing the reliability of phenotype as a guide to its breeding value. The expected response to selection is also called genetic advance (GA). High genetic advance 3 coupled with high heritability estimates the most effective condition for selection. Genetic improvement of crop is largely depending on the scale of genetic variability and the degree to which desired traits are inherited (Dinsa *et al.*, 2018).

A multi-location yield trial is crucial for variety adaptation due to genotype and environment interactions. It allows plant breeders and agronomists to study genotype effects and environmental factors affecting traits. Therefore, cultivars and genotypes that are least affected by different environmental conditions and are stable are suitable for wide cultivation (Roy *et al*., 2021).

The potential of a genotype in any environment is determined by the effect of environmental (E), genotype (G) or interaction (GE or GEI) factors. Because the breeders need quite stable cultivars in different environmental conditions and main some traits for agronomic, new varieties must have reliable results in a wide range of environments (Solonechnyi *et al*., 2015). The reason for the basic differences in the performance of genotypes in wide environments is due to the interaction of the genotype with the environment (Kendal and Aktas, 2016; Neisse *et al*., 2018). Genotype environmental interaction (GEI) analysis is of primary importance, as is the case for other crops inn barley breeding and many other intermediate studies (Kilic, 2014).

The GGE biplot analysis (i.e., the genotype main effect (G) and the genotype × environment interaction (G×E) (Frutos *et al*. 2014; Hossain *et al*. 2018) is a useful tool for plant breeders and

geneticists to find out the maximum yield and stable genotypes across the multiple locations; as well as to find out the most favorable location for a specific genotype through acquiring a graphical form (Gabriel 1971; Yan and Kang 2002; Koutis *et al*. 2012). The stability obtained by this method is widely used to characterize a genotype, performing a relatively stable yield and not even affected by altering the environmental conditions (Kiliç 2014). In all of the barley improvement activities and research, GEI (genotype × environment interaction) is of major importance as well as for other crops (Voltas *et al*. 2002). The stability methods can be categorized as parametric (univariate and multivariate) and non-parametric stability measures. Univariate and nonparametric stability statistics cannot generate an accurate picture of the complete response pattern, due to the multivariate nature of the genotype's response under different environments (Kılıç 2014). The AMMI model provides more information to researchers about the stability of genotypes in terms of grain yield, while GGE biplot on the relationships between genotypes and properties (Mohammadi *et al*., 2016). The vital characteristic of this analysis is that the adjustment is accomplished by information from other locations to perfect the estimates within a given locations (Sadeghi *et al.* 2011).

The plant's responses to stress are very complex, which are about acclimation, adaptation, and tolerance. These responses vary with the life stage and plant type, and the plant's continuity of life under stress conditions is linked to its ability to hold the line against stress (Al-Ashkar *et al*., 2023; Liu *et al*., 2022 and Khan *et al*., 2003). The development of cultivars that combine the best qualities of high productivity and stability under varying abiotic stress levels is one of the top priorities of plant breeders and the greatest goal in modern breeding programs (Hashem *et al*., 2023 and Al-Ashkar *et al*., 2023). The GY is influenced by both genetic and environmental factors. So, the multi-trait genotype ideotype distance index (MGIDI) model the magnificent efforts in modern breeding programs for the valid and reliable selection of genotypes. The reliability of prediction (the expected value is close to the visible value) is crucial for an appropriate genotype recommendation and delimitation of mega-environments (Al-Ashkar *et al*., 2022; Olivoto *et al*., 2022 and Olivoto *et al*., 2019).

According to Gauch and Zobel (Balla *et al*., 2019), to increase the accuracy of prediction in METs, researchers must utilize statistical models that have high prediction abilities (Olivoto *et al*., 2019). Plant breeders rely primarily on the genetic stability of traits. In the present study, we have shown

how the advantages of the AMMI model and the performance of multi-indices may be combined to increase the reliability of MET analysis. A study evaluating rice has shown that the estimates using the AMMI model were closer to the "true" value (Han *et al*., 2020), so predictive precision deserves special attention for model diagnosis in MGIDI analysis (Olivoto *et al*., 2019 and Zeng *et al*., 2002).

Phenotypic characters are not 100% authentic to analyze environmental variations. Morphological markers may employ to assess genetic variation and a viable substitute for morphological criteria since DNA markers are environmental-independent (Pirseyedi *et al*., 2006). Before releasing a variety, a DNA fingerprint is imperative. In this scenario, microsatellite markers or SSRs are alternative choice. Simple sequence repeats (SSRs) markers based on the polymerase chain reaction (PCR) have high polymorphism, co-dominant inheritance, highly reproducibility, locus specificity, and random genome distribution (Russell *et al*., 1997), thus suitable markers are effective for assessing genetic variation, genetic relationship, and phylogenetic development. Using SSRs technique as powerful tool for genetic studies in barley breeding for identify variation have been frequently confirmed in several investigations (Fantahun *et al.*, 2023; Jabbari *et al.*, 2022; Mariey *et al.*, 2022; Lakew *et al*., 2012; Vahideh *et al*., 2015; Hellal *et al*., 2018 and Heiba *et al*., 2019), So, the research hypothesis might be the identification of high-yielding and stable genotypes through multi-location trials and to identity distinguishing genotypes of barley applying molecular approaches.

Therefore, the major objectives of the study are to figure out the acclimatization of barley genotypes using the AMMI, stability, GGE and MGIDI models and determine distinctness among genotypes using molecular characterizations. The specific objectives are:

1) To evaluate mean performance and genetic parameters of barley genotypes for yield and yield contributing characters grown under different environmental condition across three locations,
2) To identify high-yielding and stable genotype using MGIDI, AMMI, parametric and non-parametric stability statistics and GGE analyses,
3) To assess the genotypic variation among barley genotypes using SSR markers and
4) To recommend the superior genotype across three locations as a promising variety for cultivation in Bangladesh.

CHAPTER II

REVIEW OF LITERATURE

The review of literature encompasses a comprehensive examination of previous research and scholarly works related to barley genotypes, their origin and distribution, performance, stability, and methods of identification. This section aims to provide a solid foundation by synthesizing existing knowledge and highlighting key findings from studies that have investigated barley cultivation, genotype-environment interactions, molecular techniques, and approaches to enhancing yield and stability. By analyzing and synthesizing the existing body of literature, this review sets the stage for the subsequent investigation into high-yielding and stable barley genotypes across diverse locations using molecular methodologies.

2.1. Origin and Distribution

Wallace *et al*. (2019) found that Barley, a long-standing cereal landrace in northern Scotland, is now one of Europe's oldest cereals. Geometric modern morphometric (GMM) analysis reveals morphological differences between barley and other British and Scandinavian landraces, as well as barley from Orkney and the Western Isles. This identifies barley as a living heritage and secures its commercial future, demonstrating the potential of GMM for tracing cereal landrace ancestry and exchange in the archaeological record.

Zeng *et al*. (2018) reveals that contemporary qingke, the main cereal on the Tibetan Plateau, is derived from eastern domesticated barley and introduced to southern Tibet between 4,500 and 3,500 years ago. The low genetic diversity of qingke suggests Tibet's exclusion as a center of origin or domestication. The study also supports a feral or hybridization origin for Tibetan weedy barley.

Wang *et al*. (2015) analyzes the origin and evolution of cultivated barley, focusing on its genetic diversity and changes worldwide. It found that Tibetan wild barley diverges from Near Eastern barley, indicating Tibet as a hub for domestication. The study also found gene flow between Eastern and Western barley populations, suggesting the Silk Road played a significant role.

2.2. Mean comparisons of different morpho-physiological traits studied in barley:

Fekadu *et al.* (2022) examined thirty barley genotypes in nine environments in a randomized full block design with three replications to determine genotype-by-environment interaction and stability and adaptation of high-yielding genotypes. Environment (54.61%), genotype (10.69%), and G × E interaction (34.70%) explained the variances. IPCA1 (45.48%), IPCA2 (24.65%), and IPCA3 (13.02%) explained 70.13% of the G × E interaction sum of squares. The current investigation suggested 3514-A, 24,990, and 17,148 for breeding line identification and variety development.

Angassa and Mohammed (2022) evaluated that in 2019, southern Ethiopia examined 64 landrace barley accession and 3 released varieties for eight quantitative features. Each block had three standard tests in an enhanced block design experiment. Except for days to 75% maturity and plant height. Grain yield is 20.72–57.33 quintals ha^{-1}. Chefo produced the most grain (released variety). However, 43 of the farmer's varieties had grain yields higher than the two enhanced types. The study revealed farmers' variety accessions' hidden yield-boosting potential by using conserved germplasm.

Elbasyoni *et al.* (2022) worked that 426 barley lines under deficit irrigation (DI) and full irrigation (FI) in 2019 and 2020. Number of days to flowering (NDF), Chlorophyll content (CH), Canopy temperature (CAN), grain filling duration (GFD), plant height (PH), and Yield were measured under DI and FI. 24 significant indicators were barley genes. Most of these genes affected plant drought responses. Nine important indicators were previously reported, whereas 27 were novel. This study identified markers that could predict DI and FI-optimal barley accessions.

Usubaliev *et al*. (2020) evaluated five agronomic traits of 29 barley accessions in different agro-environmental conditions. Accessions represented cultivars from Kyrgyzstan, Ukraine, and the Nordic and Baltic countries as well as landraces from northeastern and eastern Russia. The field experiments were carried out in two countries (Latvia and Kyrgyzstan) in order to select the suitable genotypes or cultivars as a source for Kyrgyz barley breeding programs. Among the accessions studied, we found material that can be used in Kyrgyz breeding as potential sources of earliness, spike length and TKW. Among the cultivars, Cecilia‟ from Sweden showed 9 an attractive agronomic performance, and had constant behavior under Kyrgyz climatic conditions

during two years of trials. Other cultivars like “Saana”, “Sencis” and “Mette” can also be included in future breeding due to their earliness, plant height, spike length and number of kernels.

Rodrigues *et al*. (2020) conducted a breeding process focused on selection for grain yield, disease resistance and malting quality. The objective of this work was to quantify the genetic gain in barley grain yield from 1968 and 2008 in Brazil and to identify the physiological characteristics associated with the increase of grain yield. Field experiments with five 2-row barley cultivars were tested from 2011 to 2013 in the absence of biotic and abiotic stresses and with mechanical restriction to lodging. The ANOVA showed no genetic gain until 1980 with average grain yield of 4.632 kg/ha. After 1980, there was a productivity increase of 59.9 kg/ha/year. The main component associated with grain yield was the number of grains, due to the higher number of spikes associated to a greater contribution of the tillers in the modern cultivars.

Yadav *et al*. (2019) evaluated forty-nine exotic and indigenous genotypes along with four check varieties of barley for yield and its contributing traits. Analysis of variance revealed significant differences among the genotypes for all the traits viz. days to 50% flowering, days to maturity, plant height (cm), flag leaf area (cm^2), peduncle length (cm), ear length (cm), productive tillers/ plant, 1000 seed weight (g), biological yield/ plant, grain yield/ plant and harvest index (%) and exhibited high amount of variability among all the genotypes. The mean plant height was 73.21 cm with a range of 51.85-89.85 cm. They observed mean grain yield/ plant 10.03 g with a range of 7.74-11.54 g.

Sayd *et al*. (2019) carried out a study with 69 barley genotypes from 2012 to 2014, under irrigation in the Cerrado. Six agronomic characteristics were assessed: grain yield, plumpness kernel, thousand seeds weight, plant height, degree of plant lodging and days to heading. Analysis of variance was performed and significant effects were observed for genotypes, years and the G x E interaction. The Colombian accession MCU363PI402112 8 stood out for its agronomic characteristics. Genotype selection based on the phenotypic evaluations was possible due to their good experimental accuracy and precision. Precocious genotypes with high grain yields and homogeneous grain sizes were selected. Due to the environmental influence on the grain yield, additional studies concerning the components of yield in this environment are necessary to facilitate the selection of more productive genotypes.

Hagenblad *et al*. (2019) conducted an experiment with morphologically and genetically characterized 57 landraces collected during the twenty first century and conserved in gene banks. The majority of accessions were of the six-row type. Accessions from the easternmost islands were genetically distinct from those from the central and western islands. Accessions from the western islands often had a mixed genetically composition, suggesting more recent exchange of plant material with the central islands.

Hajiagha *et al*. (2019) evaluated both morphological and physiological traits followed by classification of 18 barley inbred lines based on RCBD design with four replications. Results of descriptive statistics revealed vast range of variation for most of studied traits. Among the studied traits, seed yield with standard deviation of 614.06 possessed maximum divergent, hectoliter weight and grain yield had the lowest and the highest coefficients of variation values of 0.58 and 8.88, respectively. Univariate analysis of variance depicted remarkable genetic variation among studied genotypes based on morphological and physiological traits. They postulated that line number 3 with high peduncle length (7.18 cm), 1000 kernel weight (42.2 gr), yield (5172.5 kgh-1), dry matter percentage (31.3 %), fresh weight (0.463 gr) and also another moderate characteristics could considerable as promising one for arid and semi-arid region after regional field trials.

Al-Sayaydeh *et al*. (2019) worked with the agronomic performance of 10 Jordanian barley landraces and three local cultivars were evaluated in two locations for two growing seasons. Clear significant variations for all studied traits were observed among the selected genotypes, environments, and their interactions, local cultivar Rum and Baladi landrace showed the best yield performance, while Herawi and Nabawi landraces produced the lowest yield across all environments.

2.3. Genetic variability

Gupta *et al*. (2022) examined that 28 F1 s were developed by crossing 8 commercial varieties, germplasm, or local lines as per diallel mating design (excluding reciprocals) and laid out in randomized block design with parents and three replications during rabi 2019-20 to study genetic variability parameters, correlation, and path analysis. All characters had enough genetic variety. Variability estimates showed substantial phenotypic and genotypic coefficients of variation for biological and seed yield per plant. 1000 grain weight had excellent heritability and genetic

progress, indicating additive gene action and good selection potential. Seed yield per plant was positively correlated with biological yield, spike length, tillers per plant, grains per spike, 1000 grain weight, plant height, days to maturity, and harvest index. Path analysis showed that factors including biological yield per plant, harvest index, 1000 grain weight, number of grains per spike, and peduncle length affected barley seed yield and could be selected to improve it. Biological yield per plant and harvest index were useful selection factors that may be used in barley breeding programs to boost seed production.

Verma *et al.* (2022) studied that two hundred ten barley germplasm lines (144 exotic and 66 indigenous) and six standard check varieties (HBL 113 (Vimal), HBL 713 (Him Palam Jau 1), HBL 804 (Him Palam Jau 2), BHS 400 (Pusa Sheetal), BHS 352 (Himadri), and VLB 118 (VLJau 118)) were evaluated for yield and yield-related traits. This group of experimental barley genotypes showed strong PCV and GCV (>20%) for grain yield/plant, number of effective tillers/plant, biological yield/plant, and number of grains/spike, showing high selection response. For barley grain yield improvement, number of grains/spike, biological yield/plant, and grain yield/plant had strong heritability and genetic progress as a percent of mean.

Avanish and singh (2021) examined that barley yield and contributing characters were analysed for genetic variability, heritability, correlation coefficient, and path analysis. Seed yield, biological yield, number of tillers, harvest index, number of seeds per spike, 100-seed weight, ear length, and plant height had higher genotypic and phenotypic coefficients. All characters had strong heritability and genetic progress. Biological yield per plant, harvest index, number of tillers per plant, number of grains per spike, and 100-seed weight are the most important yield contributing features.

Dyulgerov and Dyulgerova (2020) showed that twenty barley types were arranged in a complete block design with four replications. Studying grain yield variability, heredity, and genetic advance. All attributes, including grain yield, differed between kinds. Grain yield had 10.10% heritability and spike length 94.60%. Spike length and 1000-grain weight had high heritability and genetic progress as percent of mean. These features also showed minimal genotypic and phenotypic variances. Selection might easily increase these features. Veslets yielded 5.27 t/ha and Izgrev 5.09 t/ha. PA86-49-95 (6.43 t/ha), Bojin (6.01), and Express (5.90) outperformed the checks in grain yield. Thus, breeding winter feed barley with these cultivars may increase grain production.

Shiferaw *et al*. (2020) assessed the genetic variability and to assess the associations among morpho-agronomic characters, three hundred and twenty Ethiopian barley genotypes were evaluated in 2017 main-season at Holetta Agricultural Research Centre using 20 × 16 Alpha Lattice design. The analysis of variance showed that there were significant differences among the genotypes in all traits except for days to emergence, indicating the presence of genotypic variation among the studied genotypes. Grain yield, biomass yield and kernels per spike had high phenotypic and genotypic coefficients of variation. The estimates of broad sense heritability and genetic advance were high for days to heading and maturity and thousand kernels weight. Therefore, there is a high possibility of developing new varieties from these genotypes.

Dido *et al*. (2020) evaluated that the genetic variability, direct and indirect effects of some metric characters on grain yield of 585 barley landraces and 10 cultivars under augmented complete block design, the current study was carried out in 2018 and 2019 at Sinana Agricultural Research Center (on-station) and Goba (on-farm), South-east Ethiopia. The mean, range, components of variation, broad sense heritability, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), and genetic progress were evaluated for yield and yield linked characters using the data on twelve quantitative characters. The studied barley accessions showed significant variance for all features, according to the results. Plant height, number of viable tillers per plant, spike length, number of seeds per spike, and weight of 1000 seeds showed strong heritability whereas grain yield per plant showed moderately high heredity along with high genetic progress. The development of high yielding barley varieties in the future crop improvement programme can be based on those quantitative characteristics that have a significant and favourable direct effect on grain yield/plant.

Matin *et al*. (2019) was carried out a study to investigate the correlation coefficient, path analysis and genetic variability among some barley varieties for nine characters in a Randomize Block Design (RBD) with three replications in three environments of Bangladesh. High genotypic coefficient of variation (GCV) was obtained from grain/ spike (29.89 %), yield/ plant (28.72%) and effective tiller/plant (21.86 %) and spike length (13.56 %). The characters with high GCV indicated high potential for selection. The highest heritability (Hb) was observed for 1000 seed weight (95.09) followed by yield/ plant (93.98), grain/ spike (92.09) and spike length (69.93), days to heading (72.65) but the lowest Hb was identified for effective tiller/plant (22.41) followed by

the plant height (34.21). Those traits with higher heritability may be considered for selection. Grain/ spike had the highest positive direct effect (5.65) on yield followed by 1000 seed weight (4.65), spike length (1.26), yield/ plant (0.66), days to heading (0.55) and days to maturity (0.34). These parameters were identified as direct selection. Direct negative effect on yield was shown by plant height (-0.32) and effective tiller/plant (-0.74). This was an indication of indirect selection.

Dinsa *et al*. (2018) worked on a study that was made to estimate the level of genotypic and phenotypic variability among yield and yield-related traits of food barley. Twenty- five food barley genotypes were tested in 5 × 5 Lattices Design with three replications. The analysis of variance shown there were highly significant ($p \leq 0.01$) difference among the studied genotypes for days to first heading, days to heading, grain filling period, days to maturity, number of kernels per spike, plant height, spike length, awn length, grain yield, thousand kernel weight and harvest index. Significant ($p \leq 0.05$) difference among genotypes was also observed for peduncle length. From the total phenotypic variance (δ2p) the portion of genotypic variance (δ2g) was greater than environmental variance (δ2e) for days to first heading, days to heading, days to maturity, number of kernel per plant, grain yield, 1000-kernel weight and harvest index, while lower for the others. The genotypic coefficient of variation (GCV) ranged from 3.94% to 22.90% while phenotypic coefficient of variation (PCV) ranged 4.98% to 30.34%. The broad sense heritability estimate varied from 22.26% to 70.50%. The expected genetic advance as percentage of mean of traits ranged from 6.43% to 35.61%. Moderate high heritability complemented with moderate genetic advance was observed for days to first heading, days to heading and 1000-kernel weight suggesting, these traits were highly heritable and governed by additive gene action. Selection of genotypes to top 5% intensity under one cycle of selection for these traits could result in genetic advance of more than 10% over the respective population mean.

2.4. Character association correlation analysis

Aklilu *et al*. (2020) showed that the phenotypic and genotypic correlation was positive and highly significant for grain yield with plant height, number of seeds per spike, biological yield and harvest index.

Shiferaw *et al*. (2020) conducted the study among morpho-agronomic characters of three hundred and twenty Ethiopian barley genotypes and found that grain yield exhibited positive and highly

significant correlations with days to heading and maturity, number of kernels per spike, biomass yield, harvest index, thousand kernels and hector litre weights.

Negash *et al*. (2019) conducted a study to determine the inter relationship among yield and yield-related agronomic characters and their effect on grain yield. Grain yield showed positive and significant genotypic correlations with grain weight per spike (rg = 0.36), spike weight per plant (rg = 0.38), 1000-seed weight (rg = 0.66), biological yield (rg = 0.83), awn length (rg = 0.34) and plant height (rg = 0.23). This suggests that simultaneous improvement in these characters might be possible.

Laidig *et al*. (2017) observed the positive phenotypic relation between grain yield and malting quality can be attributed to a shift of selection and environmental effects, but genetic correlations showed a negative association. Genetic effects of protein concentration and malting quality were not correlated indicating that both were not genetically linked. Considerable yield progress and improvement of malting quality were achieved despite of their weak to moderate negative genetic dependence.

Amardeep *et al*. (2017) conducted an experiment involving fifty genotypes/strains of barley during rabi 2014 at Crop Research Farm, Nawabganj, of Chandra Shekhar Azad University of Agriculture and Technology, Kanpur in a Randomized Block Design with three replications. Grain yield per plant had positive correlation with biological yield, number of productive tillers per plant, harvest index, number of grains per spike, days to maturity and plant height at the genotypic level.

Hailu *et al*. (2016) estimated the analysis of variance (ANOVA) revealed that there was a significant difference (p<0.001) among the sixty-four genotypes for all the characters studied except for 1000-kernel weight at Quiha which was significant (p<0.05) and plant height was non-significant at Atsbi and Ofla. Grain yield had positive and highly significant phenotypic and genotypic correlation with 1000-kernel weight and biological yield in all environments except harvest index at Ofla. Grain yield had positive and highly significant phenotypic and genotypic correlation with 1000-kernel weight and biological yield in all environments except harvest index at Ofla. On the other hand, grain yield had negative and highly significant correlation at genotypic level with days to heading and days to maturity only at Ofla.

Vitrakoti *et al*. (2016) conducted a study in 2014- 2015 to evaluate the barley genotypes for their yield attributing traits and correlation and causation. Thousand grains weight (0.333) had positively highest significant correlation with grain yield per hectare followed by spike length (0.310). Grain yield per hectare showed negative highly significant correlation with days to flowering (-0.796) followed by days to heading (-0.761) and days to booting (-0.663). Peduncle length (0.229), plant height (0.226), biological weight (0.181) and flag leaf area (0.032) were positively correlated with grain yield per hectare while flag leaf-1 area (-0.029) was negatively correlated. Thus, selection for genotypes with higher thousand grain weight and spike length accommodating earlier days to flowering, heading and booting is a prerequisite for attaining improvement in grain yield per hectare.

2.5. Stability analysis of AMMI & the genotype main effect (G) and the genotype × environment interaction (G×E) model

Karaman (2022) examined the relationship between physiological characteristics and grain yield in barley cultivars. Significant differences were found between varieties, except for the GS77 period Normalized Difference Vegetation Index. High yielding barley cultivars were found, with Bozlak being the most stable. A positive correlation was found between grain yield and NDVI during early dough formation and dough formation periods. The GGE biplot model showed a negative correlation between canopy temperature and grain yield.

Hilmarsson *et al*., (2021) evaluated a study that the yield and thousand-kernel weight of 32 spring barley genotypes in seven Icelandic environments. It found that six-row genotypes showed better response in high fertility soils and a divergent response along a temperature gradient. The study recommends defining one mega-environment for Icelandic barley cultivation and breeding, as yield is the main trait of interest. The study identified promising genetic material for both traits and highlighted the superiority of six-row genotypes for yield. The study suggests using all genotypes and interpreting AMMI results using the LMM for better modeling of GxE. Iceland is suggested as a single mega-environment for barley cultivation and breeding, with TKW as the main breeding trait.

Alireza *et al*., (2021) worked on salinity stress significantly reduced growth and physiological traits in seedling plants, but some salt-tolerant genotypes showed minimal reduction. Multivariate analysis grouped measured traits and genotypes into different clusters. G12, G14, G6, G7, and G16

were selected as the most salt-tolerant genotypes in the early growth stage. In a multi-environment trials experiment, AMMI analysis showed that grain yields were influenced by environment, genotype, and GE interaction. G7, G8, G14, and G16 were selected as superior genotypes. The MGIDI and WAASB indices revealed that G7, G14, and G16 can be recommended as new genetic resources for improving and stabilizing grain yield in barley programs in moderate climate and saline regions of Iran.

Moustafa *et al*. (2021) A field experiment in Egypt's Western Sinai Peninsula found that dual-purpose barley can produce high-quality forage and acceptable grain yields in marginal arid regions. The study found that early sowing in late October yielded higher forage yields than intermediate sowing in mid-November and late sowing in early December. Some genotypes performed better, suggesting higher adaptation capacity. The study concluded that dual-purpose barley is favorable for grain and forage production in similar environments.

Karahan *et al*. (2020) carried out a study of selection of barley *(Hordeum vulgare)* genotypes by GYT (GENOTYPE × YIELD× TRAIT) biplot technique and its comparison with GT (GENOTYPE × TRAIT). In this study, the yield and other target traits were combined using the GYT biplot approach to assess the strengths and weaknesses of each genotype, which was then compared with GT (genotype × trait). There were variations between the GYT and GT biplot approaches in terms of each genotype's stability and overall adaptability. Advanced lines (3, 9, 12) and the Altıkat variety were good genotypes according to the GT biplot approach, but only the Altıkat variety was the best genotype based on yield \ characteristics combinations according to the GYT biplot method.

Kendal *et al*. (2019) worked a study used twelve barley genotypes in trials in 2012-13 and 2013-14. The results showed that G4 was the most adaptable genotype, with high yield and stable adaptation. G2, G3, G6, G7, G8, and Altikat were the best genotypes with high yield. G4 was recommended for release as HEVSEL in 2017, while G7 and G6 were protected. The AMMI analysis showed that environments, GE, and genotypes affected the major treatment sum of squares.

Negash *et al*. (2019) conducted a field experiment in Egypt's Western Sinai Peninsula found that dual-purpose barley can produce high-quality forage and acceptable grain yields in marginal arid regions. The study found that early sowing in late October yielded higher forage yields than

intermediate sowing in mid-November and late sowing in early December. Some genotypes performed better, suggesting higher adaptation capacity. The study concluded that dual-purpose barley is favorable for grain and forage production in similar environments.

Maniruzzaman *et al*. (2018) conducted a study to Evaluation of yield stability of seven barley (Hordeum vulgare L.) genotypes in multiple environments using GGE biplot and AMMI model. The GGE biplot and AMMI model are effective methods for evaluating genotypes for sustainable barley production and climate change. An experiment was conducted in Bangladesh to analyze seven barley genotypes across three different environmental conditions. After two years, all genotypes were found to be highly significant due to environment variation, genotypic variability, and interaction. The first two principal component axes contributed to 89.65% of the total GE interaction. Locations Jamalpur, Ishurdi, and Gazipur were in two sectors. Ishurdi was the best location for all genotypes, with Gazipur and Jamalpur unfavorable. 'E7' performed best for average grain yield, followed by 'E3', 'E2', and 'E4'. E2 had the highest yield in Jamalpur. Genotypes 'E3', 'E4', and 'E1' were more stable, and recommended for commercial cultivation in Bangladesh and South-Asia.

Laidig *et al*. (2017) analyzed 187 varieties tested in German trials from 1983 to 2015, focusing on environmental variability and trait association. Results showed a significant increase in grain yield by 43% in VCU trials and 35% on farm compared to 1983, with all components contributing significantly. Malting quality improved by 2.3% for extract content and 25.1% for friability, largely due to new varieties. The study found significant differences between phenotypic and genetic correlation coefficients for grain yield and protein concentration with malting traits.

Yusuf *et al*. (2016) worked out identifying of relationship between traits and grain yield in spring barley by GGE biplot analysis. The purpose of the study was to use GGE Biplot analysis to assess the association between grain yield and other characteristics of 25 spring barley genotypes in one site over the course of two years. Three replications and a fully randomized block design were used for the trials. It was discovered that the factors G, GE, and GEI had a highly significant ($P < 0.01$) impact on grain yield. Three groups of features were identified using the GGE Biplot: the first group included the following: thousand grain weight, protein content, crude cellulose, and cold damage; the second group included the following: hectoliter weight, lodging, plant height, and heading time; the third group included grain yield and seed humidity. Furthermore, the

investigation revealed a negative link between characteristics lacking seed humidity and grain production. The AMMI model and GGE Biplot results showed that while G2, G6, G19, and G1 are desirable origins for quality and other agronomic traits to choose for advance stage and use in barley breeding program, G12, G13, G16, and G18 are appropriate for grain yield.

KENDAL *et al*. (2016) evaluated the yield performance of 12 spring barley genotypes in multi-environment trials using AMMI analysis. Results showed that environments, GE, and genotypes significantly impacted grain yield. The analysis revealed that E3 and E5 were more stable and high-productive environments, while E6 and E7 were unstable and nominally efficient. The stability variance showed that genotypes G1, G3, G6, and G9 were more productive and stable, while G4 and G5 were low-productive and stable. G2 was the best productive in all environments without E2, and the G × E model recommended it as Kendal due to its wide adaptability and high performance in all environments.

2.6 Multi trait genotype ideotype distance (MGIDI) index

Ibrahim *et al*. (2023) found that wheat production is negatively impacted by multiple abiotic stresses, and to increase productivity by 60% by 2050, it is crucial to develop stress-tolerant genotypes. Multivariate analysis techniques, including the AMMI model, linear discriminant analysis, MGIDI, and WAASB index, were used to test twenty wheat genotypes under multiple abiotic stresses. G01, G12, G16, and G02 were selected as stable genotypes, and G01 is considered a novel genetic resource for improving productivity and stabilizing wheat programs under multiple abiotic stresses.

Dariush *et al*. (2023) carried out this study investigated genotype by environment interaction (GEI) in 18 sugar beet genotypes over two years. The additive effects analysis of the additive main effects and multiplicative interaction model revealed significant effects on root yield, white sugar yield, sugar content, and extraction efficiency. Stable genotypes with optimal performance were identified through biplot analysis. G3 and G4 were deemed suitable for RY and WSY, while G15 obtained high mean values for SC and ECS. The GGE biplot method classified environments into four and three mega-environments, with G15, G10, G6, and G1 being the most ideal genotypes.

Farhad *et al*. (2023) found on farmers in northern and central India prefer early wheat planting to take advantage of soil moisture and extend the time frame for spring wheat. A study at BISA in

2017 aimed to identify wheat lines suitable for early establishment by analyzing agro-morphological traits and genetic mapping of associated genes or quantitative trait loci (QTLs). Advancing the planting schedule by two-three weeks provided longer crop growth duration and reduced irrigation needs. The study found multiple QTLs linked to early adaptation in yield and contributing traits, including 44 novel QTLs and 44 novel QTLs.

Alireza *et al*. (2023) worked on Climate change is causing increasing temperatures and water deficit stress in tropical and subtropical regions, impacting barley, the fourth most important cereal crop. A study investigated 56 promising barley genotypes and four local varieties in four locations to identify high-yielding and adapted genotypes in Iran's warm climate. The analysis showed significant effects of genotypes, environments, and their interaction on traits. G18, G24, G29, and G57 were identified as desirable genotypes, while G6, G11, G22, G24, G29, G38, G52, and G57 were identified as superior genotypes. G24, G29, and G57 are well-adapted to warm Iran regions.

Tebra *et al*. (2023) carried out a study that found pearl millet, a cross-pollinated crop, was evaluated for breeding cultivars that adapt to drought conditions in southern Tunisia. The study evaluated grain yield and yield-related traits using 27 landraces. The results showed a wide range of variability and the possibility of genetic selection for advantageous traits. Broad sense heritability ranged from 24.10% for GY to 57.11% for spike length, with high genetic advance as a percentage of the mean. The phenotypic coefficient of variation was higher than the genotypic coefficient.

Ashok *et al*. (2023) aimed to estimate variance components, genetic parameters, inter-trait relations, and expected selection gains (SGs) across soil moisture regimes for 75 tropical pre-released maize hybrids. Twelve traits, including grain yield, were studied at two locations. Positive and negative SGs were estimated across moisture regimes, including drought, waterlogging, and optimal moisture conditions. Eleven genotypes were selected, with two hybrids, ZH161289 and ZH161303, common across all moisture regimes. The selected hybrids showed desired genetic gains, such as positive gains for grain yield and negative gains in flowering traits. The MGIDI was found to be a robust and easy-to-handle multi-trait selection process.

2.7. Molecular characterization of barley genotypes using SSR markers

Abebe *et al*. (2023) studied that breeders use genetic variability to select barley accessions. Given the germplasm's heterogeneity, Ethiopia's gene bank does not archive genetic information about barley landraces. Hence, 20 barley accessions comprising 194 plants were studied for genetic diversity. Accessions come from 10 Ethiopian regions at various heights. 15 SSR markers tested the accessions (SSRs). Barley plants' genetic diversity was shown by the SSR markers' 57 alleles, ranging from two (GBM1042) to seven (Bmac0040). Ethiopian barley accessions from mid- and low-altitudes had higher allelic diversity than high-altitude accessions. The biggest molecular variance was within accessions (64.39%) and altitude classes (64.04%). Geographic and altitude-based genetic structure study found two subpopulations. Altitude influences barley genetic diversity, as shown by individual assignment by collection altitude. This study reveals that each barley line among the accessions should be considered a possible genetic material source and treated separately during germplasm collection and use.

Degu *et al*. (2023) examined that Ethiopia's fifth-most-important food crop is barley. Barley genetics and population structure investigations are limited to gene bank collections. So, this study examines the selection, consumption, economic worth, genetic diversity, and population structure of farm-collected barley from the Gumer district of the Gurage Zone, which has been neglected. Semi-structured interviews and questionnaires collected barley use data in the research region. Population structure research showed Gumer barley landraces diverged from Japan and the US. US barley was the farthest from Gumer barley in principal component analysis. The markers' allele frequencies ranged from 0.10 to 0.50, averaging 0.28. The samples had substantial genetic diversity based on Nei's gene diversity (0.38) and polymorphism information content (0.30). Accessions were not clustered geographically. Because ethnic groups influenced Ethiopian barley selection and use, significant genetic variation necessitates further barley diversity research.

Gungor *et al*. (2022) recognizing that crop plant genetic structure and variety improves quantitative attributes. To assess population structure and genetic diversity, 54 barley cultivars issued from 1963 to the present by Turkish and Bulgarian institutes were screened with 18 iPBS and four SCoT markers. The results showed 530 polymorphic bands out of 560 total (438 and 92 amplified bands for iPBS and SCoT markers, respectively). Polymorphic band number averaged 24.09. The average polymorphism information content (PIC) value was 0.48 for iPBS and SCoT markers.

0.50 was the highest PIC. The iPBS2271 marker had the most effective number of alleles, Shannon's information index, and Nei's genetic diversity at 1.61, 0.52 and 0.35, respectively. The SCoT-71 marker had the highest values at 1.55, 0.32 and 0.48. Structure analysis of 530 amplified bands in 54 barley cultivars revealed a value of k=5 for the subpopulations. Average anticipated heterozygosity and fixation indices were 0.234 and 0.322. Martı and Zahir barley varieties had 75% genetic closeness, whereas Özdemir and Karatay 94 and Tosunpaşa and Konevi had 73%. Bayrak and Avcı-2002 have the maximum genetic diversity at 19.9%. Hence, barley cultivars released in Turkey and Bulgaria varied, and genetic diversity and statistical index analysis showed that iPBS and SCoT markers are powerful markers for genetic diversity study.

Marzougui (2021) examined that barley breeding operations need genetic dissimilarity and population structure data to conserve genetic diversity and generate new cultivars. SSR-type molecular markers were used to analyse Tunisian barley (*Hordeum vulgare* L.) genetic variation. 11 SSR markers had 30 alleles. The 16 barley accessions had 2 to 4 alleles per locus, averaging 2.7, and the average polymorphic information content (PIC) was 0.54. STRUCTURE programme detected 4 subpopulations with allele frequencies from 0.0119 to 0.0597 and fixation indices (Fst) from 0.29 to 0.43. Higher allelic frequencies differentiate subpopulation members. Barley collection genetic diversity analysis will aid cultivar development and genetic resource usage.

Ahmed *et al.* (2021) studied that Fertile Crescent barley (*Hordeum vulgare* L. subsp. vulgare) was one of the first domesticated crops. Germplasm conservation and breeding programmes benefit from primary gene pool and landrace genetic diversity studies. Eighty-two barley genotypes—22 Iranian improved cultivars and 60 landrace accessions from different regions of Iran—were used for genetic diversity analysis using three gene targeted markers, start codon targeted (SCoT) polymorphism, conserved DNA-derived polymorphism (CDDP), and CAAT box-derived polymorphism (CBDP) in comparison with sequence-related amplified polymorphism (SRAP) markers. 40 primers (10 from each marker) revealed barley genotype genetic polymorphism. SRAP markers had more polymorphic bands (67) than SCoT, CDDP, and CBDP markers. SCoT, CDDP, CBDP, and SRAP averaged 0.33, 0.37, 0.37, and 0.31 PIC values. SRAP and CBDP markers have higher MI than SCoT and CDDP. CDDP, CBDP, and SRAP markers clustered barley genotypes into three groups, while SCoT markers split them into four. Similarity matrices from each marker type correlated positively. Cluster analysis and STRUCTURE analysis using combined data sorted barley genotypes into three groupings that strongly matched their origins.

Genotypes from west and north-west Iran clustered apart from those from north-east and centre Iran. This is the first complete report of gene-targeted molecular marker genetic diversity analysis in barley. These markers were effective for barley genetic diversity study and genomic diversity and germplasm conservation.

Kashyap *et al*. (2020) studied that Ustilago hordei's genome was mined for microsatellite distribution and genetic variation. Using simple sequence repeats, 59 fungal isolates from two Indian agro-ecological zones were studied to determine U. hordei's genetic structure and variation (SSRs). Bioinformatic methods revealed 100,239 and 137,442 microsatellites from 20.13 and 26.94 Mb of assembled genomic sequences of U. hordei isolates Uh364 and Uh4857-4. Penta- and tri-nucleotides were the most common in both genomes. 15 polymorphic microsatellites with conservancies in both genomes were chosen to study U. hordei population genetics. With band sizes from 180 to 850 bp, microsatellite markers averaged two alleles. Polymorphic information content (PIC) was 0.095–0.37. UPGMA clustering and population structure analysis (K = 2) divided 59 isolates into two groups with 65% genetic similarity. The population has moderate gene flow (Nm = 1.009). AMOVA showed 87% genetic variation within groups and 13% between populations. Linkage disequilibrium (LD) analysis showed LD with epidemic population structure in both population groups (SIA = 0.181). In conclusion, the newly created neutral SSR markers are highly polymorphic within U. hordei and will help disclose evolutionary history, population dynamics in India, and CS of barley management techniques.

El-Awady *et al.* (2012) examined that KSA, which is losing biodiversity due to landrace replacement, knows nothing about barley landrace genetic diversity and morphological variability. A clear and reproducible band profile of 13 RAPD and 7 SSR primers was produced from 20 and 10 primers, respectively. 54.6 percent of 111 RAPD bands were polymorphic. 5-15 alleles per primer averaged 8.54. ISSR found 53 alleles, 16 of which (30.2%) were polymorphic. 5-10 alleles per SSR primer averaged 7.57. RAPD markers had a mean PIC of 0.45 and SSR markers 0.37. SSR detects barley landrace genetic diversity better than RAPD. RAPD and SSR results match, and SSR results are more accurate for the geographical distribution of the six barley landraces. This work may improve barley data for KSA national barley programmes.

CHAPTER III

MATERIALS AND METHODS

In the materials and methods section of this study, the detailed procedures and methodologies employed in the identification of high-yielding and stable barley genotypes across three locations and using molecular approaches are elucidated. This section serves as a roadmap, outlining the systematic framework utilized to conduct the research, including the selection of barley genotypes, experimental design, data collection methods, molecular techniques, and statistical analyses. By providing a clear and concise overview of the methodology, this section aims to ensure transparency, reproducibility, and reliability in the experimental procedures, ultimately facilitating the accurate interpretation of results and the validation of findings.

3.1 Location

The present study was carried out in three multiple environments of Bangladesh: (i) at the experimental field of the Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur (25.6987° N, 88.6562° E); (ii) at the Taraganj upazila, Rangpur (25° 48' 41.23"N, 89° 1' 3.95"E) and (iii) at the Debiganj upazila, Panchagarh (26°07′07″N, 88°45′33″E).

3.2 Environmental condition during growing season

The experimental site belongs to the Rabi season (December-April, 2022-23). During the growth period of this crop the atmospheric temperature was decreased as the rabi season proceed with occasional gusty winds.

3.3 Soil

In Dinajpur, the experimental field was a medium high land belonging to the non-calcarious dark gray floodplain soil under the agro-ecological zone (AEZ-1) of Old Himalayan piedmont plain. The soil is sandy loam under the order Inceptisol. The soil pH condition was 4.7. So, it denoted the acidic condition of soil. The Panchagarh field was a medium high land belonging to the non-calcarious dark gray floodplain soil under the agro-ecological zone (AEZ-1,3) of Old Himalayan piedmont plain and Tista Meander Floodplain. Its soil is sandy, alluvial and bears close affinity with the soil of the older Himalayan basin. The soil pH condition was 5.1-6.0. So, it denoted the

acidic condition of soil. In Rangpur, the experimental field was under the agro-ecological zone (AEZ-3) of Tista Meander Floodplain. The soil composition is mainly alluvial soil (80%) of the Teesta River basin and the remaining is barind soil. The soil pH condition was 5.5-6.5. So, it denoted the acidic condition of soil.

3.4 Experimental design and layout

The experiment was conducted in a Randomized Complete Block Design (RCBD) with three replications. Here, genotypes were assigned randomly in plot. The plot size is 3.75m². The row-to-row distance was 25 cm and the plant-to-plant distance was 15 cm. The inter block distance was 50 cm.

3.5 Plant Materials

In the research work, 9 barley genotypes were used. The six (6) barley genotypes collected from Germplasm Centre, Plant Breeding Institute (PBI), University of Sydney, Australia and three (3) check barley varieties were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. These genotypes and their origin are presented in Table 1.

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

SL No.	Genotype	Name	Origin
1	EEB_18	Chernishevka	Russia
2	EEB_91	Zero	Russia
3	EEB_114	Ciho 2632	Russia
4	EEB_152	31259a	Hungary
5	EEB_409	Auksinjai	Armenia
6	EEB_450	Atlas	Czech Republic
7	BARI_7	BARI Barley-7	Bangladesh
8	BARI_8	BARI Barley-8	Bangladesh
9	BARI_9	BARI Barley-9	Bangladesh

3.6 Land preparation

The land was first ploughed by two ploughing with a tractor mounted disc plough during first time. During second time, the land was ploughed by a power tiller. After a few days the land was further ploughed and cross ploughed with the country plough followed by laddering to get a good puddle condition. Weeds and stubbles were removed from the field prior to the sowing of seed. Manures and fertilizers were applied as per the recommended dose.

3.7 Sowing of seeds

The seeds of nine barley genotypes were sown between 3th - 10th December 2022 in Rangpur, Dinajpur and Panchagarh on experimental seed beds.

Dinajpur Panchagarh Rangpur

Figure 1: Sowing of seeds and land preparation

3.8 Fertilizer application

The following fertilizer, manure and other materials doses were applied in the field

Types of Fertilizer	**Recommended dose ha^{-1}**
DAP	156 kg
MoP	156 kg
Boron	104 kg
Monovit(sulphur)	10 kg
Furadan	10 kg

Here, 1/2 doses of nitrogen (N) & full doses of phosphorus (P), potassium (K), Sulphur (S) and zinc (Zn) was applied as basal during final land preparation & remaining ½ doses of the nitrogen was top dressed in two equal splits at 30-35 days after sowing (DAS) and 55-60 DAS.

3.9 Intercultural operations

After sowing care were taken against birds up to 15 days. For protecting the field from animal, netting was given. Some important intercultural operations were accomplished during the cropping period for the better growth and development of the plants.

3.9.1 Gap filling

The gap fillings were made where it was necessary.

3.9.2 Weeding

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

3.9.3 Irrigation

Three irrigations were given at the experimental plot. First irrigation was given lightly at 10 DAS for crown root initiation. Second irrigation was given at the hard drought stage of the control field only and adequate measures were taken to keep the pest and disease infestation to 29 a minimum level at 25 to 30 days after sowing. Third irrigation was given at panicle emergence at 65-75 days after sowing on the control field only. During irrigation, precautions were taken to remove the stagnancy of water.

3.9.4 Harvesting

The maturity of crops was determined when 80% of the barley seeds became physiologically mature. It should be harvested the right time to prevent from over ripening to avoid breaking of spikes. The crop was harvested and the yields of grain were recorded after thoroughly drying in sun.

3.9.5 Processing

Barley grain has a property of absorbing moisture from the atmosphere so the crop was stored in dry place after harvesting. Then the spikes were taken in an oven for proper drying. From oven the spikes were taken for threshing. Grains were collected to record the grain yield per plant.

3.10. Collection of data

The data on different yield and yield contributing characters were recorded considering each plant of the plots. Among the characters studied days to 50% flowering was recorded at different times from the plots. Here, five plants from each row were tagged after germination and the data were recorded on the following characters:

i) Plant height in cm (PH)

ii) Flag leaf length in cm (FLL)

iii) Chlorophyll content (CC)

iv) Spike length in cm (SL)

v) Number of spikes per plant (NSPP)

vi) Number of tillers per plant (NTPP)

vii) Number of effective tillers per plant (ETPP)

viii) Number of grains per spike (NGPS)

ix) Grain weight per spike in gram (GWPS)

x) Shoot dry weight in gram (SDW)

xi) Days to flowering (DF)

xii) Days to maturity (DM)

xiii) Thousand grain weight in gram (TGW)

xiv) Yield per plant in gram (YPP)

xv) Yield per plot in gram (YPPLOT)

3.11. Procedure of recording data on morpho-physiological traits

A brief outline of the data recording procedure is given below:

i) Plant height (cm)

Plant height was measured from the base of the plant to the top of the spikes with the help of measuring scale and was expressed as centimeter (cm).

ii) Flag leaf length (cm)

Flag leaf length measure from the tip of the entire leaf down to the base of the lowest leaflets where they meet the leaf stem for the leaf length measuring scale and was expressed as centimeter (cm).

iii) Chlorophyll content

This trait was measured in fully expanded sunlight flag leaves by using self-calibrating Minolta chlorophyll meter (Model: SPAD-505, Minolta Co. Ltd., Japan) and expressed as SPAD (Soil Plant Analyses Development) unit.

iv) Spike length (cm)

Spike length was measured from the base of the lowest spikelet to the top of the highest spikelet measuring slide caliper's and was expressed as centimeter (cm).

v) Number of spikes per plant

For recording the number of spikes per plant, the effective tiller which emerges the spikes that were counted from the individual plant at the reproductive growth stage.

vi) Number of tillers per plant

For recording the number of tillers per plant, the tiller which emerges the spikes or not that were counted from the individual plant at the reproductive growth stage.

v) Number of spikes per plant

For recording the number of spikes per plant, the effective tiller which emerges the spikes that were counted from the individual plant at the reproductive growth stage.

Figure 2: Measuring plant height, chlorophyll content and shoot dry weight

vi) Number of tillers per plant

For recording the number of tillers per plant, the tiller which emerges the spikes or not that were counted from the individual plant at the reproductive growth stage.

vii) Number of effective tillers per plant

For recording the number of effective tillers per plant, the tiller which emerges the spikes that were counted from the individual plant at the reproductive growth stage.

viii) Number of grains per spike

Number of grains in every spike of the individual plants are counted and averaged to measure number of grains per spike.

ix) Grain weight per spike (g)

After collecting number of seeds per spike, all seeds weight was weighted with an electric balance.

x) Shoot dry weight (g)

Shoot dry weight of individual plant was counted.

xi) Days to heading

Days to 50% flowering were calculated by counting the number of days from the date of sowing till the emergence of 50% spike (eye estimation).

xii) Days to maturity

Days to physiological maturity were calculated by counting the number of days from the date of sowing till 90% peduncle and spike become completely yellow.

xiii) Thousand grain weight (g)

After collecting number of thousand seeds from each genotype, thousand seed weight was weighted with an electric balance.

xiv) Yield per plant (g)

It was recorded as weight of clean dry seeds of each genotype harvested after full maturity and was expressed as grams.

xv) Yield per plot (g)

It was recorded as weight of clean dry seeds of each plot harvested after full maturity and was expressed as grams.

3.12 Statistical analysis

The data obtained for different characters were recorded first on MS excel sheet. Afterwards, the data were analyzed using the software R studio of version 4.3.2 (R Core Team 2023). Several parametric and nonparametric stability statistics were calculated using a web-based STABILITYSOFT program (Pour-Aboughadareh *et al*. 2019). The AMMI, GGE and MGIDI analyses was performed in R software 4.3.2 using package ‘metan’ (Olivoto and Lucio 2020).

3.12.1. Analysis of variance

The analysis of variance was computed using the software package R version 4.2.3 (R Core Team 2023). For the experiment of RCBD method, the structure of ANOVA was as follows:

The structure of ANOVA (two factor)

Source of variation	d.f.	M.S.S.	Expected values of M.S.S.
Replication (r)	r-1	M1	-
Genotypes (g)	g-1	M2	σ^2_e+ r. σ^2_g
Envionment (e)	t-1	M3	σ^2e+ r. σ^2_y
Genotype × Envionment	(r-1) (gy-1)	M4	σ^2e+ r. σ^2_{gy}
Error	(r-1) (g-1)	M5	σ^2e
Total	(rg-1)	M1 + M2 + M3+ M4+ M5	-

The structure of ANOVA (one factor)

Source of variation	d.f.	M.S.S.	Expected values of M.S.S.
Replication (r)	r-1	Mr	-
Genotypes (g)	g-1	Mg	σ^2_e+ r. σ^2_g
Error	(r-1) (g-1)	Me	σ^2e
Total	(rg-1)	Mr + Mg + Me	–

Where, r = number of replications

g = number of genotypes

e = environment

3.12.2 Estimation of genetic parameters

3.12.2.1 Genotypic and phenotypic variances

Genotypic and phenotypic variances were estimated based on method whether the genotype, 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

3.12.2.2 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.

3.12.2.3 Estimation of genetic advance

Estimation of genetic advance was done following formula given by Johnson *et al.* (1955) and Allard (1960).

$$\text{Genetic advance (GA)} = h^2_{b.}\, K.S_p$$

Where,

h^2_b = Heritability in broad sense;

K = Selection intensity which is equal to 2.06 at 5% level; and

S_p = Phenotypic standard deviation

3.12.2.4 Estimation of genetic advance in percentage of mean, GA (%)

Genetic advance in percent of mean was calculated by the formula of Comstock and Robinson (1952) as follows

$$\text{GA\%} = \frac{\text{GA}}{\text{X}}$$

Where,

GA = Genetic advance; and

x = Population mean

3.12.3 Estimation of correlation co-efficient

Simple correlation co-efficient (r) among 11 important barley characters were estimated with the following formula (Miller *et al.* 1958 and Singh and Chaudhary, 1985).

$$r = \frac{\sum xy - \frac{\sum x \sum y}{N}}{\sqrt{\{\sum X^2 - \frac{(\sum X)^2}{N}\}\{\sum X^2 - \frac{(\sum X)^2}{N}\}}}$$

Where,

Σ = Summation;

x and y = Two variables correlated; and

N =Number of observations

3.13. Stability analysis

In the context of agricultural research, assessing the stability and adaptability of crop genotypes across diverse environmental conditions is crucial for informing breeding programs and enhancing crop productivity. In this study, stability analysis serves as a pivotal component in the evaluation of high-yielding and stable barley genotypes. Various methods, including StabilitySoft, Additive Main effects and Multiplicative Interaction (AMMI), Genotype Plus Genotype by Environment (GGE), and Multi-Trait Genotype Ideotype Distance Index (MGIDI) analyses, are employed to comprehensively examine the performance and stability of barley genotypes across multiple locations. These diverse analytical approaches offer valuable insights into the genotype by environment interaction (GEI) and help identify genotypes with consistent performance across different environments. By integrating multiple stability analysis techniques, this study aims to provide a robust assessment of barley genotype stability, facilitating informed decision-making in barley breeding programs and contributing to sustainable agricultural practices.

3.13.1 Parametric and non-parametric stability statistics

StabilitySoft analysis is a online based software method used to assess the stability of yield and performance in response to changing conditions, management techniques, or genetic factors. It focuses on factors related to resilience, productivity, and crop development. Researchers use

statistical techniques like regression analysis to examine environmental variables' impacts on crop yield and assess crop types performance across different factors. Quantitative analysis helps researchers make informed decisions to improve productivity in crops.

Parametric measures

These parametric measures provide valuable insights into the stability of crop yields and help researchers to select stable and high-yielding agricultural productivity and resilience.

3.13.1.1 Regression coefficient (bi)

The regression coefficient (bi) is the response of the genotype to the environmental index that is derived from the average performance of all genotypes in each environment (Finlay and Wilkinson, 1963). If bi does not significantly differ from 1, then the genotype is adapted to all environments. A bi>1 indicates genotypes with higher sensitivity to environmental change and greater specificity of adaptability to high-yielding environments, whereas a bi<1 describes a measure of greater resistance to environmental change, thereby increasing the specificity of adaptability to low-yielding environments.

3.13.1.2 Deviation from regression (S^2di)

In addition to the regression coefficient, variance of deviations from the regression (S^2di) has been suggested as one of the most-used parameters for the selection of stable genotypes. Genotypes with an $S^2di = 0$ would be most stable, while an $S^2di > 0$ would indicate lower stability across all environments. Hence, genotypes with lower values are the most desirable (Eberhart and Russell, 1966).

3.13.1.3 Wricke's ecovalence stability index (Wi^2)

Wricke (1962) proposed the concept of ecovalence as the contribution of each genotype to the GEI sum of squares. The ecovalence (Wi) of the i th genotype is its interaction with the environments, squared and summed across environments. Thus, genotypes with low values have smaller deviations from the mean across environments and are more stable.

3.13.1.4 Environmental coefficient of variance (CVi)

The coefficient of variation is suggested by Francis and Kannenberg (1978) as a stability statistic through the combination of the coefficient of variation, mean yield and environmental variance. Genotypes with low CVi, low environmental variance (EV) and high mean yield are considered to be the most desirable.

Non-parametric measures

These non-parametric measures offer flexible and robust approaches to stability analysis in agricultural crop research, allowing breeders to assess stability without stringent distributional assumptions.

3.13.1.5 Nassar and Huhn's non-parametric statistics

Huhn (1990) and Nassar and Huhn (1987) suggested four non-parametric statistics. We use during this study two parameters: (1) S(1), the mean of the absolute rank differences of a genotype over all tested environments; (2) S(6), the sum of squares of rank for each genotype relative to the mean of ranks. The lowest value for each of these statistics reveals high stability for a certain genotype.

3.13.1.6 Thennarasu's non-parametric statistics

Four NP (1-4) statistics are a set of alternative nonparametric stability statistics defined by Thennarasu (1995). We use just two parameters (NP(2) and NP(4)). These parameters are based on the ranks of adjusted means of the genotypes in each environment. Low values of these statistics reflect high stability. The data were analyzed by the using of the online software (STABILITYSOFT) developed by Pour-Aboughadareh *et al*. (2019).

3.13.2 AMMI analysis

The results were processed by additive main effect and multiplicative interaction (AMMI) analysis, with each year by treatment combination being considered as an environment. The model is based on the number of axes of the main components and it is displayed graphically in the form of biplots. AMMI1 biplot is comprised from the main effects shown on the abscissa and the first principal component shown on the ordinate, while AMMI2 biplot illustrates the first (PC1) and second (PC2) principal component ratio (GAUCH, 2006; GAUCH *et al.*, 2008).

3.13.2.1 AMMI Stability Value (ASV)

The ASV is the distance from the coordinate point to the origin in a two-dimensional of IPCA1 scores against IPCA2 scores in the AMMI model (Purchase *et. al.*, 2000). Because the IPCA1 score contributes more to the GE interaction sum of square, a weighted value is needed. This weight is calculated for each genotype and each environment according to the relative contribution of IPCA1 to IPCA2 to the interaction SS as follows:

$$ASV_i = \sqrt{\left[\frac{SS_{IPCA1}}{SS_{IPCA2}}(IPCA1score)\right]^2 + (IPCA2score)^2}$$

Where, SS_{IPCA1}/SS_{IPCA2} is the weight given to the IPCA1-value by dividing the IPCA1sum of squares by the IPCA2 sum of squares.

The larger the IPCA score, either negative or positive, the more specifically adapted a genotype is to certain environments. Smaller IPCA score indicate more stable genotype across environments.

3.13.2.2 The Genotypic Stability Index

In the traditional AMMI model usage, when the proportion of the variance explained in IPCA1 is relatively low, there may be a biased interpretation regarding the stability of the genotypes using the AMMI1 biplot since GEI patterns are still explained in the remaining IPCA axis. To handle this problem, we propose a new stability index called WAASB, that is the Weighted Average of Absolute Scores from the singular value decomposition of the matrix of BLUPs for the GEI effects generated by an LMM, estimated as follows:

$$WAASB_i = \sum_{k=1}^{p} \left| IPCA_{ik} \times EP_k \right| \Big/ \sum_{k=1}^{p} EP_k$$

Where, $WAASB_i$ is the weighted average of absolute scores of the i^{th} genotype (or environment); $IPCA_{ik}$ is the score of the i^{th} genotype (or environment) in the k^{th} IPCA, and EP_K is the amount of the variance explained by the *k*th IPCA. The genotype with the lowest WAASB value is considered the most stable, that is, the one that deviates least from the average performance across environments. Aiming at identifying highly productive and stable genotypes, we propose swapping the well-known AMMI1 biplot by a biplot with the abscissa represented by the WAASB values

and the ordinate by the response variable. This biplot has the advantage of using all the estimated IPCA axes to identify the stability in a bi-dimensional plot. To identify whether and how the ranks of genotype are altered when different numbers of IPCA are used in the WAASB estimation, the genotype's ranks were obtained considering the WAASB estimated with 1, 2,..., p IPCA. When using only one IPCA, WAASB = |IPCA1|. The ranking was increasing; so, the genotype with the smallest WAASB value had the first-order rank. A heatmap graph was used to show the ranks of the genotypes in the different scenarios of WAASB estimation.

3.13.2.3 A Superiority Index that Allows Weighting between Performance and Stability

To select genotypes that combine high performance and stability we introduced the WAASBY index, which is a superiority index that allows weighting between performance (in our example, GY) and stability (WAAS index). The first step is rescaling both GY and WAASB to 0 to 100 so that they can be directly compared. Since the best values for GY is the maximum value and for WAASB is the lowest value, the transformations were performed according to the following equations:

$$rG_i = \frac{100-0}{G_{\max}-G_{\min}} \times (G_i - G_{\max}) + 100$$

and

$$rW_i = \frac{0-100}{W_{\max}-W_{\min}} \times (W_i - W_{\max}) + 0$$

where rG_i and rW_i are the rescaled values for GY and WAAS, respectively, for the i^{th} genotype; G_i and W_i are the response variable (GY) and the WAAS values for i^{th} genotype. Then the WAASBY index was calculated according to:

$$\text{WAASBY}_i = \frac{(rG_i \times \theta_Y) + (rW_i \times \theta_S)}{\theta_Y + \theta_S}$$

where, WAASBY_i is the superiority index for the i^{th} genotype that weights between performance and stability, and θ_Y and θ_S are the weights for response variable and stability assumed to be 65 and 35 in this study, respectively. In addition, 21 scenarios varying θ_Y and θ_S (100/0, 95/5, 90/10,...,

0/100) were planned. For each scenario, the first-order rank was attributed to the genotype with the highest WAASBY value. The objective here is to show how the ranking of genotypes is altered depending on the weight assigned to the stability and response variable. To assist with intuitive interpretation, a heat map graph was produced.

3.13.2.4 Relationship between Stability Measures

In this section the indexes WAAS and WAASY (considering a fixed-effect model), and the indexes WAAS and WAASBY (considering a mixed-effect model) were compared in terms of genotypes' ranking with the following five AMMI derived stability indexes, namely: (i) absolute values of the first principal component axis, (ii) AMMI stability value (Purchase *et al*., 2000) (iii) sums of the absolute value of the IPCA scores, and (iv) averages of the squared eigenvector values described by Sneller *et al.* (1997), where P is the number of IPCA retained via F tests; and (v) the absolute value of the relative contribution of IPCAs to the interaction (Zali *et al.*, 2012).

3.13.3 Genotype Environment Interaction

To explain the G×E interaction, the multivariate stability analysis was performed graphically based on GGE biplot and AMMI using R studio (a simplified version of R statistical software) developed by the R Core Team. The GUI package of R studio was used for GGE biplots while the Agricole package was used for AMMI36, involving two concepts, the biplot concepts (Gabriel *et al*., 1971 and Yan *et al*., 2007) and the GGE concept (Yan *et at*., 2000). The GGE biplots and AMMI are graphical images to exemplify G×E interaction and genotype ranking based on mean and stability. The graph generated is based on multi environment evaluation (which-won-where pattern), Genotype evaluation (mean versus stability), and tested environment raking (discriminative versus representative). The ranking of genotypes was allocated in increasing order of each stability parameter. The biplots were based on singular-value partitioning=2, transformed (transform=0), environment-centered (centering=2), and standard deviation-standardized (scaling=0).

3.13.4.1 Multi-trait genotype ideotype distance (MGIDI) Index

The ideotype has the highest rescaled value (100) for all the analyzed desirable traits. Thus, the ideotype can be defined by a l × p vector I such that I = [100,100 . . . ,100]. The fourth step of the analysis is to estimate the multi-trait stability index (Olivoto *et al*. 2019) using the Equation

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

Where D_{ij} is the distance between the ith ith genotype (row) and the ID for the jth factor. Low contributions of a factor specify that the traits within that factor are similar to the ideotype designed.

Where the MTSI is the multi-trait stability index for the ith genotype, F_{ij} is the jth score of the ith genotype, and F_j is the jth score of ideotype. The genotype with the lowest MTSI is then closer to

$$\text{MSTI}_\text{i} = \left[\sum_{j=1}^{f} \left(F_{ij} - F_j\right)^2\right]^{0.5},$$

the ideotype and, therefore, presents a high mean performance and stability (MPE) for all variables analyzed. The functions WAAS and MTSI of the "metan" package (Olivoto *et al*. 2020) were used to compute the MTSI index.

3.13.4.2 Mean Performance of Multiple Traits within Environments

The multi-trait genotype-ideotype distance index (MGIDI) proposed by Olivoto and Nardino (2020) was used to identify genotypes that perform well for most of the traits within each environment. The MGIDI has the same theoretical foundation as the MTSI (rescaling the trait, computing the factor analysis, and the distance from each genotype to the ideotype), with a key difference that the re-scaled matrix used to compute the factor analysis in the MGIDI is obtained with the BLUP for genotype (mean performance), rather than the WAASBY (mean performance and stability), in the MTSI. It is important to note that, if the weight for the mean performance (θY) in the MTSI is 100 for all traits under study, the genotype classification by the MTSI will become identical to the MGIDI since stability will not take into account. The genotype with the lowest MGIDI is then closer to the ideotype representing desired values for all the assessed traits within each environment. The proportion of the MGIDI index of the ith genotype explained by the jth factor (ωij) was used to show the strengths and weaknesses of genotypes within each environment, and it was computed as:

where d_{ij}^2 is the distance between the i^{th} genotype and the ideotype for the j^{th} factor. Factor with low contributions represents that the traits will be closer to ideotype. The functions gamem and MGIDI of the "metan" package (Olivoto et al. 2020) were used to compute the MGIDI index.

3.13.4.3 Estimation of Selection Differential Heritability

Selection Differential (S) It is computed as the difference between the mean of the selected parents (PS) and mean of the population before selection (PO) and is symbolized as (S). It is a measure of the phenotypic superiority of the selected parents over the population from which the parents were selected (Falconer et al. 1996).

S = mean of PS—means of PO.

3.14 Experiment-2. Molecular characterization and diversity analysis in barley using SSR markers

3.14.1 Genomic DNA Isolation

The 2-4 pieces of young leaves were collected in Eppendorf tube and were dried for 6-7 days in silica gel. Young leaves are 21 days older seedling stage. 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 spent five minutes at room temperature after being removed from the water bath. Isoamyl alcohol in 600 µl of chloroform 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 10,000 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 10,000 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.

3.14.2 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).

3.14.3 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 µLof 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.

3.14.4 Microsatellite/Simple Sequences Repeat (SSR) Markers

A total of 7 microsatellite (SSR) markers primer pairs (sigma Aldrich, Germany) covering all 10 chromosomes were selected for the genetic diversity analysis of the 9 Eastern European barley genotypes of as showed in the Table 2. 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 chromosomal location, annealing temperature and primer sequences to SSR markers are shown in Table 2.

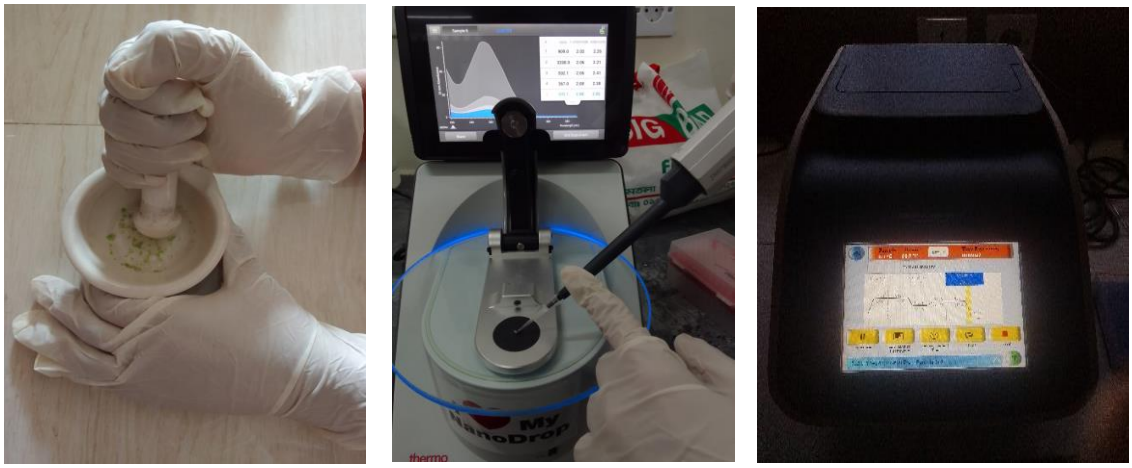


Figure 3: Leaf extraction, measuring concentration and amplification of polymerase chain reaction

(a) (b)

Figure 4: Polyacrylamide gel electrophoresis (a) and gel washing (b)

Table 2. SSR markers used for molecular characterization in barley genotypes

SSR Loci	Forward primer(5′–3′)	Reverse primer (5′–3′)	Annealing temperature (degree centigrade)
BMAG808	TCATAGACTACGACGAAGATG	TCATAGACTACGACGAAGATG	52
Bmag125	AATTAGCGAGAACAAAATCAC	AGATAACGATGCACCACC	52
Bmag006	TTAAACCCCCCCCTCTAG	TGCAGTTACTATCGCTGATTTAG	50
Scssr25691	ACGAGCTGATATCCCACGAG	TCCGAGCTTCTTATCTTTGG	55
Bmac0113	TCAAAAGCCGGTCTAATGCT	GTGCAAAGAAAATGCACAGATAG	54
GMS027	CTTTTTCTTTGACGATGCACC	TGAGTTTGTGAGAACTGGATGG	55
Bmag337	ACAAAGAGGGAGTAGTACGC	GACCCATGATATATGAAGATCA	55

3.14.5 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.

Model-based clustering program STRUCTURE V2.3.4 (Pritchard *et al.* 2000) was employed to deduce the population structure of all the 9 accessions. Number of populations (K) was determined with a burn-in period of 100000 and Markov Chine Monte Carlo of 100000. Five independent runs were performed for each K varying from 1 to 10. In this model, several subpopulations (K) were assumed to be present, each of which was characterized by a set of allele frequencies for each locus. Individuals in the sample were assigned to subpopulations or jointly to two or more subpopulations if their genotypes were admixed. The model choice criterion to detect the most likely value of K was ΔK, an ad hoc quantity related to the second-order change in the log probability of data (LnP[D]) with respect to the number of clusters inferred by Structure (Evanno *et al.* 2005). An individual was assigned to a subpopulation group if >80% of its genome fraction value was derived from that group. Most probable K-value was defined based on DK method (Evanno *et al.* 2005) by running the Structure Harvester software (Earl and von Holdt 2012). Every band was considered as a single locus. All the scorable Loci were considered for generation of bivariate 1-0 data matrix and for estimation of genetic diversity dendrogram was constructed using the software Metabo Analyst (Online Version) (Chong and Xia, 2018).

CHAPTER IV
RESULTS AND DISCUSSION

In the results and discussion section of this thesis, the culmination of extensive research and analysis on the identification of high-yielding and stable barley genotypes using molecular approaches is presented and deliberated upon. This section serves as the platform for the interpretation and synthesis of empirical findings derived from the experimental procedures outlined in the materials and methods section. The results encompass a comprehensive evaluation of barley genotype performance, stability, and adaptability across multiple locations, shedding light on the efficacy of different analytical methodologies employed. Through rigorous statistical analyses and comparison with existing literature, the discussion delves into the implications of the findings, elucidating the significance of identified genotypes, their molecular characteristics, and their potential impact on barley breeding programs and agricultural sustainability. By integrating empirical evidence with theoretical insights, this section aims to provide a nuanced understanding of the research outcomes, fostering discourse and generating valuable insights for the advancement of barley cultivation practices and crop improvement strategies.

4.1 Analysis of variance among different quantitative characters in barley

The analysis of variance for 15 morpho-physiological contributing characters of 9 barley genotypes were recorded and presented in table 3. Analysis of variances showed highly significant differences among the genotypes for all the characters including yield. Analysis of variance was also calculated combinedly with three environment (Table 3). The interaction effects of different between environment were also found significant for all the characters. In table 3, the sources of variation included genotype, replication, error and CV% for individual environments. The trends of variations among the genotypes were more or less similar in all environments, which could be observed by the CV values (Table 3). The analysis of variances revealed a highly significant difference among the genotypes for all traits viz. plant height (cm) (PH), Flag leaf length (cm) (FLL), chlorophyll content (CC), spike length (cm) (SL), number of spike per plant (NSPP), number of tillers per plant (NTPP), number of spike per plant (NSPP), number of Effective tillers per plant (ETPP), number of grain per spike (NGPS), Grain weight per spike (GWPS), shoot dry

weight (gm) (SDW), days to fifty percent flowering (DFF), days to maturity (DM), thousand-grain weight (g) (TGW), yield per plant (g) (YPP) and yield per plot (YPPLOT). The genotypes studied all were significant at 0.1% level of probability. The CV values which ranged from 1.08% for yield per plot in Panchagarh environment to 15.68% for number of spikes per plant in Rangpur environment.

Angassa & Mohammad (2022) studied that the analysis of variance was performed for plant height, thousand-grain weight, number of grains per spike, number of spikes per plant, days to maturity of barley and the treatment effect was also highly significant for all the studied trait. Karkee *et al*. (2020) reported that the analysis of variance was performed for days to flowering and maturity, plant height, number of grains per spike, and 1000 kernel weight in barley crop. The interaction between genotype and treatment revealed also highly significant differences for all the studied traits. Similar results were reported by Iyem *et al*., (2021) and Chaudhary *et al*., (2020).

Table 3. Analysis of variance of 14 important characters of barley genotypes under three environmental conditions

Characters	Environment	Source of variation with Mean sum square			
		Genotype	Replication	Error	CV%
PH	Dinajpur	150.83***	1.75	4.80	2.16
	Rangpur	418.14***	8.49	16.81	4.52
	Panchagarh	380.17***	0.20	0.89	0.91
SL	Dinajpur	6.21***	1.66	0.31	5.98
	Rangpur	4.84***	0.37	0.14	4.48
	Panchagarh	5.19***	0.02	0.51	8.45
CC	Dinajpur	21.71***	0.55	1.27	2.56
	Rangpur	99.40***	6.75	1.74	2.78
	Panchagarh	46.46**	6.6	11.02	7.89
FLL	Dinajpur	0.44***	9.47	0.74	6.52
	Rangpur	1.66***	19.32	1.53	8.00
	Panchagarh	3.69*	1.77	1.04	6.96
NTPP	Dinajpur	47.41***	3.02	6.75	11.26
	Rangpur	198.49***	17.45	11.72	14.37
	Panchagarh	46.57***	22.60	3.79	8.07
NSPP	Dinajpur	38.08***	1.21	4.758	11.93
	Rangpur	98.96***	5.25	6.1659	15.68
	Panchagarh	23.92**	23.92	4.1881	9.87
NGPS	Dinajpur	1268.01***	4.14	1.26	2.55
	Rangpur	538.394***	2.52	0.888	2.58
	Panchagarh	397.746***	2.12	4.187	4.35
GWPS	Dinajpur	279.60***	27.43	8.260	7.01
	Rangpur	147.46***	0.36	0.962	4.14
	Panchagarh	153.74***	4.04	7.907	6.79
SDW	Dinajpur	165.92***	8.10	5.874	7.30
	Rangpur	853.35***	5.84	3.333	5.12
	Panchagarh	991.33***	1.73	3.610	4.36
DFF	Dinajpur	124.51***	0.48	2.981	2.48
	Rangpur	123.98***	0.48	1.981	1.99
	Panchagarh	116.83***	1.44	1.903	1.97
DM	Dinajpur	210.59***	1.44	3.07	1.59
	Rangpur	0.44***	210.91	2.61	1.46
	Panchagarh	219.93***	3.59	2.05	1.29
TGW	Dinajpur	149.49***	6.61	9.64	7.98
	Rangpur	129.12***	4.61	12.18	9.30
	Panchagarh	149.49***	6.61	9.64	7.98
YPP	Dinajpur	564.81***	20.14	5.26	9.28
	Rangpur	401.76***	17.97	8.71	11.38
	Panchagarh	468.05***	13.26	7.41	8.99
YPPLOT	Dinajpur	11788.0***	372.6	368.3	16.24
	Rangpur	4931.64***	12.62	3.52	1.51
	Panchagarh	8239.17***	0.07	2.91	1.08

Legends, CV (%) = Coefficient of variation (%), ***, ** and * = significant at 0.1%, 1% and 5% levels of probability, respectively and NS = non-significant respectively. Here, PH= plant height, FLL= flag leaf length, CC= chlorophyll content, SL= spike length, NTPP= number of tillers per plant, ETPP= effective tillers per plant, NGPS= number of grains per spike, GWPS= grain weight per spike, SDW= shoot dry weight, DFF= days to flowering, DM= days to maturity, TGW = thousand grain weight, YPP= yield per plant and YPPLOT= yield per plot.

4.2 Environment comparisons of 9 barley genotypes on 14 morpho-physiological characters

The differences between the three environmental conditions on different yield-related parameters were shown in heat maps (Figure 5). The function is used to predict the outcome variable of a two-way table according to the AMMI model, judging by the number of multiplicative terms used, which regression plots were made with predicted value lines i.e. Dinajpur, Panchagarh and Rangpur condition with average value that was indicated in different characters.

Figure 5 presents the plotting of the genotypes' performance comparing the studied values of the indices. Fourteen traits (PH, FLL, SL, CC, NTPP, ETPP, NGPS, GWPS, DM, DFF, TGW, SDW, YPP and YPPLOT) showed clear differences between the mean value and average value.

The highest value was observed in genotype EEB 114 in the plant height (PH) trait; maximum difference between EEB 18, BARI Barley-8 and BARI Barley-9. Minimum difference between EEB 450, EEB 91 genotypes. For flag leaf length (FLL), EEB 18 and EEB 114 were highest mean value and there was not show any difference. In ETPP, EEB 450 was highest mean value and BARI Barley-8 indicated highest number of grains per spike (NGPS).

In, Figure 5. plant height (PH) was higher in the Panchagarh than the Dinajpur and Rangpur environment. Also, days to maturity (DM), Number of spikse per plant (NSPP), effective tillers per plant (ETPP), yield per plant (YPP) and yield per plot were contained comparatively higher mean values in Panchagarh than Dinajpur and Rangpur environment. This indicated that the time required for maturity, plant height, number of spikes per plant, effective tillers per plant, yield per plant and yield per plot most of the plant in optimum temperature were higher than others environment (Figure 5). Days to flowering also considered as a decisive stage for improving yield and yield components (Cuesta-Marcos *et al.,* 2009; Pasam *et al.,* 2012). Fine adjustment of flowering date is important for understanding other developmental traits such as plant height, tillering and grain number (Alquadah *et al.,* 2016).

Chlorophyll content (CC) and flag leaf length (FLL) were contributed comparatively higher values in Rangpur condition (Figure 5.). But spike length (SL) and number of grains per spike (NGPS) were higher in Dinajpur than Rangpur and Panchagarh optimum environmental condition. Some characteristics like leaf chlorophyll content and canopy temperature depression may be correlated with field performance, especially under drought stress (Jin *et al.,* 2012; Wu *et al.,* 2015). Shoot

dry weight (SDW) were equal in Panchagarh and Rangpur and greater than Dinajpur. Thousand grain weight (TGW) and grain weight per spike (GWPS) were equal in Panchagarh and Dinajpur greater than Rangpur. Number of tillers per plant (NTPP) and days to fifty percent maturity (DFF) were shown comparatively lower values in Panchagarh condition than Dinajpur and Rangpur condition.

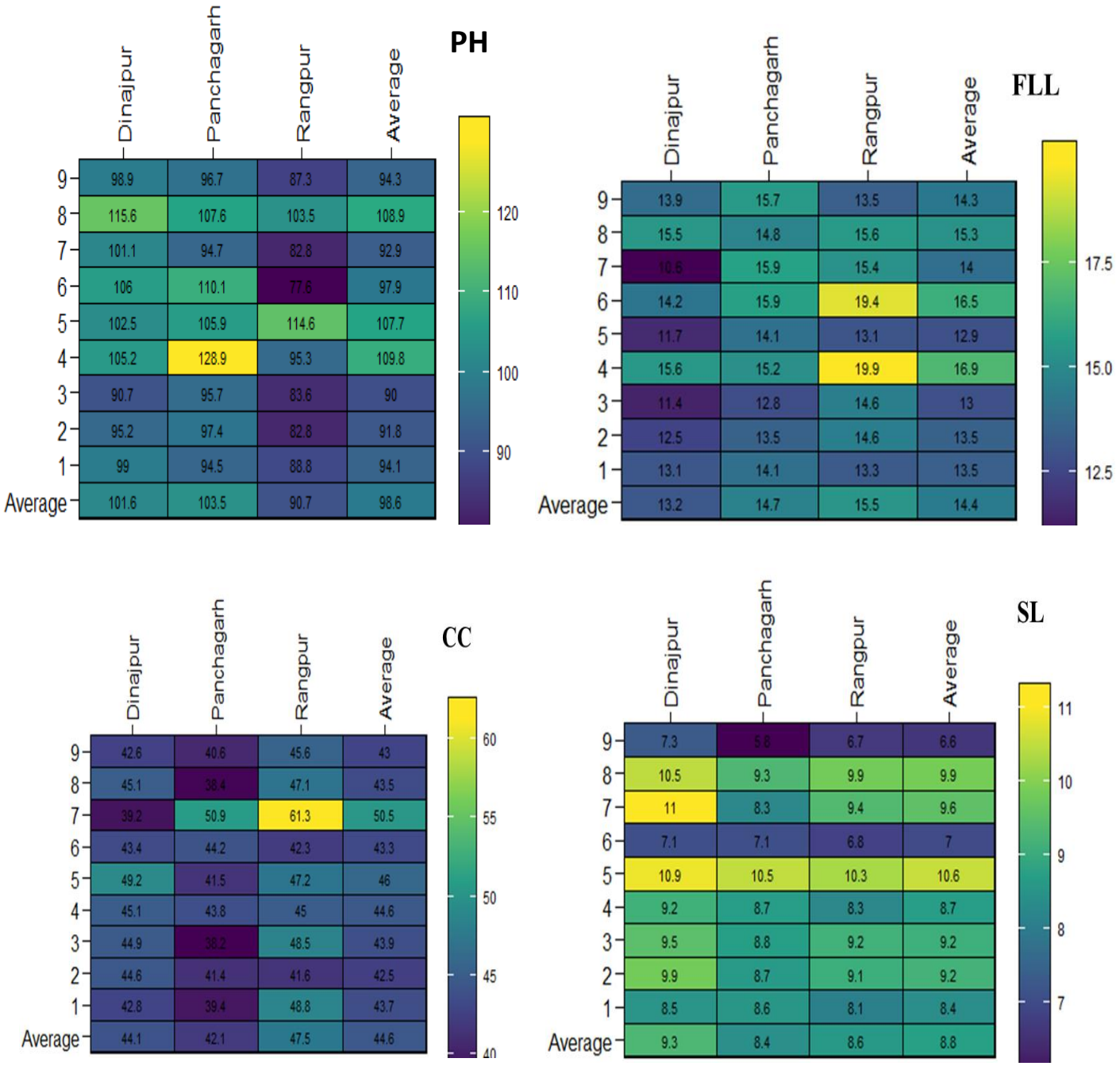


Figure 5: Heat map showing the environmental comparison between three environments for plant height (PH), flag leaf length (FLL), Chlorophyll content (CC) and spike length (SL); here, 1= BARI Barley-7,2= BARI Barley-8, 3= BARI Barley-9, 4= EEB_18, 5 = EEB_ 91, 6= EEB_ 114, 7= EEB_ 152, 8= EEB_ 409 and 9= EEB_ 450 (continued)

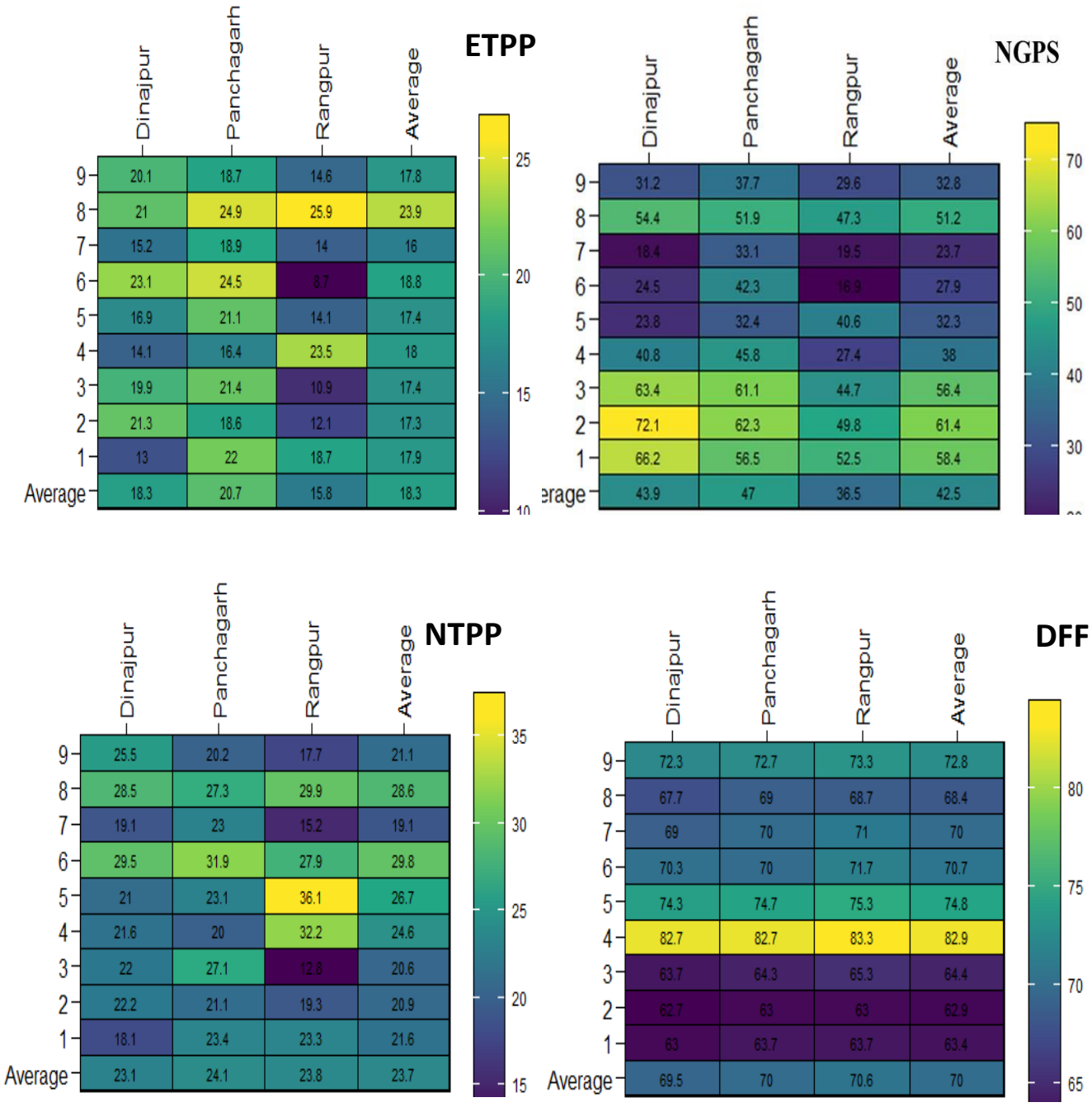
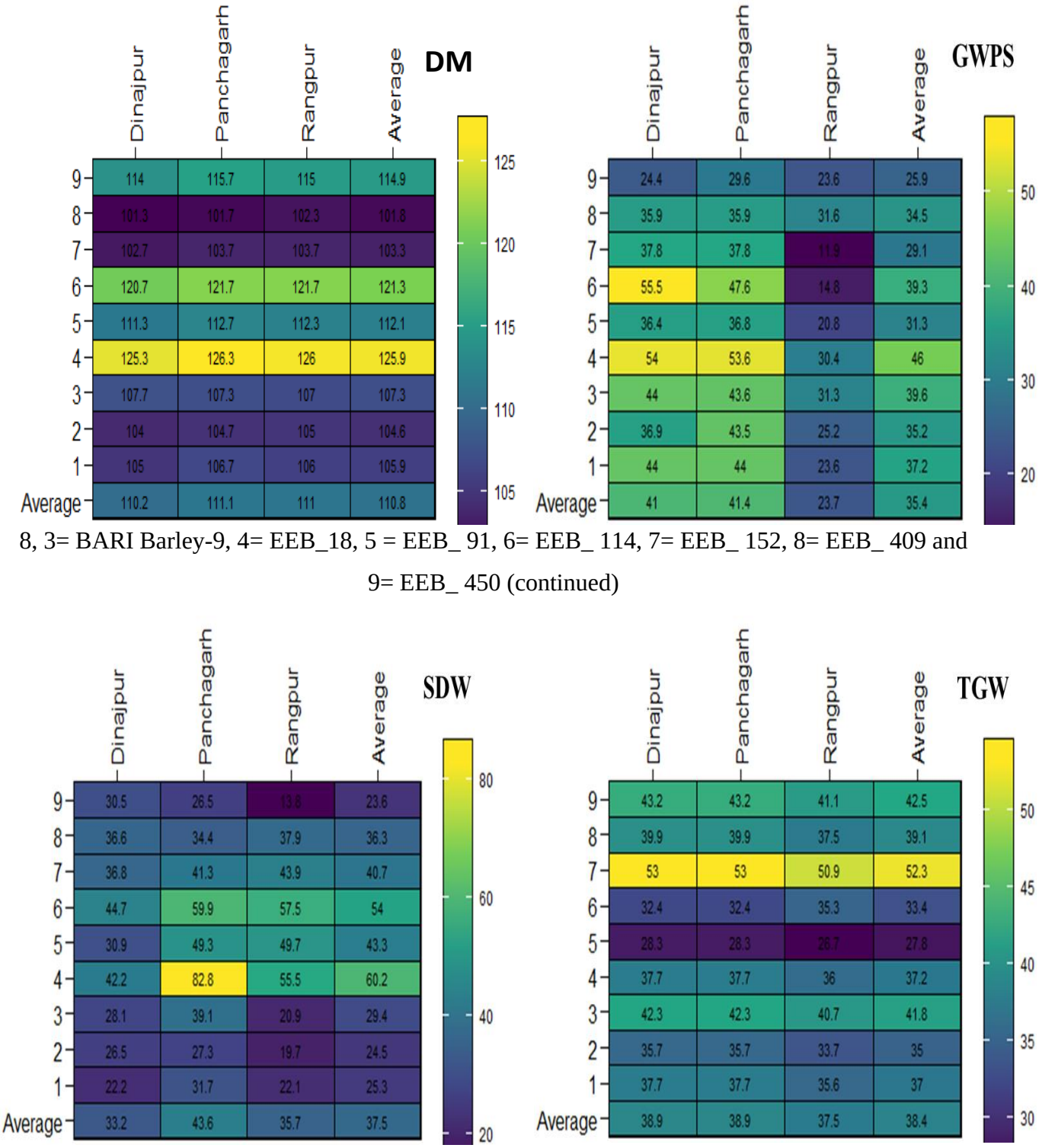


Figure 5: Heat map showing the environmental comparison between three environments for number of effective tillers per plant (ETPP), number of grains per spike (NGPS), number of tillers per plant (NTPP) and days to flowering (DFF); here, 1= BARI Barley-7,2= BARI Barley-



8, 3= BARI Barley-9, 4= EEB_18, 5 = EEB_ 91, 6= EEB_ 114, 7= EEB_ 152, 8= EEB_ 409 and 9= EEB_ 450 (continued)

Figure 5: Heat map showing the environmental comparison between three environments for days to maturity (DM), grain weight per spike (GWPS), shoot dry weight (SDW) and thousand grain

weight (TGW); ; here, 1= BARI Barley-7,2= BARI Barley-8, 3= BARI Barley-9, 4= EEB_18, 5 = EEB_ 91, 6= EEB_ 114, 7= EEB_ 152, 8= EEB_ 409 and 9= EEB_ 450 (continued)

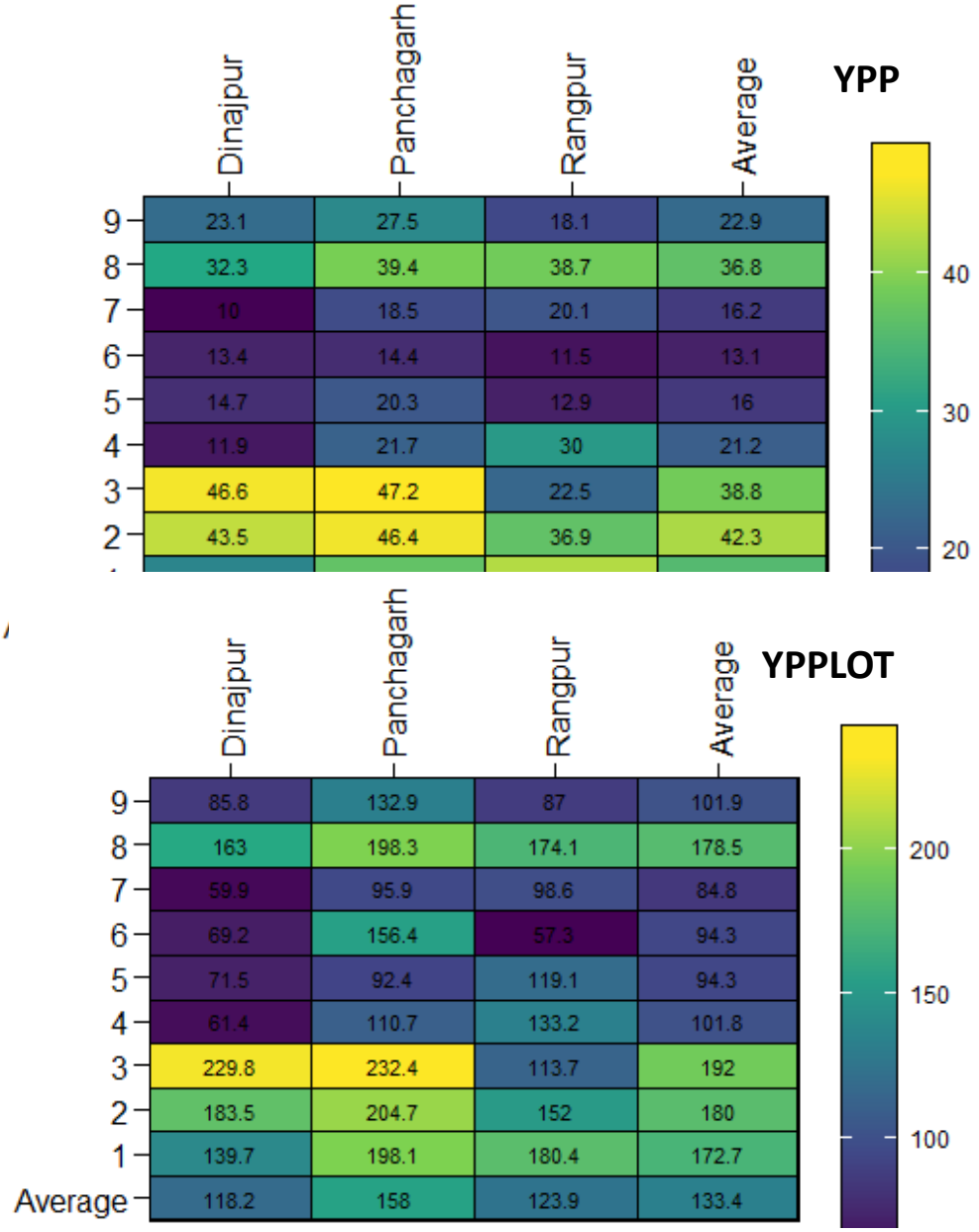


Figure 5: Heat map showing the environmental comparison between three environments for yield per plant (YPP) and yield per plot (YPPLOT); here, 1= BARI Barley-7,2= BARI Barley-8, 3= BARI Barley-9, 4= EEB_18, 5 = EEB_ 91, 6= EEB_ 114, 7= EEB_ 152, 8= EEB_ 409 and 9= EEB_ 450.

4.3 Mean performances of 9 barley genotypes for morpho-physiological characters

The mean performance on table 4 to 9, the grand mean and range value indicated that there was a wide range of variation among the studied genotypes for the quantitative traits.

4.3.1 Plant height (cm)

The variability of the traits found significant differences among the nine genotypes. The data on plant height (cm) of nine barley genotypes were recorded table 4. The highest plant height was 130.40 cm exhibited in EEB_114 at Panchagarh while the lowest plant height was 76.22 cm in EEB_18 at Rangpur. The mean ranges of plant height were 98.60 ± 1.27 with HSD value was 8.42. Mirosavljević *et al*. (2015) found the highest average plant height in genotype G12 and the lowest average plant height in genotype G17.

4.3.2 Flag leaf length (cm)

In response of flag leaf length, the highest flag leaf length 22.46 cm was measured in Rangpur EEB_114 and the lowest 10.00 cm was recorded in EEB_409 in Dinajpur. The flag leaf lengths of nine genotypes were statistically different from each other cultivars. The range of flag leaf length was 10.00 - 22.46 cm and HSD value was 3.19. The average mean was 14.44 cm. In barley, QTL underlying net photosynthetic rate has been analyzed in two DH populations (Wójcik-Jagła *et al*., 2013). According to Jiang *et al*. (2006) stomatal conductance significantly affected net photosynthetic rate, and is a key parameter to assess limitation of photosynthesis in barley. Rybiński *et al*. (2004) found significant linear relationship between transpiration rate and net photosynthetic rate in different irradiated times under laser light. However, the QTLs underlying stomatal conductance, intercellular CO_2 concentration and transpiration rate have not been reported in barley. 38 QTLs on chromosomes 1H, 2H, 3H, 4H, 6H, and 7H explained 6.53%-31.29% phenotypic variation, associated with net photosynthetic rate, stomatal conductance, flag leaf area, length, width, and chlorophyll content (Lipan *et al*., 2015).

4.3.3 Spike length (cm)

The spike length estimated at mature stage of the plant. The effect of different genotypes on spike length per plant were determined and presented in table 4. The mean value and HSD value were

1.99 ± 0.16 and 0.34, respectively. The range of spike length at mature stage was 5.50 - 11.67 cm. the highest spike length 11.67 cm was recorded in Dinajpur EEB_152 whereas the lowest 5.50 cm was found in Panchagarh EEB_91 genotypes. The data reveled significant difference from one another genotypes. As discussed by Sreenivasulu and Schnurbusch (2012), grain number enhancement can be theoretically obtained through modifications of the spike fertility and morphology. Due to the implications in the grain production and yield, the genetic dissection of the developmental plan of this storage sink is therefore of relevance when designing the cereal for the future.

Table 4: Performance of plant height (cm), flag leaf length (cm) and spike length (cm) for three different environments of nine barley genotypes

Genotype	PH			FLL			SL		
	Dinajpur	Rangpur	Panchagarh	Dinajpur	Rangpur	Panchagarh	Dinajpur	Rangpur	Panchagarh
BARI_9	90.73i-l	83.59lm	95.67g-k	11.43ef	14.59b-e	12.83b-f	9.52a-c	9.20a-d	8.76b-e
BARI_8	95.17g-k	82.78lm	97.43f-i	12.47c-f	14.59b-e	13.54b-f	9.87a-c	9.13a-d	8.65b-f
BARI_7	99.03e-i	88.78j-l	94.53h-k	13.13b-f	13.27b-f	14.05b-e	8.54b-f	8.10c-f	8.56b-f
EEB_450	115.60b	103.48c-g	107.57b-d	15.53bc	15.64bc	14.82b-d	10.49ab	9.86a-c	9.33a-c
EEB_409	101.13d-h	82.85lm	94.72h-k	10.63f	15.35bc	15.94b	11.02a	9.40a-c	8.27c-f
EEB_152	102.47c-h	114.56b	105.93c-e	11.67d-f	13.06b-f	14.09b-e	10.90a	10.31ab	10.51ab
EEB_114	105.17c-f	95.33 g-k	128.93a	15.57bc	19.89a	15.24bc	9.18a-d	8.28c-f	8.71b-e
EEB_91	98.93e-i	87.27kl	96.73g-j	13.93 b-e	13.45b-f	15.65bc	7.32d-g	5.84g	6.69fg
EEB_18	106.00c-e	77.64m	110.06bc	14.23b-e	19.43a	15.91b	7.12e-g	6.83e-g	7.12e-g
Mean ± SE	98.60 ± 1.27			14.44 ± 0.24			8.79 ± 0.16		
Range	76.22 - 130.40			10.00 - 22.46			5.50 - 11.67		
HSD	8.42			3.19			1.99		

Here, PH= plant height, FLL= flag leaf length, SL= spike length and HSD= Honest significant difference.

4.3.4 Chlorophyll content

Chlorophyll plays a vital role for photosynthesis. Photosynthesis rate depends on chlorophyll content (CC). The higher chlorophyll content, the more amounts of food storage and it will supply more energy. In three environmental conditions, the mean values of chlorophyll content giving a range 34.66 - 63.60 with mean value 44.55 ± 0.56 and the LSD value was 7.08. The maximum chlorophyll content was 63.60, revealed for the genotype EEB_409 in Rangpur and the minimum chlorophyll content exhibited in genotype BARI_7 in Panchagarh 34.66. Sidko *et al*. (2016) showed that the high efficiency of chlorophyll photosynthetic potential in combination with traditional spectrophotometric measurements to assess chlorophyll content of crops.

4.3.5 Days to fifty percent flowering

Days to 50% flowering varied from 61.00 - 85.00 where the earlier flowering 61 days was in BARI_7 in Dinajpur genotype. While the late flowering needed 85 days was in Panchagarh EEB_114 genotype. Regarding this trait, the 61 days and all nearest flowering genotypes were superior including the statistically similar resulted genotypes. The grand mean of this character was 70.04 ± 0.69 and HSD value was 2.96. Poudyal *et al*. (2019) observed the mean of days to flowering was 79 days. The days to flowering for normal sown condition was about 83 days and it was significantly higher than days to flowering for late sown condition (60 days).

4.3.6 Days to maturity

In three environmental conditions, the mean values of days to maturity having a range 99.0 - 127.0 with mean value 110.79. The maximum days required for maturity was exhibited in the Panchagarh EEB_114 genotype. The minimum days required for maturity was revealed for the genotype EEB_450 in Panchagarh. Adhikari *et al*. (2018) observed that days to 90% maturity from days to sowing ranged from 125.3 to 133 days. Early maturing types can select as an early type variety or can be used as parent to develop early maturing cultivars.

Table 5: Mean performance of 9 genotypes for yield and different yield contributing characters (chlorophyll content, days to fifty percent flowering and days to maturity) in barley (Contd.)

Genotype	CC			DFF			DM		
	Dinajpur	Rangpur	Panchagarh	Dinajpur	Rangpur	Panchagarh	Dinajpur	Rangpur	Panchagarh
BARI_9	44.89b-g	48.50b-d	38.27g	63.67k	65.33jk	64.33k	107.67f	107.00fg	107.33fg
BARI_8	44.67b-g	41.60d-g	41.35eg	62.67k	63.00k	63.00k	104.00h-k	105.00f-j	104.67g-j
BARI_7	42.84c-g	48.77bc	39.41fg	63.00k	63.67k	63.67k	105.00f-j	106.00f-i	106.67f-h
EEB_450	45.13b-g	47.10b-e	38.40g	67.67ij	68.67hi	69.00g-i	101.33k	102.33jk	101.67k
EEB_409	39.15fg	61.30a	50.97b	69.00g-i	71.00e-h	70.00f-i	102.67jk	103.67i-k	103.67i-k
EEB_152	49.23bc	47.20b-e	41.54d-g	74.33b-d	75.33b	74.67bc	111.33e	112.33de	112.67de
EEB_114	45.07b-g	45.03b-g	43.79c-g	82.67a	83.33a	82.67a	125.33a	126.00a	126.33a
EEB_91	42.63c-g	45.63bc-f	40.64e-g	72.33c-f	73.33b-e	72.67b-f	114.00c-e	115.00 cd	115.67c
EEB_18	43.43c-g	42.27c-g	44.24b-g	70.33f-i	71.67d-g	70.00f-i	120.67b	121.67b	121.67b
Mean ± SE	44.55 ± 0.56			70.04 ± 0.69			110.79 ± 0.90		
Range	34.66 - 63.60			61.00 - 85.00			99.0 - 127.0		
HSD	7.08			2.96			2.84		

Here, CC= chlorophyll content DFF= days to flowering, DM= days to maturity and HSD= Honest significant difference.

4.3.7 Number of spikes per plant

The number of spikes per plant was estimated after harvesting of plant. The highest spikes per plant 29.60 was found in Panchagarh for EEB_450 while the second highest number of spikelets 29.2 was found in Rangpur for genotype EEB_14. EEB_450 and EEB_14 genotypes were significantly higher and statistically similar. The lowest count, 7.60, was observed in Rangpur for genotype EEB_18. The range of spikes per plant varied 7.60 to 29.60. The data reveled significant differences among the genotypes.

4.3.8 Number of tillers per plant

Vinesh *et al*. (2018) observed the high mean number of tillers per unit area and low variance for number of tillers could be used in selecting varieties with consistently high yield at varying environments. The highest number of tillers per plant 38.00 was recorded in EEB_152 genotype while the lowest value 11.20 was found EEB_409 genotype Rangpur in both respectively. The range of tillers per plant and HSD were 11.20 - 38.00 and 10.34 respectively. The result revealed that EEB_152 genotype had higher number of tillers per plant over nine genotypes.

4.3.9 Number of effective tillers per plant

Number of effective tillers per plant is an important yield contributing traits. The highest number of effective tillers per plant 29.60 was recorded in Panchagarh EEB_450 genotype while the lowest value 7.60 was found in Rangpur EEB_18 genotype. The range of effective tillers per plant was 7.60 - 29.60. The result reveled that EEB_450 genotype produce higher number of effective tillers that ensure higher yield.

Table 6: performance of number of spikes per plant, number of tillers per plant and number of effective tillers per plant of nine barley genotypes in three different locations (contd.)

Genotype	NSPP			NTPP			ETPP		
	Dinajpur	Rangpur	Panchagarh	Dinajpur	Rangpur	Panchagarh	Dinajpur	Rangpur	Panchagarh
BARI_9	19.87a-h	10.87ij	21.40a-f	22.00b-i	12.80i	27.07a-g	19.87a-h	10.87ij	21.40a-f
BARI_8	21.27a-f	12.13h-j	18.60a-i	22.20b-i	19.33e-i	21.13d-i	21.27a-f	12.13h-j	18.60a-i
BARI_7	13.00g-j	18.73a-i	22.00a-e	18.13g-i	23.33b-h	23.40b-h	13.00g-j	18.73a-i	22.00a-e
EEB_450	21.00a-f	25.87a	24.87a	28.53a-f	29.87a-d	27.33a-g	21.00a-f	25.87a	24.87a
EEB_409	15.20d-j	14.00f-j	18.93a-h	19.07f-i	15.20hi	23.00b-i	15.20d-j	14.00f-j	18.93a-h
EEB_152	16.93b-i	14.13e-j	21.13a-f	21.00d-i	36.13a	23.07b-i	16.93b-i	14.13e-j	21.13a-f
EEB_114	14.13e-j	23.47a-c	16.40c-j	21.60c-i	32.20ab	20.00d-i	14.13e-j	23.47a-c	16.40c-j
EEB_91	20.13a-g	14.60e-j	18.73a-i	25.53b-h	17.67g-i	20.20d-i	20.13a-g	14.60e-j	18.73a-i
EEB_18	23.07a-d	8.73j	24.47ab	29.53a-e	27.93a-g	31.93a-c	23.07a-d	8.73j	24.47ab
Mean ± SE		18.28 ± 0.54			23.67 ± 0.66			18.28 ± 0.54	
Range		7.60 - 29.60			11.20 - 38.00			7.60 - 29.60	
HSD					10.34			7.91	

Here, NTPP= number of tillers per plant, ETPP= effective tillers per plant, NGPS= number of spikes per plant and HSD= Highest significant difference.

4.3.10 Number of grains per spike

The number of grains per spike revealed the ranges of mean were 16.20 - 72.80 with mean value 42.45. The maximum number of grains per spike were revealed for the genotype BARI_8 (72.80) and the minimum number of grains per spike were revealed for the genotype EEB_18 (16.2) followed by EEB_409 (12.25). Ahmadizadeh *et al*. (2013) reported that the range of number of grains per spike had between 49.73 (line 14) to 29.63 (line 15) in barely genotypes. Total means of number of grains per spike was 41.11 in 20 lines studied. The data reveled significant difference from one another genotypes.

4.3.11 Shoot dry weight

Shoot dry weight was estimated after harvesting of plant. The highest weight 85.80g was found in EEB_114 genotype which was statistically at EEB_18 genotype. The range of shoot dry weight was 12.80 - 85.80g. The lowest 12.80g was found in Rangpur EEB_91. The mean and HSD were 37.48 ± 1.66 and 6.37, respectively.

4.3.12 Grain weight per spike

Grain weight per spike was estimated after harvesting of plant. The highest weight 59.76 was found in Panchagarh EEB_114 whereas the second highest weight 59.60 was found in Dinajpur EEB_18 genotype. EEB_114 and EEB_18 genotypes were significantly higher and statistically similar. The lowest 10.88 was found in Rangpur EEB_409 genotype. The range of grain weight per spike was 10.88 - 59.76. The data reveled significant difference from one another genotypes. The HSD and mean value were 6.78 and 35.35 ± 1.27, respectively.

Table 7: performance of number of grains per spike, shoot dry weight and grain weight per spike of nine barley genotypes in three different locations (contd.)

Genotype	NGPS			SDW			GWPS		
	Dinajpur	Rangpur	Panchagarh	Dinajpur	Rangpur	Panchagarh	Dinajpur	Rangpur	Panchagarh
BARI_9	63.40bc	44.67i-k	1.13cd	28.13k-m	20.93no	39.13e-h	43.96cd	31.26g-j	43.62c-e
BARI_8	72.07a	49.80f-h	62.27bc	26.53l-n	19.67op	27.27l-n	36.87e-h	25.24j-m	43.54c-f
BARI_7	66.20b	52.47ef	56.47de	22.20m-o	22.13m-o	31.67i-l	43.99cd	23.60lm	43.99cd
EEB_450	54.40ef	47.30g-i	51.87e-g	36.60g-j	37.93f-i	34.40h-k	35.93g-i	31.57g-j	35.93g-i
EEB_409	18.40r	19.53qr	33.13mn	36.83g-j	43.87d-f	41.33e-g	37.77d-g	11.85o	37.77d-g
EEB_152	23.80pq	40.60kl	32.40n	30.87j-l	49.67cd	49.27cd	36.44gh	20.84mn	36.75f-h
EEB_114	40.80kl	27.40op	45.80h-j	42.20e-g	55.53bc	82.80a	53.99ab	30.40h-k	53.65ab
EEB_91	31.20no	29.60no	37.73lm	30.53j-l	13.80p	26.53l-n	24.42k-m	23.61lm	29.56i-l
EEB_18	24.53p	16.87r	42.33j-l	44.73de	57.53b	59.87b	55.50a	14.84no	47.63bc
Mean ± SE	42.45 ± 1.73			37.48 ± 1.66			35.35 ± 1.27		
Range	16.20 - 72.80			12.80 - 85.80			10.88 - 59.76		
HSD	4.88			6.37			6.78		

Here, NGPS= number of grains per spike, GWPS= grain weight per spike, SDW= shoot dry weight and HSD= highest significant difference.

4.3.13 Thousand grain weight

The mean ranges of thousand grain weight were 25.14 - 54.10 with mean value 38.45. The maximum number of thousand grain weight were revealed for the genotype EEB_409 (54.10) in Panchagarh and the minimum number of thousand grain weight were exhibited in the genotype EEB_152 (25.14) in Rangpur. The mean value and HSD were 4.72 and 3.02 respectively. Mirosavljević *et al*. (2015) found the highest thousand grain weight in genotypes G4, G10 and G19 while the lowest thousand grain weight was found in G18.

4.3.14 Yield per plant

In case of yield per plant, it was counted that the highest yield per plant 49.05g was found in BARI_8 in Rangpur and the lowest yield per plant EEB_152. The second yield per plant was 8.52g found in EEB_409. The mean range was 8.30 - 49.05g and average mean was 26.98. The HSD was 9.28. The data reveled significant difference from one another genotype.

4.3.15 Yield per plot

In three environmental conditions, the mean ranges of yield per plot were 56.64 - 233.05g with mean value 133.37g. Maximum values for yield per plot was revealed for the genotype BARI_9 (233.05g) in Panchagarh and the minimum yield per plot were exhibited in the genotype EEB_18 (57.32g) in Rangpur followed by EEB_152 (92.42g) in Dinajpur. The HSD was 35.63. Adhikari *et al*. (2018) observed that the genotypes COQ/K1/DESC11, MATICO"S" produced higher yield than other genotypes.

Table 8: performance of thousand grain weight, yield per plant and yield per plot of nine barley genotypes in three different locations

Genotype	TGW			YPP			YPPLOT		
	Dinajpur	Rangpur	Panchagarh	Dinajpur	Rangpur	Panchagarh	Dinajpur	Rangpur	Panchagarh
BARI_9	42.28bc	40.75b-e	42.28bc	46.61a	22.48f-j	47.20a	229.81a	113.72h-j	232.39a
BARI_8	35.67f-h	33.74gh	35.67f-h	43.54ab	36.93b-d	46.43a	183.47b-d	151.97d-g	204.67ab
BARI_7	37.67c-g	35.64f-h	37.67c-g	26.94e-h	42.74ab	37.03b-d	139.65e-h	180.39b-d	198.13a-c
EEB_450	39.88b-f	37.55d-g	39.88b-f	32.32c-e	38.69a-d	39.38a-c	163.03c-f	174.11b-e	198.28a-c
EEB_409	53.05a	50.89a	53.05a	10.05m	20.05g-l	18.52g-m	59.91m	98.60i-k	95.90j-l
EEB_152	28.33ij	26.67j	28.33ij	14.70i-m	12.88k-m	20.33g-l	71.55k-m	119.06g-j	92.42j-m
EEB_114	37.74c-g	36.05e-h	37.74c-g	11.94lm	30.04d-f	21.67f-k	61.39lm	133.21f-i	110.67h-j
EEB_91	43.22b	41.06b-d	43.22b	23.11e-i	18.06h-m	27.52e-g	85.78j-m	86.97j-m	132.91f-i
EEB_18	32.39hi	35.31f-h	32.39hi	13.38j-m	11.46lm	14.36i-m	69.24km	57.32m	156.40df
Mean ± SE		38.45 ± 0.79			26.98 ± 1.38			133.37 ± 5.97	
Range		25.14 - 54.10			8.30 - 49.05			56.64 - 233.05	
HSD		4.72			9.28			35.63	

Here, TGW= thousand grain weight, YPP= yield per plot and YPPLOT= yield per plot and HSD= Honest significant difference.

4.4 Estimation of Genetic parameters

Different parameters such as genotypic variance (σ^2g), phenotypic variance (σ^2p), environmental variance (σ^2e), genotypic coefficient of variation (GCV%), phenotypic coefficient of variation (PCV%), environmental coefficient of variation (ECV%), heritability (%), genetic advance (GA) and genetic advance as percent of mean (GAM%) of 14 characters were estimated to observe the variability existed among the characters. The results presented in (Table 9.) revealed that the phenotypic variances were higher than genotypic variances for all the characters.

Highest phenotypic and genotypic variances were recorded with yield per plot 4174.89 and 3806.56 in Dinajpur respectively. The low values of phenotypic and genotypic variances were observed with the characters spike length and flag leaf length (1.71 and 0.88) respectively and spike length 1.56 and flag leaf length 1.92 respectively. Highest environmental variances were recorded with yield per plot 1646.22 and the lowest value was recorded with spike length 0.14 in Rangpur.

The phenotypic coefficients of variance (PCV) were ranged from 7.19% for plant height to 72.04% for days to maturity, whereas genotypic coefficients of variance (GCV) were ranged from 6.40 % for flag leaf length to 55.22% for yield per plant in Panchagarh and Dinajpur, respectively. Vinesh *et al*. (2018) reported that the phenotypic coefficients of variation (PCV) values were higher than genotypic coefficients of variation (GCV) values for all the traits studied by them. Medium phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) were recorded for days to flowering, effective tillers per plant, spike number per plant, row type, growth habit and plant height. Medium phenotypic coefficients of variation (PCV) and low genotypic coefficients of variation (GCV) values were displayed for days to flowering, effective tillers per plant, spike number per plant, row type, growth habit and plant height. Low phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) values were recorded for days to maturity which suggests the limitation of selection for these traits. Fantahun *et al.* (2023) studied that phenotypic variance (σ^2p) and phenotypic coefficient variance (PCV) were higher than theirs corresponding genotypic variance (σ^2g) and genotypic coefficient of variance (GCV) respectively for all the characters studied, indicating that the expression of these characters was influenced by environment. Genotypic coefficient variance (GCV) were ranged from 6.01% for days to maturity to 91.64% for yield per plant. Vinesh *et al*. (2018) reported that

the phenotypic coefficients of variation (PCV) values were higher than genotypic coefficients of variation (GCV) values for all the traits studied by them. Medium phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) were recorded for days to flowering, effective tillers per plant, spike number per plant, row type, growth habit and plant height. Medium phenotypic coefficients of variation (PCV) and low genotypic coefficients of variation (GCV) values were displayed for days to flowering, effective tillers per plant, spike number per plant, row type, growth habit and plant height. Low phenotypic coefficients of variation (PCV) and genotypic coefficients of variation (GCV) values were recorded for days to maturity which suggests the limitation of selection for these traits. Fantahun *et al.* (2023) studied that phenotypic variance (δ^2p) and phenotypic coefficient variance (PCV) were higher than theirs corresponding genotypic variance (δ^2g) and genotypic coefficient of variance (GCV) respectively for all the characters studied, indicating that the expression of these characters was influenced by environment.

Heritability analysis estimates the relative contributions of differences in genetic and non-genetic factors to the total phenotypic variance in a population. It is an important concept in quantitative genetics, particularly in selective breeding. The heritability estimation varied from 45% to 99.99%, respectively for flag leaf length to plant height, yield per plot. Heritability for yield per plant was much higher (93-97%). All the characters revealed more than 50% heritability. The highest heritability (H^2b) was observed for grains per spike followed by yield/ plant, tiller number/plant, thousand grain weight but the lowest H^2b was identified days to flowering followed by shoot dry weight. Those traits with higher heritability may be considered for selection for further analysis. The result is supported by Matin *et al.* (2019) the high value of GA% was recorded with number of grains per spike (46.00) and the low (1.72) with growth habit.

Genetic advance (GA) had a general range between 1.31% for flag leaf length and followed by 121.36% for yield per plot. Genetic advance as percent mean expected (GAM) had a general range between 12.11% for chlorophyll content and followed by 112.19% for yield per plot. Among the characters high values of GAM (>20%) were recorded for all the characters except plant height, chlorophyll content, flag leaf length, effective tillers per plant, days to fifty percent flowering and days to maturity. Characters with a high genetic advance as a percent of mean allow the improvement of this character through selection (Vinesh *et al.* 2018). Heritability and the genetic

advance are also important selection parameters. It is more useful as a selection tool when considered jointly with heritability. Heritability coupled with high genetic advance was observed for characters biomass per plant, grain yield and number of tillers per plant indicating that selection for these characters could be more effective due to additive gene action (Addisu *et al.* 2015). Thus, this study revealed the presence of sufficient variability among the barley landraces in the country that can be exploited for germplasm enhancement. The result is supported by Matin *et al.* (2019) the high value of GA% was recorded with number of grains per spike (46.00) and the low (1.72) with growth habit.

Table 9: Estimation of genetic variability parameters on 14 characters in nine barley genotypes

Traits	Location	PH	SL	CC	FLL	NTPP	ETPP	NGPS	GWPS	SDW	DFF	DM	TGW	YPP	YPPLOT
$\sigma^2 g$	Din	48.67	1.96	6.81	2.91	13.55	11.10	422.25	90.44	53.34	40.50	69.17	46.61	186.51	3806.56
	Ran	133.77	1.56	32.55	5.93	62.25	30.9	179.16	48.83	283.33	40.66	69.4352	38.97	131.01	1642.70
	Pan	0.89	1.56	11.81	0.88	14.26	6.57	131.18	48.61	329.23	38.31	72.62	46.61	153.54	2745.41
$\sigma^2 p$	Din	53.48	2.27	8.08	3.64	20.30	15.86	423.50	98.70	59.22	43.49	72.24	56.26	191.77	4174.89
	Ran	150.58	1.71	34.29	7.46	73.97	37.09	180.05	49.79	286.67	42.64	7.64	51.16	139.72	3.52
	Pan	127.31	2.06	22.83	1.92	18.05	10.76	135.37	56.51	332.84	40.21	74.67	56.26	160.95	2748.33
$\sigma^2 e$	Din	4.80	0.31	1.27	0.73	6.75	4.75	1.25	8.25	5.87	2.98	3.06	9.64	5.26	368.33
	Ran	16.81	0.14	1.73	1.53	11.72	6.16	0.88	0.96	3.33	1.98	2.61	12.18	8.71	1646.22
	Pan	126.42	0.50	11.01	1.04	3.79	4.18	4.18	7.90	3.61	1.90	2.05	9.64	7.40	2.91
GCV%	Din	6.86	15.03	5.91	12.94	15.96	18.22	46.84	23.20	22.01	9.15	7.54	17.54	55.22	52.19
	Ran	12.75	14.46	12.01	15.73	33.1	35.11	36.70	29.49	47.18	9.03	7.50	16.64	44.14	32.70
	Pan	10.86	14.84	11.36	6.40	15.65	12.37	24.36	16.84	41.63	8.84	7.66	17.54	40.93	33.16
PCV%	Din	7.19	16.18	6.44	14.49	19.53	21.77	46.91	24.24	23.19	9.48	7.71	19.27	56.00	54.66
	Ran	13.53	15.13	12.33	17.65	36.09	38.45	36.79	29.78	47.46	9.25	72.04	19.06	45.59	32.74
	Pan	10.90	17.08	8.17	9.45	17.61	15.83	24.74	18.16	41.85	9.05	7.77	19.27	41.91	33.18
ECV%	Din	2.15	5.97	2.55	6.51	11.26	11.92	2.55	7.01	7.30	2.48	1.58	7.98	9.27	16.23
	Ran	4.52	4.47	2.77	8.00	14.36	15.67	2.58	4.14	5.11	1.99	1.45	9.30	11.38	1.5
	Pan	0.91	8.44	7.89	6.95	8.07	9.87	4.35	6.79	4.35	1.96	1.28	7.98	8.99	1.08
h^2b	Din	0.91	0.86	0.84	0.79	0.66	0.70	0.99	0.91	0.90	0.93	0.95	0.82	0.97	0.91
	Ran	0.88	0.91	0.94	0.79	0.84	0.83	0.99	0.98	0.98	0.95	0.96	0.76	0.93	0.99
	Pan	0.99	0.75	0.51	0.45	0.78	0.61	0.96	0.86	0.98	0.95	0.97	0.82	0.95	0.99
GA	Din	13.71	2.68	4.93	3.13	6.19	5.74	42.26	18.75	14.28	12.65	16.76	12.80	27.74	121.36

	Ran	22.45	2.45	11.45	4.47	14.91	10.46	27.50	14.25	34.47	12.82	16.85	11.22	22.83	83.40
	Pan	23.08	2.23	5.09	1.31	6.91	4.13	23.22	13.32	37.17	12.44	17.31	12.80	24.93	107.88
GAM	Din	13.49	28.78	11.19	23.82	26.86	31.41	96.35	45.75	43.03	18.20	15.21	32.89	112.19	102.67
	Ran	24.76	28.45	24.11	28.89	62.57	66.05	75.41	60.17	96.63	18.17	15.18	29.92	88.06	67.30
	Pan	22.29	26.57	12.11	8.93	28.65	19.92	49.40	32.18	85.29	17.77	15.57	32.89	82.36	68.29

Here, σ^2g= genotypic variance, σ^2p= phenotypic variance, σ^2e= environmental variance, GCV= genotypic coefficient variance, PCV= phenotypic coefficient variance, ECV= environmental coefficient variance, h^2b= heritability in broad sense, GA= genetic advance and GAM= Genetic Advance as percentage of mean

Din= Dinajpur, Ran= Rangpur and Pan= Panchagarh

4.5 Estimation of correlation coefficient of fourteen characters of barley genotypes in three locations

The simple correlation coefficient among fourteen important characters viz. days to flowering, days to maturity, plant height**,** flag leaf length, spike length, tiller number per plant, effective tillers per plant, chlorophyll content, number of grains per spike, thousand-grain weight, shoot dry weight, grain weight per spike, yield per plant, and yield per plot were analyzed for 9 barley genotypes. The correlated characters and the values of the correlation coefficient on combined and three different locations are presented in Figure 6.

Plant height has significant positive association with number of tillers per plant, number of effective tillers per plant, grain weight per spike and days to flowering and negative with thousand grain weight and yield per plant. Flag leaf length has significant positive association with shoot dry weight, days to fifty percent flowering and days to maturity and negative with thousand grain weight, yield per plant, number of grains per spike and yield per plot. Spike length has significant positive association with grain weight per spike, number of spikes per plant, yield per plant and yield per plot and negative with shoot dry weight, days to fifty percent flowering and number of tillers per plant.

Number of effective tillers per plant has significant positive association with grain weight per spike, yield per plant and yield per plot and negative with shoot dry weight, days to fifty percent flowering, days to maturity and thousand grain weight. Shoot dry weight has significant positive association with days to fifty percent flowering, yield per plant and yield per plot and negative with thousand grain weight.

Chlorophyll content has a significant positive association with flag leaf length but plant height has a significant negative association. In case of flowering days have significant positive association with days to maturity, yield per plant and yield per plot and have a significant negative association with thousand grain weight. Days to maturity has a significant positive correlation with yield per plant, thousand grain weight and yield per plot. Yield per plant has a significant positive correlation with number of grains per spike and significant negative with number of tillers per plant. In number of tillers per plant has positive correlation with number of effective tillers per plant and negative correlation with number of grains per spike and thousand grain weight.

Number of grains per spike has significant positive association with grain weight per spike yield per plant and yield per plot and negative with shoot dry weight, days to fifty percent flowering and days to maturity.

Firoozabadi *et al.* (2022) revealed that biological yield and thousand grain weight had the most effects on grain yield. Ramazani & Abdipour (2019); Roljević-Nikolić *et al.* (2021); Nazari *et al.* (2020) also found similar kinds of results.

Figure 6: correlation coefficient analysis in barley genotypes

4.6 Selection of superior genotypes using mean yield and stability parameters by using Eberhart and Russell model (StabilitySoft) analysis

Huhn (1990) and Nassar and Huhn (1987) suggested four non-parametric statistics: (1) $S^{(1)}$, the mean of the absolute rank differences of a genotype over all tested environments, (2) $S^{(2)}$, the variance among the ranks over all tested environments, (3) $S^{(3)}$, the sum of the absolute deviations for each genotype relative to the mean of ranks, and (4) $S^{(6)}$, the sum of squares of rank for each genotype relative to the mean of ranks. To compute these statistics, the mean yield data have to be transformed into ranks for each genotype and environment, and the genotypes are considered stable if their ranks are similar across environments. The lowest value for each of these statistics reveals high stability for a certain genotype. So, BARI_7 and EEB_450 were highly stable (table 10).

Four $NP^{(1–4)}$ statistics are a set of alternative non-parametric stability statistics defined by Thennarasu (1995). These parameters are based on the ranks of adjusted means of the genotypes in each environment. Low values of these statistics reflect high stability.

Wrickle (1962) proposed the concept of eco-valence as the contribution of each genotype to the GE interaction sum of squares. The eco-valence (Wi) of the i^{th} genotype is its interaction with the environments, squared and summed across environments. Thus, genotypes with low values have smaller deviations from the mean across environments and are more stable. Shukla (1972) suggested the stability variance of genotype i as its variance across environments after the main effects of environmental means have been removed. According to this statistic, genotypes with minimum values are intended to be more stable. In this case EEB_91 was more stable (table 10).

In addition to slope regression, variance of deviations from the regression (S^2_{di}) has been suggested as one of the most-used parameters for the selection of stable genotypes. Genotypes with an $S^2_{di} = 0$ would be most stable, while an $S^2_{di} > 0$ would indicate lower stability across all environments. Hence, genotypes with lower values are the most desirable. In this case EEB_91 was more desirable (table 10). The slope regression (b_i) is the response of the genotype to the environmental index that is derived from the average performance of all genotypes in each environment (Finlay and Wilkinson, 1963). If b_i does not significantly differ from 1, then the genotype is adapted to all environments. A $b_i > 1$ indicates genotypes with higher sensitivity to environmental change and greater specificity of adaptability to high-yielding environments, while a $b_i < 1$ describes a

measure of greater resistance to environmental change, thereby increasing the specificity of adaptability to low-yielding environments. The coefficient of variation is suggested by Francis and Kannenberg (1987) as a stability statistic through the combination of the coefficient of variation, mean yield, and environmental variance. Genotypes with low CVi, low environmental variance (EV), and high mean yield are considered to be the most desirable. In this case EEB_409 was more desirable (table 10).

Kang's rank-sum (Kang, 1988) uses both yield and σ^2_i as selection criteria. This parameter gives a weight of one to both yield and stability statistics to identify high-yielding and stable genotypes. The genotype with the highest yield and lower σ^2_i are assigned a rank of one. Then, the ranks of yield and stability variance are added for each genotype and the genotypes with the lowest rank-sum are the most desirable. So, in this case EEB_450 was the most stable and desirable genotype (table 10).

Alireza *et al*. (2022) aimed to determine the effectiveness of the WAAS index in identifying ideal barley genotypes. Results showed a similar ranking pattern for at least one genotype to all stability statistics except $S^{(1)}$, $S^{(2)}$, $S^{(6)}$, $NP^{(1)}$, $NP^{(4)}$) and b_i allowing selection of high-yielding and stable genotypes. The study uses all IPCAs to rank productive and broadly adapted genotypes, allowing for the recognition of non-retained GEI patterns. Three environments, DAB2, GON1, and MOG1, significantly influence GEI, while stable genotypes with low WAAS values and high grain yield are identified.

Radia *et al*. (2021) found that Wahbi, Bidi17, Cirta, and Gta dur genotypes had the highest grain yield. The parametric index and non-parametric index classifications also indicated these genotypes as stable. The principal component analysis classified Wahbi and Bidi17 in the dynamic stability group, while Cirta, Bousselem, and OTB4 were classified in the static stability group.

Table 10: The mean yield (GY) and stability statistics values for 9 barley genotypes across three tested environments

Genotype	Y	$S^{(1)}$	$S^{(2)}$	$S^{(3)}$	$S^{(6)}$	$NP^{(2)}$	$NP^{(4)}$	W_i^2	σ^2_i	s^2d_i	b_i	CVi	$\theta_{(i)}$	θi	KR
BARI_7	52.48(5)	1.90(1)	2.71(1)	7.58(1)	4.09(3)	0.30(1)	0.41(2)	2052.43(3)	174.11(3)	244.00(4)	1.13	85.85(7)	205.63(3)	204.31(7)	8(2)
BARI_8	52.94(3)	3.21(6)	7.98(7)	25.03(8)	7.86(8)	0.76(8)	0.77(8)	2998.17(8)	267.65(8)	367.13(8)	1.15	87.54(8)	193.94(8)	245.23(2)	11(6)
BARI_9	54.55(2)	3.05(5)	6.62(5)	17.20(5)	6.00(4)	0.57(7)	0.61(4)	3731.63(9)	340.19(9)	419.52(9)	1.20	89.14(9)	184.87(9)	276.97(1)	11(6)
EEB_114	52.56(4)	2.44(2)	4.59(2)	8.71(2)	3.83(1)	0.33(2)	0.36(1)	2132.79(5)	182.06(5)	292.46(7)	0.93	72.28(2)	204.64(5)	207.79(5)	9(3)
EEB_152	46.65(7)	3.26(7)	7.76(6)	20.48(6)	7.10(6)	0.50(5)	0.66(7)	1855.81(2)	154.67(2)	226.86(2)	0.88	76.39(4)	208.06(2)	195.80(8)	9(3)
EEB_18	47.67(6)	3.38(9)	8.75(9)	22.11(7)	7.33(7)	0.43(4)	0.66(6)	2311.96(6)	199.78(6)	280.94(6)	0.87	74.41(3)	202.42(6)	215.54(4)	12(8)
EEB_409	44.44(9)	3.35(8)	8.53(8)	28.24(9)	8.87(9)	1.02(9)	0.85(9)	2748.88(7)	242.99(7)	252.73(5)	0.77	71.88(1)	197.02(7)	234.45(3)	16(9)
EEB_450	55.48(1)	2.48(3)	4.77(3)	10.33(3)	4.00(2)	0.34(3)	0.41(3)	2072.65(4)	176.11(4)	228.55(3)	1.16	82.58(6)	205.38(4)	205.19(6)	5(1)
EEB_91	45.32(8)	2.71(4)	5.48(4)	16.34(4)	6.03(5)	0.56(6)	0.62(5)	1117.27(1)	81.62(1)	132.83(1)	0.90	78.65(5)	217.19(1)	163.85(9)	9(3)

Here, Y= mean yield, $S^{(1)}$- $S^{(6)}$= Huhn's and Nassar and Huhn's non-parametric statistics, $NP^{(1)}$- $NP^{(4)}$= Thennarasu's non-parametric statistics, W_i^2= Wricke's ecovalence, σ^2_i= Shukla's stability variance, s^2d_i= Deviation from regression, CVi= Coefficient of variance, θi = Mean variance component, $\theta_{(i)=}$ GE variance component and KR= Kang's rank-sum.

4.7 Additive main-effects and multiplicative interaction (AMMI)

Genotypes with high productivity in various environments are the ultimate objective of plant breeders, and they work to develop genotypes to strengthen stability and stabilize yield (Al-Ashkar *et al*., 2022 and Hendawy *et al*. 2020). These results suggest that the cross-reaction gives rise to an overall response and ranking of the genotypes with the yield per plot, yield per plant and number of grains per spike indices under different environmental conditions (Table 18,19,20).

The novel WAASB model explains the GEI, bringing together the AMMI and BLUP models into a unique index to select genotypes based on both index performance and stability (Meier et al., 2019; Al-Ashkar *et al*., 2021; Aboughadareh *et al*., 2021 and Olivoto *et al*., 2019). The genotypes that have a high WAASB score compared to the WAASB grand mean were regarded as less stable (Table 18,19,20). The WAASB biplot quantifies the stability of genotypes by combining an explanation of the stability and productivity in a two-dimensional plot, taking into account all of the IPCAs of the model for GEI effects not maintained in IPCA1 (Meier *et al*., 2019 and Gupta *et al*., 2022), so the WAASB gives more reliable results. When we took a closer look at the WAASB results, we found that EEB_450, EEB_91 and EEB_114 are more stable (smaller WAASB values) according to NGPS (table 18). For YPP, EEB_18, EEB_152 and EEB_450 are more stable (smaller WAASB values) (table 19) and EEB_450, BARI_8, EEB_91 and BARI_7 is more stable (smaller WAASB values) (table 20) respectively. Eventually, further investigation focused on total comprehension of these modern statistical methods would be of benefit and make the method more consistent and useful, to obtain the best (high productivity and stability) genotypes under environmental stresses and to meet increasing demands for crop wheat because of population growth coupled with extreme climatic variations.

The interaction was further partitioned into principal components, of which the first two were highly significant for both analyzed direct yield components. In addition, since the sums of squares of the first two PCs encompassed the model with two axes was concluded as the best model. For the same reason, the same model was the best for other traits of agronomic importance, although in most cases more than two principal components were highly significant (Gauch, 2006; YAN *et al*., 2007; Gauch *et al*., 2008).

Genotypes and environments of the same sign of the same principal component (eider positive or negative) are positively associated; vice versa, the opposite sign implies the negative interaction.

The position close to the biplot origin indicates high stability. As depicted for bulb weight; out of five onion genotypes analyzed, only Jasenički crveni expressed high bulb weight stability across the range of organic and conventional fertilizer treatments applied in the two years of the experiment, while all other cultivars exhibited narrower adaptability to particular environments. However, the mentioned stable genotype coincidently had the lowest bulb weight, while the genotype Majski srebrnjak which achieved the highest bulb weight in the trial ranged the lowest with the respect to stability (Genetika *et al*., 2016). So EEB_450 was more stable than others genotype (table18 & 20) according to NGPS and YPPLOT. On the other hand, EEB_18 was more adaptable than other genotypes (table 19).

Similar ranking of genotypes in terms of means and stability was noted for the majority of the investigated traits of agronomic importance. For the EEB_91 had low mean and the high stability value while BARI _9 had high mean and the low stability value (table 20). The phenomenon is common and it was reported for the yields and yield-related traits of many agricultural plants (e.g. Girek *et al*., 2013; Lakic *et al*., 2015).

AMMI Stability Value (ASV), genotypes with least ASV scores are the most stable, whereas genotypes with high ASV score are unstable (Meier *et al*. 2019). Accordingly, genotypes EEB_450 was appeared to be among those showing low ASV and were the most stable (table 18,19 & 20). On the contrary, genotypes EEB_152 and BARI_9 revealed the highest ASV and were thus considered to be unstable respectively (table 18,19 &20). Stability by itself should, however, not be the only parameter for selection, as the most stable genotype would not necessarily give the best yield performance (Olivoto *et al*. 2021). The stable genotype was followed with mean grain yield above the grand mean and this result was in agreement with (Vaezi *et al*. 2018), who has used ASV as one method of evaluating grain yield stability of bread wheat varieties in Tigray and similar reports been made by (Mondal *et al*. 2013) in barley in Tigray and bread wheat using AMMI stability value. A genotype with the least of genotype selection index (GSI) is considered as the most stable genotype (George and Lundy 2019).

Table11: Results for WAASB estimation of 9 barley genotypes for Number of grains per spike (NGPS) assessed using 3 environmental indices

	Code	Y	PC1	PC2	WAAS		ASV	
					Value	Rank	Value	Rank
Genotypes	BARI_7	58.4	-0.96	-1.83	1.29	5	2.44	4
	BARI_8	61.4	-2.19	-0.9	1.74	7	3.81	8
	BARI_9	56.4	-1.65	0.09	1.07	4	2.77	6
	EEB_114	38	-0.85	0.95	0.89	3	1.72	3
	EEB_152	32.3	3.49	-1.37	2.70	8	6.02	9
	EEB_18	27.9	0.02	2.83	1.07	4	2.83	7
	EEB_409	23.7	1.27	1.36	1.31	6	2.54	5
	EEB_450	51.2	0.01	-1.12	0.42	1	1.12	1
	EEB_91	32.8	0.84	0.06	0.55	2	1.42	2
Environment	Dinajpur	43.9	-3.49	-1.66	2.81	3		
	Panchagarh	47	0.10	3.49	1.37	1		
	Rangpur	36.5	3.39	-1.82	2.80	2		

Table 12: Results for WAASB estimation of 9 barley genotypes for Yield per plant (YPP) assessed using 3 environmental indices

	Code	Y	PC1	PC2	WAAS		ASV	
					Value	Rank	Value	Rank
Genotypes	BARI_7	35.6	-2.02	-0.42	2.02	7	12.9	3
	BARI_8	42.3	1.07	0.31	1.07	5	14	4
	BARI_9	38.8	3.63	-0.51	3.63	9	50.1	9
	EEB_114	21.2	-2.39	0.029	2.39	8	29.2	8
	EEB_152	16	0.46	-0.35	0.46	2	23.3	7
	EEB_18	13.1	0.27	1.61	0.27	1	18.1	6
	EEB_409	16.2	-1.21	-0.33	1.21	6	16.2	5
	EEB_450	36.8	-0.71	-0.11	0.71	3	3.42	1
	EEB_91	22.9	0.9	-0.16	0.9	4	3.63	2
Environment	Dinajpur	24.7	2.93	1.09	2.93	2		
	Panchagarh	30.3	1.21	-1.45	1.21	1		
	Rangpur	25.9	-4.14	0.35	4.14	3		

Table 13: Results for WAASB estimation of 9 barley genotypes for Yield per plot (YPPLOT) assessed using 3 environmental indices

	Code	Y	PC1	PC2	WAAS		ASV	
					Value	Rank	Value	Rank
Genotypes	BARI_7	173	-2.03	-1.57	1.97	3	12.9	3
	BARI_8	180	2.20	1.53	2.11	2	14	4
	BARI_9	192	7.89	2.47	7.16	9	50.1	9
	EEB_114	102	-4.60	-0.18	4.00	8	29.2	8
	EEB_152	94.3	-3.66	2.57	3.51	7	23.3	7
	EEB_18	94.3	2.73	-5.34	3.08	6	18.1	6
	EEB_409	84.8	-2.55	0.82	2.32	5	16.2	5
	EEB_450	178	-0.53	0.55	0.53	1	3.42	1
	EEB_91	102	0.55	-0.85	0.59	2	3.63	2
Environment	Dinajpur	118	5.29	4.56	5.19	2		
	Panchagarh	158	3.62	-5.17	3.83	1		
	Rangpur	124	-8.91	0.60	7.78	3		

4.7.1 AMMI 2 biplot for selected traits

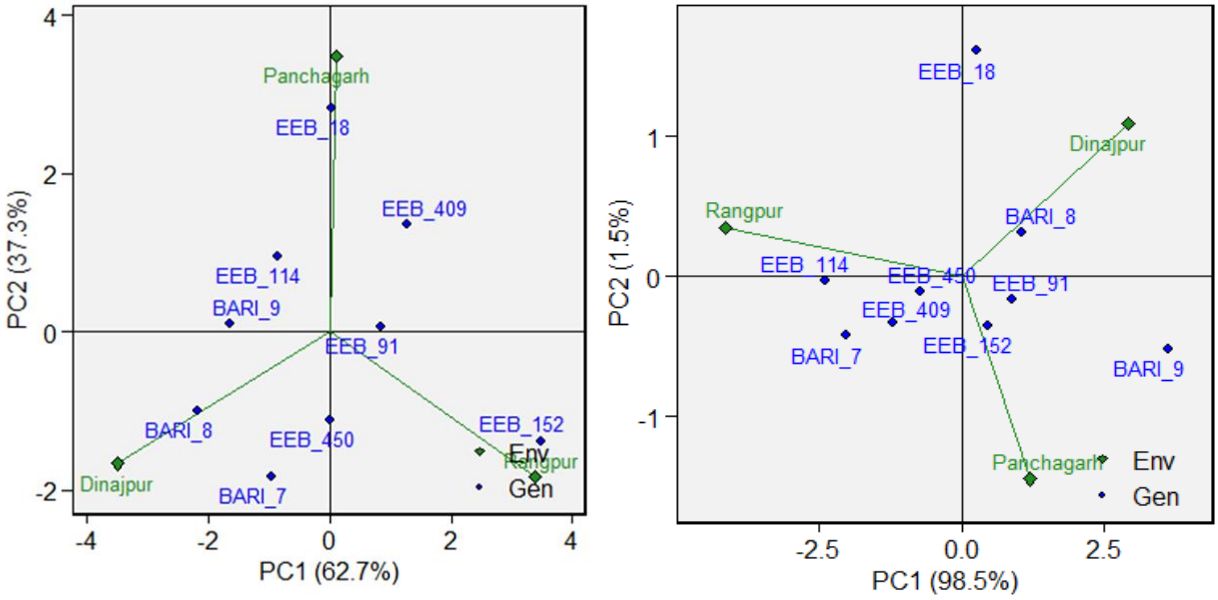
Number of grains per spike (NGPS) and yield per plant (YPP)

Two indicates Panchagarh and Rangpur had a positive correlation (the angle among them was <90°), and another one, Dinajpur indices provided the negative correlation (Figure 9). This indicates that the magnitude of the interaction effects tends to be the same and independent when applying the same abiotic stress (Figure 9,10). The WAASB statistic was used for better descriptions to select ideal genotypes based on both index performance and stability.

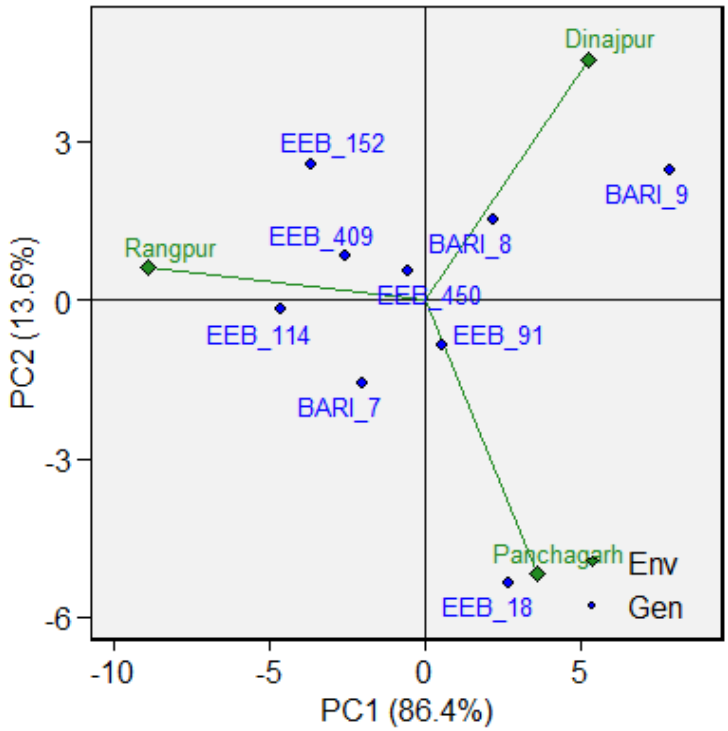
The axis indicating that the magnitude of the interaction effects tends to be the same and independent when applying the same abiotic stress (Ibrahim *et al*., 2023). The obtuse angle of vectors in Panchagarh with Rangpur and Dinajpur points out the negative association between them. A vertical projection from the genotype to the environmental vector detects the extent of the interaction with the environment (Al-Ashkar *et al*., 2022; Singamsetti *et al*., 2021 and Paderewski *et al*., 2016). The plot (Figure 9, 10) shows that 6 genotypes (EEB_18, EEB_152, EEB_409, BARI_7, BARI_8 and BARI_9) were unstable across the environments.

Yield per plot (YPPLOT)

Three indicates Panchagarh, Dinajpur and Rangpur had a positive correlation (the angle among them was <90°), (Figure 11). This indicates that the magnitude of the interaction effects tends to be the same and independent when applying the same abiotic stress (Figure 11). The WAASB statistic was used for better descriptions to select ideal genotypes based on both index performance and stability. The obtuse angle of vectors in Panchagarh with Rangpur and Dinajpur points out the positive association between them. The plot (Figure 11) shows that 4 genotypes (EEB_18, EEB_152, EEB_114 and BARI_9) were unstable across the environments.



NGPS YPP



YPPLOT

Figure 7: AMMI biplot for selected traits

4.7.2 Y×WAAS biplot of different traits

Considering the significant effect of GEI for all studied traits, multiplicative effects analysis was performed to identify stable genotypes based on AMMI's model. The biplot of mean performance versus WAAS which is called the WAAS biplot (Fig. 8), unlike AMMI model which considers only the first IPC, shows stability based on all scores of the IPCs. Therefore, WAAS considers the total variance of GEI in identifying stable genotypes. In this biplot, the vertical line in the middle of the biplot shows the total mean NGPS, YPP and YPPLOT of 3 experimental environments. Genotypes and environments on the right side of this line have a yield value higher than the total mean, and on the other hand, genotypes and environments on the left side of this line have a yield value lower than the total mean. The horizontal axis in the middle of the biplot shows the mean of the WAAS.

From the intersection of this axis with the vertical axis, the biplot is divided into four quadrants. Genotypes in different quadrants of the biplot can be classified based on their suitability for different environments. The genotypes located in the first quadrant of the biplot include EEB_152 related to NGPS, EEB_114 related to YPP and EEB_114 and EEB_152 related to the YPPLOT had a high WAAS value and yield value lower than the total mean, which indicates their highly fluctuating and unstable nature in terms of the relevant traits in different environments and a performance value lower than the mean. In general, these genotypes are not recommended for cultivation. Genotypes BARI_8 located in the second quadrant of the NGPS biplots, BARI_7 and BARI_9 located in the second quadrant of the YPP biplot, and BARI_9 located in the second quadrant of the YPPLOT biplot have high WAAS and performance value above the total mean. If the environmental conditions are favorable, the yield value of these genotypes will be high and can be recommended for cultivation in areas with ideal conditions for the growth and development of barley. Genotypes EEB_18, EEB_114, EEB_409 and EEB_91 in the third quarter of the NGPS biplot, EEB_18, EEB_114, EEB_152 and EEB_91 in the third quarter of the YPP biplot, EEB_18, EEB_409 and EEB_91 in the third quarter of the YPPLOT biplot had lower WAAS, which indicates their stability or lack of influence from environmental conditions. At the same time, these genotypes showed low performance value. Genotypes EEB_459, BARI_7 and BARI_9 located in the fourth quadrant of the biplot related to NGPS, EEB_450 and BARI_8 located in the fourth quadrant of the biplot related to YPP, and EEB_450, BARI_7 and BARI_8 located in the fourth

quarter of the biplot related to the YPPLOT had low WAAS and higher performance value than the total mean. Genotypes placed in this quadrant of the biplot are known as stable genotypes with optimal performance due to their low influence on environmental conditions as well as proper performance.

In general, the WAAS biplot can be described in a way that genotypes with WAAS values of zero or close to zero are considered the most stable genotypes; the ideal genotypes are those that have a WAAS value of zero or close to zero and a yield value higher than the total mean. Genotypes G2 and G16 in terms of RY, G16 and G2 in terms of WSY, G6, G4 and G1 in terms of SC and G8, G10 and G15 in terms of ECS, in addition to stability, had a yield value higher than the total mean, so they were selected as stable genotypes with optimal performance value (Darish at all 2023).

Despite the different stability analysis methods, the AMMI provides useful information to achieve accurate results (Mostafavi *et al*., 2021 and Sharif *et al*., 2017). The statistic was used for better descriptions to select ideal genotypes based on both index performance and stability. For that, a biplot was rendered based on the WAASB and mean index yields in four quadrants for comprehensive interpretation and a joint evaluation of the genotypes/environment (Ibrahim *et al*., 2023).

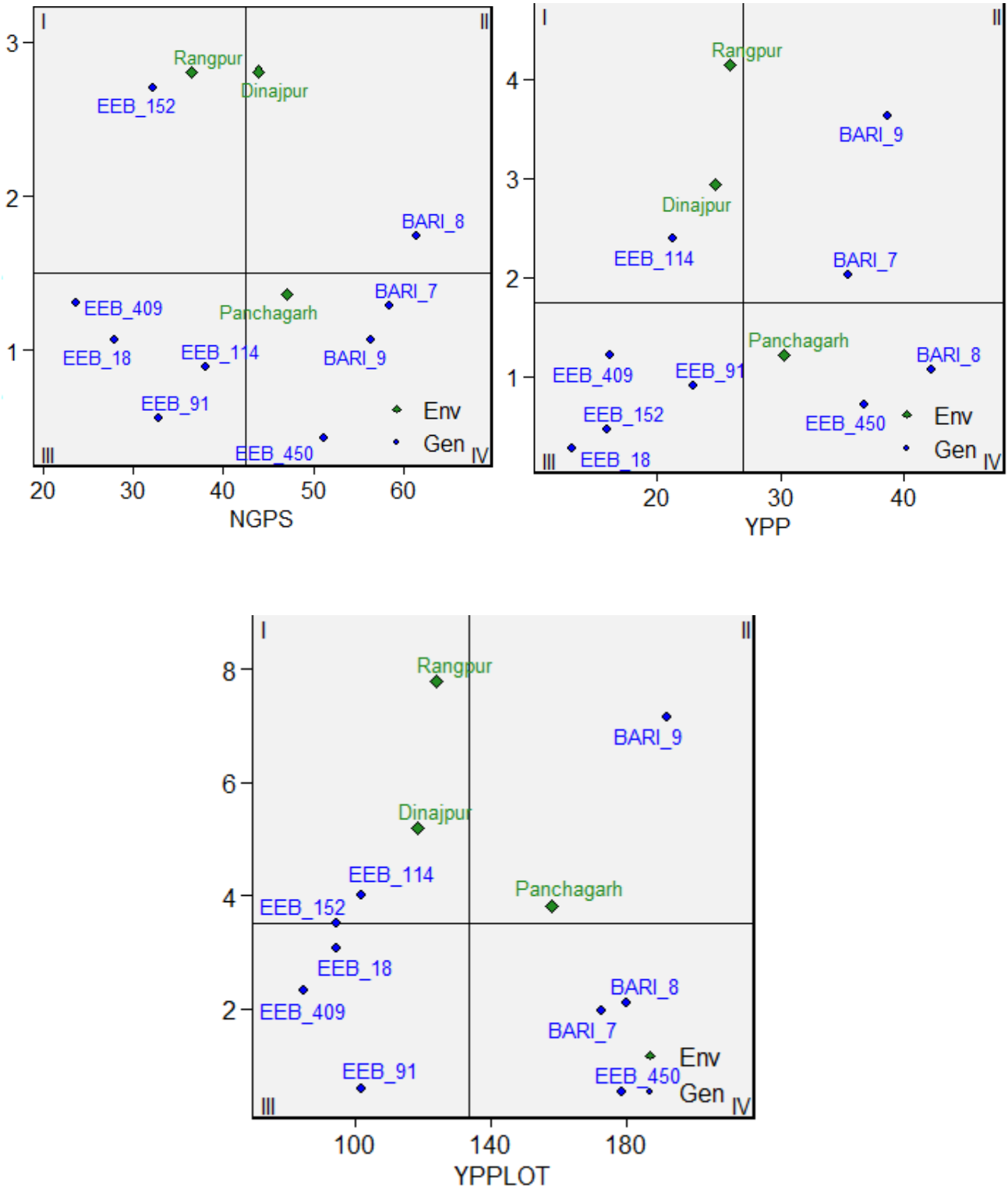
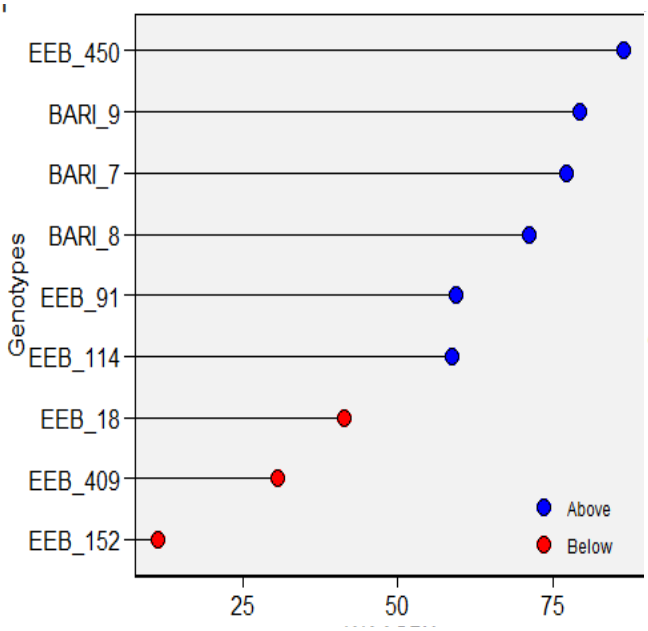


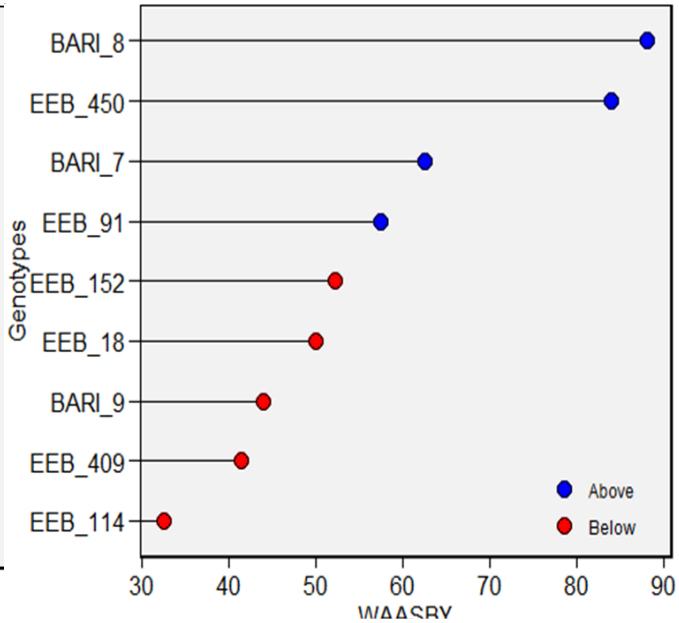
Figure 8: Y × WAAS biplot for all traits

4.7.3 Estimation value of WAASBY by selected traits

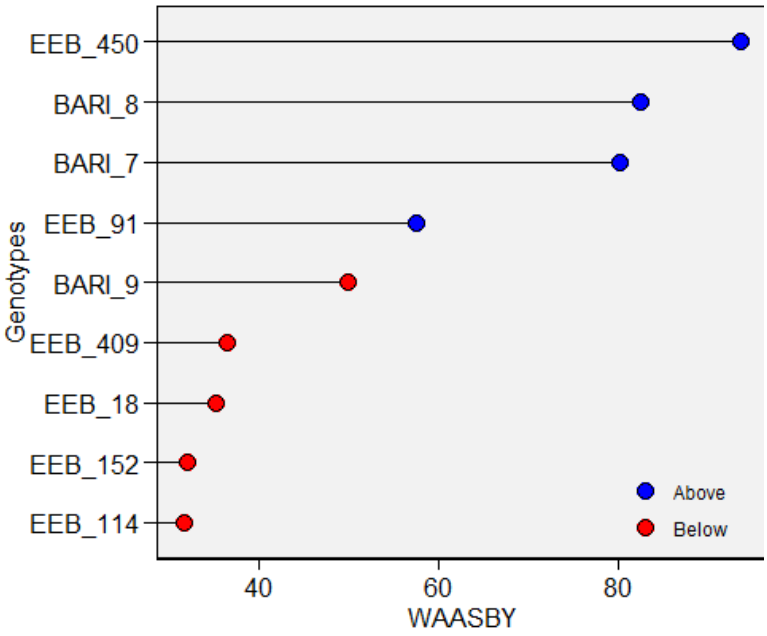
In Figure 13, it is possible to rank and select genotypes at the same time based on yield value and stability. The WASSBY is actually a combination of the WAASB and the yield value of the trait. Blue circles indicate higher than mean WAASBY and red circles indicate lower than mean WAASBY index. Regarding NGPS, EEB_450 had WAASBY index higher than the mean and BARI_9 and BARI_7 had relatively higher WAASBY index values compared with other genotypes (Fig. 13). Based on this, EEB_450 and BARI_9 was recognized as stable genotypes with high NGPS. Results of the WAASBY for the YPP showed that among 9 genotypes with an index higher than the mean, EEB_450 and BARI_8 were the best in terms of higher values compared with other genotypes. Nine genotypes for YPPLOT had high WAASBY. Genotype EEB_450 and BARI_8 was selected as the best genotype in terms of both above-mentioned traits. Stability parameters are important for breeders to ensure the stability of quality properties throughout different environments and influences (Dariusged *et at*., 2023). Olivoto *et al*. (2019) presented the WAASBY index, in which both stability and performance characteristics are considered simultaneously. Based on the results, stable genotypes could be identified successfully by this index.



NGPS



YPP



YPPLOT

Figure 9: WAASBY value of selected traits

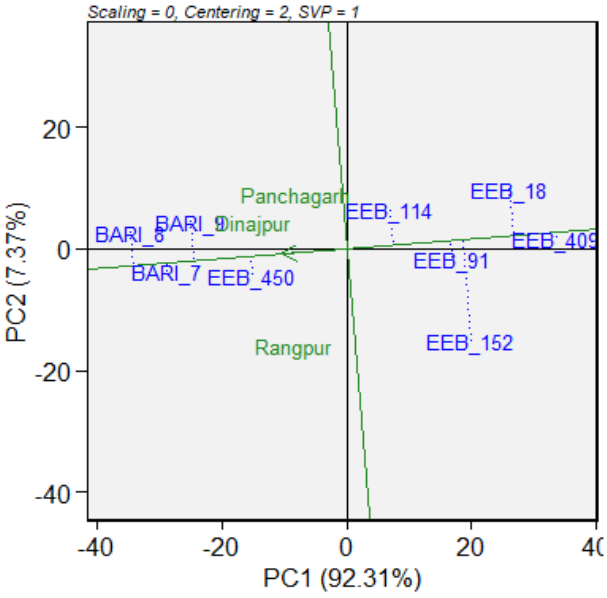
4.8 Genotype into Genotype environment interaction (GGE biplot)

4.8.1 GGE biplot pattern of 'mean vs. stability' analysis and ideal genotype assessment

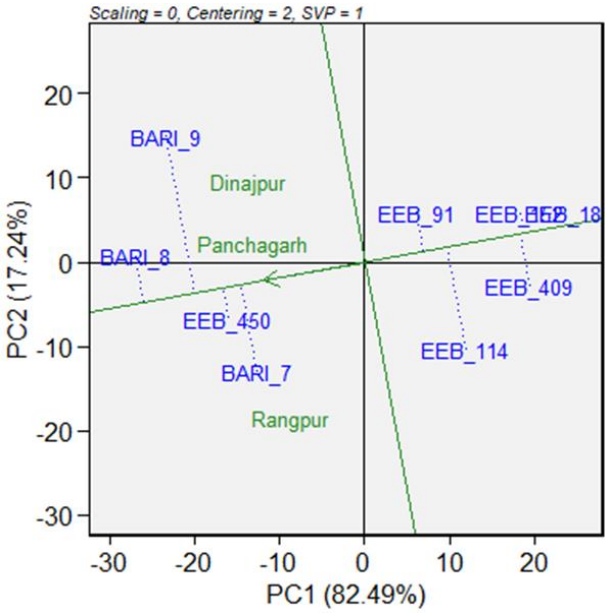
The average environment coordinate (AEC) or average environment axes (AEA) line crosses through the biplot's origin if SVP=1 (single value portioning). As a report by Yan and Rajcan (2002), the mean of PC1 and PC2 of the environmental scores is defined. The 'Mean vs. stability' view frequently stating to as AEC and SVP that helps to simplify the genotype assessment based on the mean performance and stability under a wide range of environment (Fig. 10). The two straight lines, (i) the AEC abscissa (vertical) and (ii) AEC ordinate (horizontal) comprise this biplot graph. Line one consists of a single arrow that pointed towards greater mean performance for each trait. In our investigation, the 'mean vs. stability' pattern of GGE biplot revealed 99.68% for number of grains per spike (NGPS), 99.73% for yield per plant (YPP), 97.1% for Yield per plot (YPPLOT) of G+G×E variation (Fig. 10). The arrow sign on the AEC abscissa line directed the ranking of genotypes in increasing order with a greater value of traits evaluated. However, genotype BARI-7 produced higher pods followed by EEB_450 in Rangpur while in Dinajpur and Panchagarh, the high grains producing genotype is the BARI_8 followed by BARI_9. For the trait NGPS, the genotypes BARI_7 and EEB_450 are more stable over the tested environment though these genotypes produced lower grains. The highest yield per plant was recorded for genotype EEB_450 afterward BARI_7 in Rangpur but genotype BARI_8 and BARI_9 gave higher YPP in Dinajpur and Panchagarh. Over the environment EEB_450 and BARI_8 leading to highly stable ones with lower performance. In environment Rangpur genotype EEB_450 noted for yield per plot followed by BARI_7 on the other hand genotype BARI_8 and BARI_9 showed higher YPPLOT in Dinajpur and Panchagarh though genotype EEB_450 and BARI_8 considered as highly stable across the environment.

However, these genotypes might be incorporated in the breeding strategy for crop enhancement. Aside from these, genotypes EEB_18, EEB_114, EEB_152 and EEB_409 provided somewhat desired yield but shown low stability due to their position on the biplot far from the AEC line. Similar trends of observations were recorded by Oladosu *et al*. (2017), Hashim *et al*. (2021), and Sabri *et al*. (2020). However, the stability of each genotype measure by line two which crosses over the biplot origin, and it vertical bisects the AEA abscissa. The genotype positioned into nearness to the concentrical rings, determining the best performing genotype and the projection

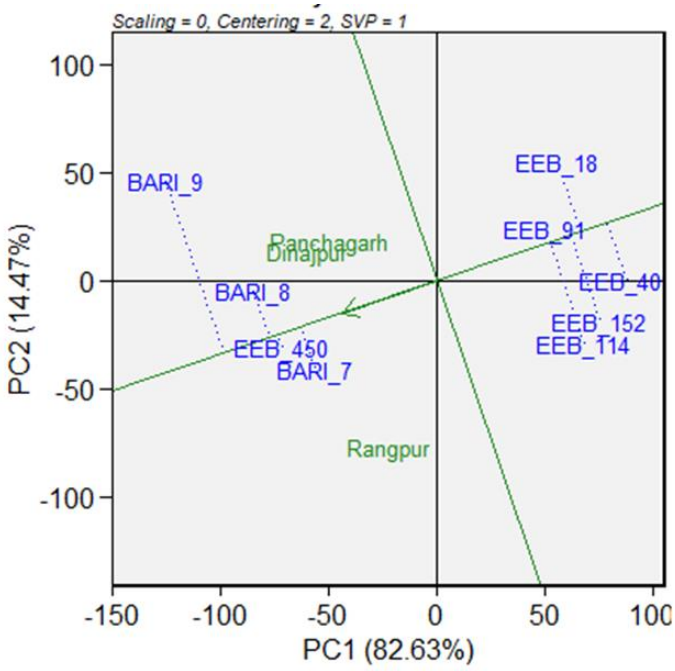
from AEA abscissa indicate the genotype stability. Genotypes consider being more stable when it placed on the horizontal axis (AEC abscissa) and had zero projection from the vertical axis (AEC ordinate) while the genotype with the lengthiest direction from the AEC abscissa is treated as unstable, a similar report was stated by Oladosu *et al*. (2017).



NGPS



YPP



YPPLOT

Figure 10: Mean vs stability biplot of selected traits

4.8.2 GGE biplot ('which-won-where' pattern)

Figure 11 illustrated the polygon view of the GGE biplot pattern for number of grains per spike (NGPS), yield per plant (YPP), yield per plot (YPPLOT). GGE biplot revealed 99.68% for number of grains per spike, 99.73% for yield per plant, 97.1% for Yield per plot of G+G×E variation (Figure 11).

This result confirming the presence of distinct interaction between genotype and environment for all the traits evaluated. Based on 9 genotypes and 3 environments the generated GGE biplot was divided into 6 clockwise fan-shaped sections for NGPS, YPP and YPPLOT respectively. The genotype BARI_8 produced a maximum number grains per spike and highly stable in Dinajpur and Panchagarh while genotypes EEB_450 and BARI_7 perform best in Rangpur. The genotype BARI_7 in Rangpur, genotype BARI_8 and BARI_9 in Panchagarh and Dinajpur were recorded as highly stable and produce more yield per plant. For yield per plot genotype EEB_450 and BARI_7 in Rangpur whereas genotype BARI_9 in Panchagarh and Dinajpur was found as highly stable and best performing line. However, the genotype EEB_450 was recorded as high yielding and stable genotype for environment.

The findings of our study are the agreement with the report stated by Hashim *et al*. (2021) considered two seasons two location and Oladosu *et al*. (2017) considered two seasons five location. The positioning of all environmental indicators into one section of biplot directed that a unique genotype performs best under all tested environments. Oppositely, different genotypes gained different environments if the environmental indicators were positioned into a different segment of biplot. Besides, the genotypes placed at the polygon vertex in a section of biplot where there is no environmental indicator are treated as poorly perform genotypes under all tested environments (Oladosu *et al*., 2017). Consequently, exposing the 'which-won-where' pattern of the GEI data matrix is a crucial feature of the GGE biplot that was extracted by the innermost assets or product of the biplot (Yan *et al*., 2002). The genotype that attached with a vertex of the polygon in a sector where environment markers drop in suggested, such genotype provided greater yield and perform best in that environment. On the contrary, a genotype that is linked with polygon vertex where no environment indicator drops in the sector indicated that such genotype is poorly performed across the environment. The genotypes placed within the polygon are less respective to the environment than the corner genotypes. However, if multi-environments acknowledge by

different winning genotypes recommends the presence of GEI in 4 environments for TNP, FPW, HSW, and Yield, this trend is validated by Gauch and Zobel (1996).

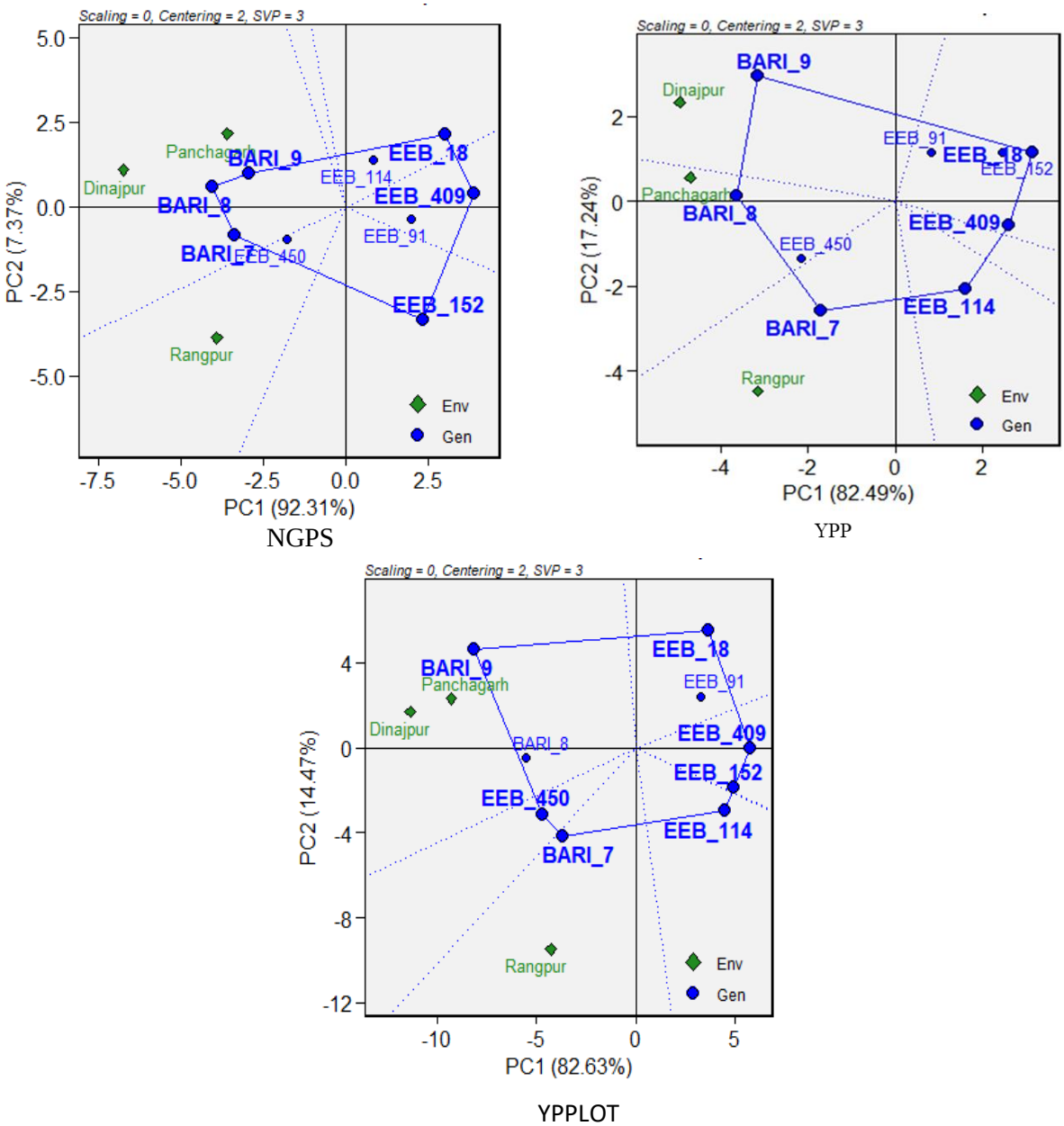


Figure 11: GGE biplot (‘which-won-where’ pattern)

4.8.3 'Descriminitiveness vs. representativeness' pattern of GGE biplot

The determination of a best suited (ideal) test environment is crucial for a successful breeding technique in the selection of superior genotypes. The two features like descriminitiveness (the ability of an environment to distinguish genotype) and representativeness (the ability of an environment to represent all other evaluated environments) signify the idealness of the tested environments (Oladosu *et al*., 2017). In our investigation Fig. 12 illustrated the 'descriminitiveness vs. representativeness' of the GGE biplot study. We recorded three environments for NGPS, YPP, YPPLOT (Fig. 12) as an independent and unique research location due to their short vector while the environment with long vector is more influential in discriminating among the barley accessions. However, the environment with a long vector that forms a shorter angle with the AEC abscissa line is idyllic for the selection of superior genotypes. Tus, environment Dinajpur for NGPS, YPP and YPPLOT had small-angle alongside long vector with AEC abscissa indicated that the test environment was greater representative and discriminative. Figure 12 represent the ranking of environment, exposed that environment, environment Panchagarh and Dinajpur are regarded as the ideal environment. Oppositely, for all traits the environment Rangpur was noted as the poorest environment to select genotype across the environment.

Thus, this study suggests that the studied genotype determined the most suitable environment to assess the mega environment based on test environments representativeness and discriminating ability. Hashim *et al*. (2021) reported one environment is ideal for genotype selection considering yield per hectare among the tested four environments. Among the five evaluated locations, three were noted as an ideal location by Oladosu *et al*. (2017). The correlation coefficient between the genotype mean value over the environment and the genotype values in that environment is approximately equal to the cosine of the angle between the average environment coordinate (AEC) often refers as the average environment axis (AEA) and the environment vector (Yan *et al*., 2007). The smaller angle between AEC abscissa and vector of test environment represents the better environment related to those generate greater angles. The arrow on the AEC abscissa line shows its direction and a small concentric circle denotes the average value of the environment while the length of the test environment vector guesses the discriminating ability. The length of each environment.

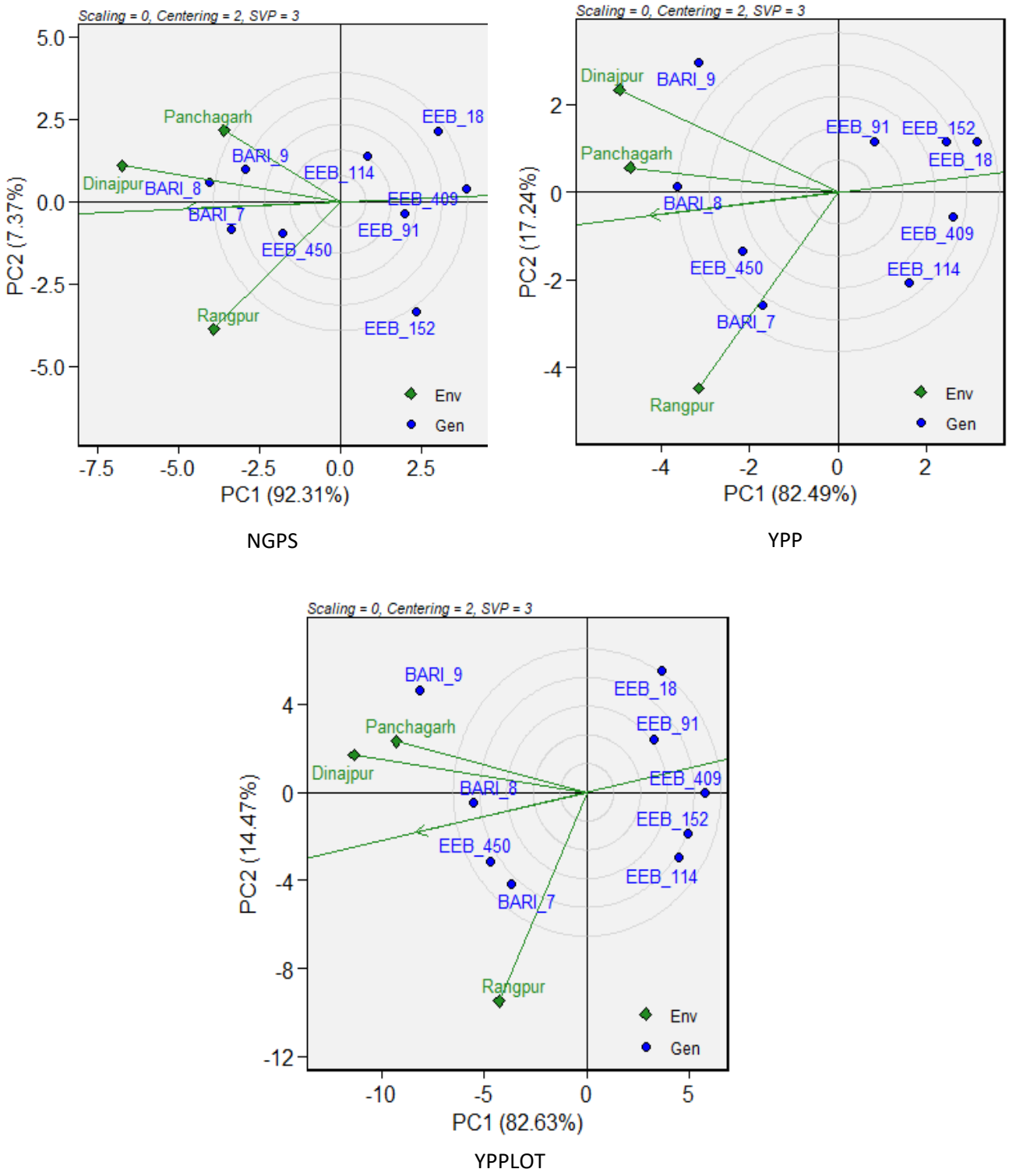


Figure 12: Descriminitiveness vs. representativeness' pattern of GGE biplot

4.8.4 Ranking environment

Figure 13 represent the ranking of environment, exposed that environment Dinajpur for the number of grains per spike (NGPS), environment Panchagarh for yield per plant (YPP), and yield per plot (YPPLOT) are regarded as the ideal environment. Oppositely, for all traits the environment Rangpur was noted as the poorest environment to select genotype across the environment. Tus, this study suggests that the studied genotype determined the most suitable environment to assess the mega environment based on test environments representativeness and discriminating ability.

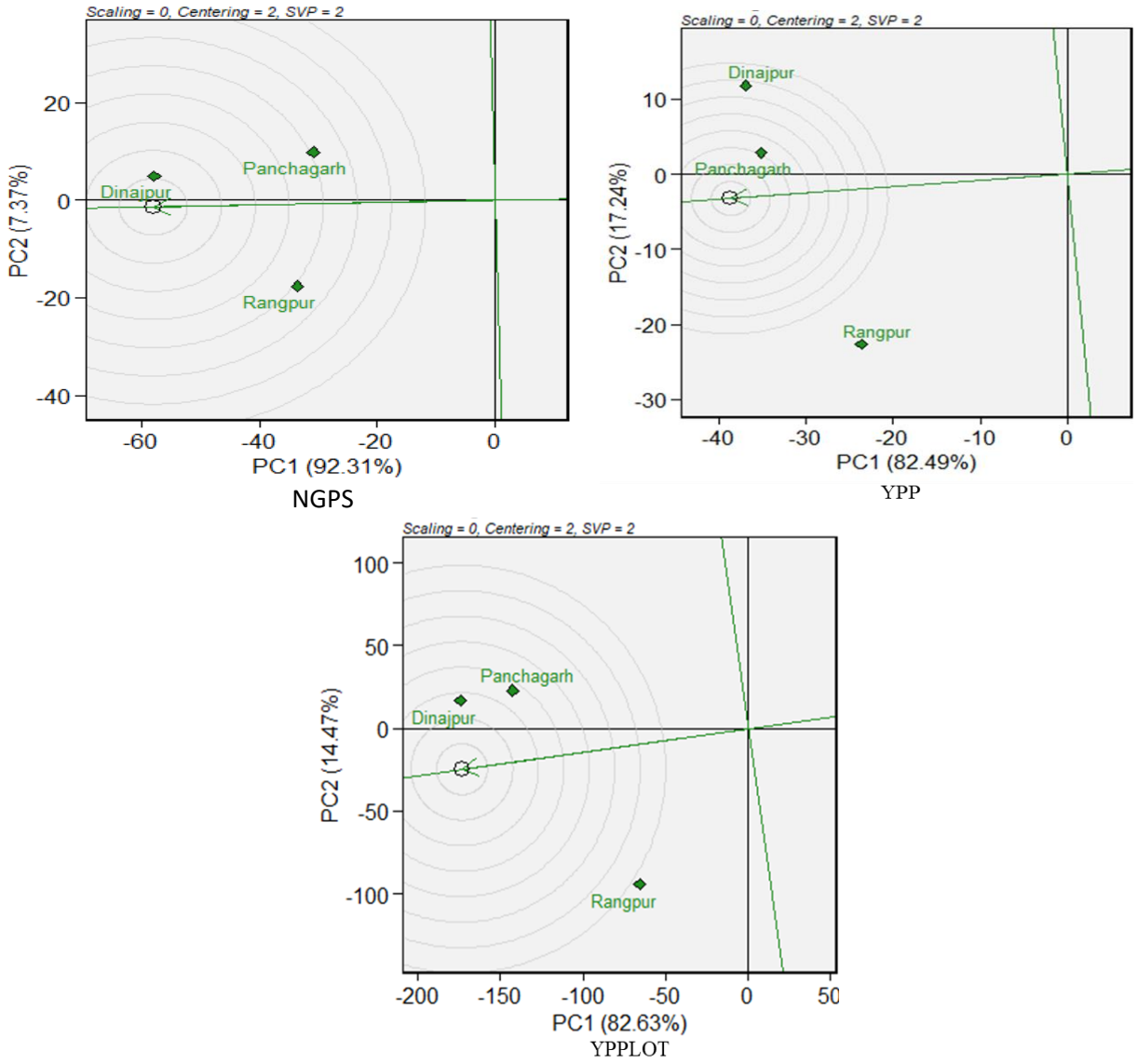
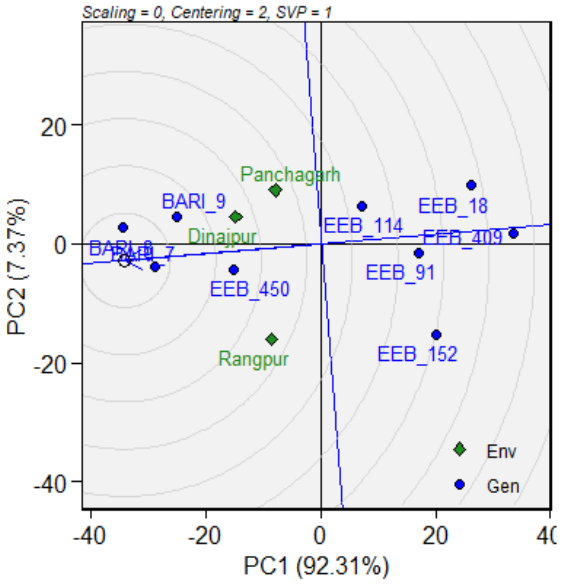


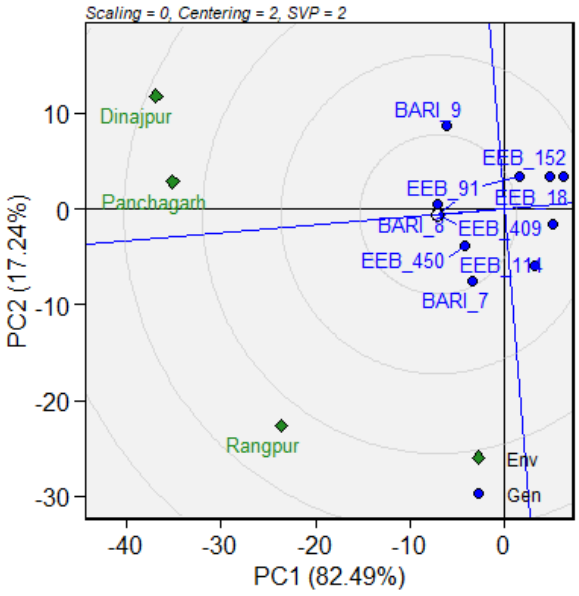
Figure 13: Ranking environment

4.8.5 Ranking genotypes

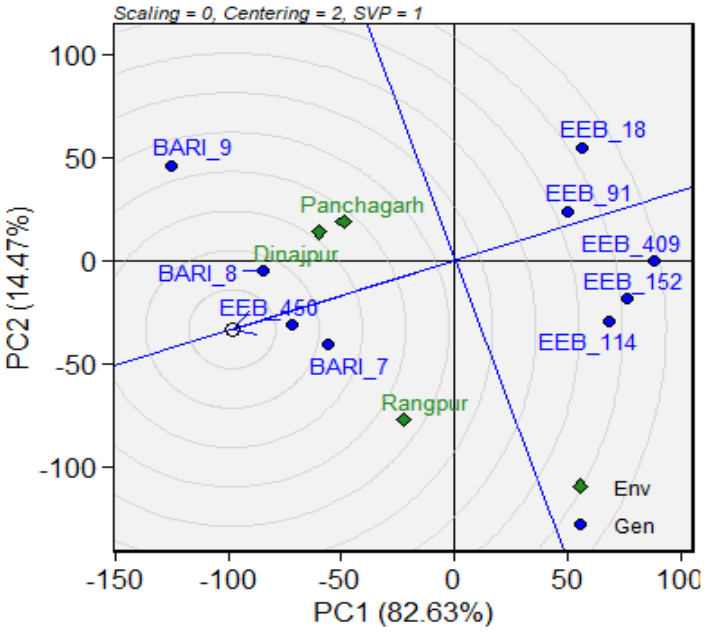
Through the genotype ranking biplot (Fig. 14) we can detect an ideal genotype in contrast to other genotypes evaluated. The genotypes BARI_7, BARI_8 and BARI_9 could be noted as the best leading genotype due to their nearness to the arrowhead in the circle for number of grains per spike (NGPS). Similarly, for yield per plant (YPP) genotype BARI_8 and EEB_450 (Fig. 3: Pattern B); for yield per plot (YPPLOT) genotype EEB_450, BARI_7 and BARI_8 regarded as best genotype due to proximity to concentric circle. Commonly, an ideal genotype is always placed into the innermost circle and relatively nearer the head of the arrow at the center of the circular ring (Fig. 14). The genotype located in the inner circle is highly desirable compared to the genotypes of the outer circle. However, in some cases no genotype was positioned inside the inner circle, consequently, genotypes next closer to the inner circle are considered to be an ideal one (Oladosu *et al*., 2017). Consequently, genotypes BARI_7 and BARI_8 for regarded as ideal genotypes across the tested environment because they were positioned closer to the centre of the biplot origin, indicating that they are stable genotypes. For an effective selection, an ideal genotype should have both high mean and stability properties (Yan *et al*., 2006). A ring at the head of the arrow on the horizontal AEC abscissa axis generally represents an ideal genotype (Oladosu *et al*., 2017) and additionally, the idealness of a genotype refers to a small circle on the AEC abscissa line. Genotypes on the left side of the vertical line often outperform the grand mean, whereas genotypes on the right side underperform the grand mean (Oladosu *et al*., 2017). Plant breeders used data from yield performance evaluations based on mean and stability to choose genotypes best suited to a specific environment within a multi-environment (Kouassi. *Et al*., 2010) while genotypes close to the ideal genotype were also more promising or appropriate. So, the genotype ranking based on ideal genotype for yield per hectare was G1> G10> G13> G5> G3> G6> G14> G17 >G11 >G12 >G8 >G2 > G4 (Mahmud *et al.,* 2023). Oladosu *et al*. (2017) found similar findings across 10 settings as evidence of our result.



NGPS



YPP



YPPLOT

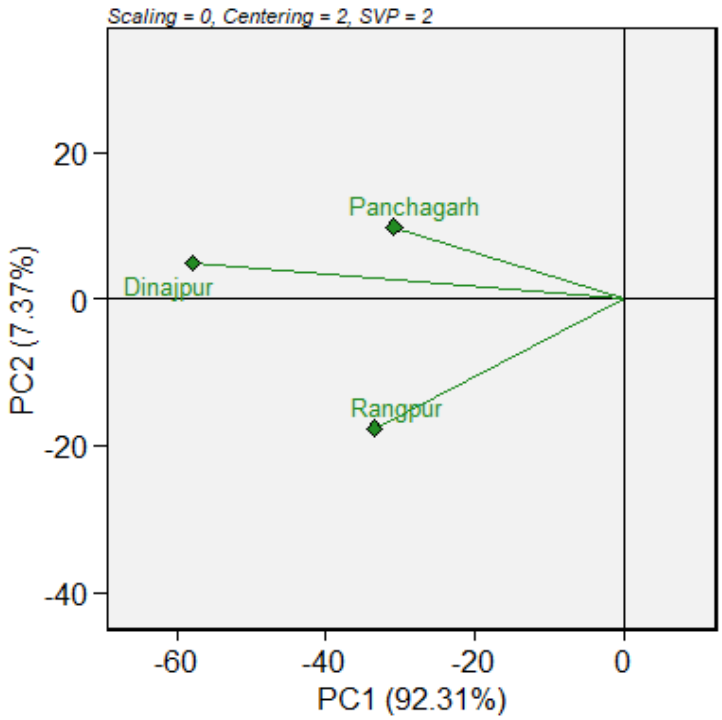
Figure 14: Ranking genotypes

4.8.6 Relationship among environment

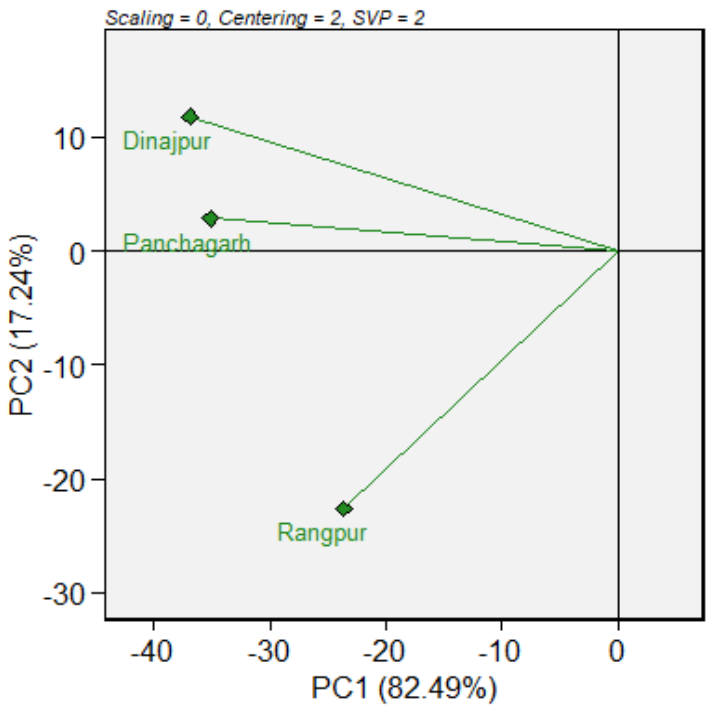
Based on the biplot graph, assessment of test environment is the next important step after multi environment identification to fix the environment discriminativeness and representativeness ability, inter-relatedness, and redundant among the environments.

Figure 15 represent the discriminativeness and representativeness of tested locations. The biplot accounted for 92.31% (PC1) and 7.37% (PC2) for number of grains per spike, 82.49% (PC1) and 17.24% (PC2) for yield per plant and 82.63% (PC1) and 14.47% (PC2) for yield per plot of G+G×E interaction variation across the tested environment. In all cases, the 1st principal components showed the maximum variation for all traits evaluated. Across the location, season are largely influenced by the genotype by environment effect. The distance among each tested environment is displayed in Fig. 15 also for all evaluated traits. As a report by Lin and Binns (1994) the effect of environment on genotype is highly influenced by unpredictable (e.g., weather) and predictable (e.g., soil) factors. The soil is a fixed factor due to its persistence from year after year and is noted as a predictable component. Contrary, the weather is a complex component because it includes predictable elements that are well-defined by the overall climatic region whereas the unpredictable components arise variation due to alternation of time (year to year) (Lin and Binns., 1994). However, it is effective and productive to take into consideration evaluating test environment due to it does represent proximate to multi environments, hence, can be a representative of a multi environment (Yan *et al*., 2007).

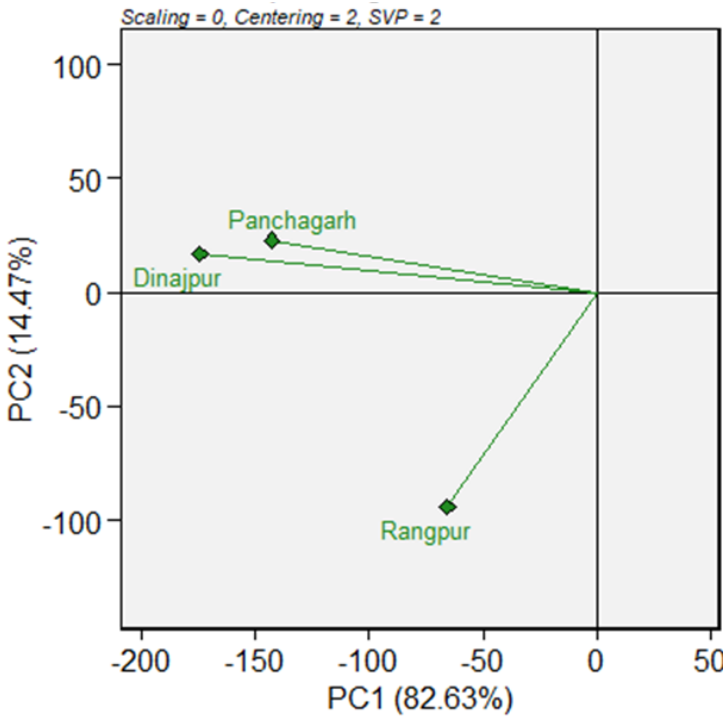
Based on our findings, we categorized Dinajpur for NGPS and YPP and Panchagarh for YPPLOT (lower angle vs. longer vector) indicated that these environments are appropriate for promising genotypes selection because of their notable representativeness and discriminating power. Rangpur for all traits (short vector) which provided little or no information on genotypes also unfit for use as a test location. Yan *et al*. (2007) categorized the environment into three principal categories based on discriminativeness and representativeness during his study. Hashim *et al.* (2021) reported one environment is suitable for genotype selection considering yield per hectare among the tested four environments.



NGPS



YPP



YPPLOT

Figure 15: relationship among environment for different traits

4.8 Likelihood Ratio Test and Overall Performance

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. The statistical significance of the likelihood ratio for genotypes (LRTg) and the likelihood ratio for the genotype and planting time interaction (LRTge) were observed in three environments for all the phenological traits. Similarly, all traits in the group were significant for the LRTg and LRTge, except for the LRTge for the SL, DFF, DM and TGW. All physiological traits except the PH, FLL, CC, NTPP and ETPP were non-significant for the LRTg. The NGPS, SDW, YPP and YPPLOT were significant in the likelihood ratio test for the LRTg and LRTge (Tables 14 and 15).

Each season, the average deviation was higher, showing a significantly larger genotypic response in the different environmental context (Table 16). Consequently, the range of the genotype selection pertaining to these traits is expanded. Most of the genotypes exhibited an increase or decrease, while sustaining a stable thousand grain weight, grain weight per spike and days to maturity across both environments. Moreover, it was observed that the genetic factors contributed significantly to the variance in most traits across all three locations. Variations in both the overall performance and variance components were detected among the different trait groups. The mean deviation observed for the SL during all locations were comparatively low in contrast to other traits (Table 16).

Farhad *et al*., (2023) revealed that the likelihood ratio test revealed significant genotype impact on all trait categories, except BTH, in seasons 2 and 3, with plant stature traits, TGW, and grain yield being significant and the average deviation was higher, indicating a significantly larger genotypic response in the early-planting context. He also found in the mean deviation observed for the EGC during early sowing was comparatively low in contrast to planting at the appropriate time.

Table 14: Likelihood ratio test for the genotypic effect showing the significance of traits in single environment analysis in barley

Traits	Dinajpur	Rangpur	Panchagarh
PH	30.3***	26.9***	70.7***
FLL	18***	17.7***	4.61*
CC	21.7***	39.2***	5.97*
SL	23.8***	30.7***	15.2***
NTPP	10.8***	21.6***	17.4***
ETPP	12.3***	20.9***	8.72***
NGPS	84.4***	76.2***	47***
GWPS	31.4***	54.5***	23.5***
SDW	28.7***	62.5***	63.7***
DFF	34.5***	40.6***	40.3***
DM	42***	44.5***	48.9***
TGW	20.4***	15.6***	20.4***
YPP	48.9***	36***	40.7***
YPPLOT	30.5***	89.6***	101***

Here, PH= plant height, FLL= flag leaf length, CC= chlorophyll content, SL= spike length, NTPP= number of tillers per plant, ETPP= effective tillers per plant, NGPS= number of grains per spike, GWPS= grain weight per spike, SDW= shoot dry weight, DFF= days to flowering, DM= days to maturity, TGW = thousand grain weight, YPP= yield per plant and YPPLOT= yield per plot; *** $p \geq 0.001$, ** $p \geq 0.01$, * $p \geq 0.05$; ns: non-significant.

Table 15: Likelihood ratio test (LRT) for the genotypic effect showing the significance of traits in the genotype-environment interaction for multi-environmental trial analysis in barley

Traits	LRTg	LRTge
PH	3.44(NS)	73.7***
FLL	3.74(NS)	22***
CC	-9.63E-09(NS)	45.5***
SL	26.2***	3.01(NS)
NTPP	0.841(NS)	40.4***
ETPP	-1.14E-13(NS)	43.3***
NGPS	16.2***	124***
GWPS	2.83(NS)	67.2***
SDW	11.4***	103***
DFF	69.1***	-5.68E-14(NS)
DM	76.7***	-6.09E-11(NS)
TGW	46.2***	-5E-12(NS)
YPP	12.5***	63.6***
YPPLOT	9.09***	73.3***

Here, PH= plant height, FLL= flag leaf length, CC= chlorophyll content, SL= spike length, NTPP= number of tillers per plant, ETPP= effective tillers per plant, NGPS= number of grains per spike, GWPS= grain weight per spike, SDW= shoot dry weight, DFF= days to flowering, DM= days to maturity, TGW = thousand grain weight, YPP= yield per plant and YPPLOT= yield per plot; *** $p \geq 0.001$, ** $p \geq 0.01$, * $p \geq 0.05$; ns: non-significant.

Table 16: The statistical measures for the traits observed in three locations: mean $\bar{x}$, confidence interval of mean (CI), and average deviation (AD) in barley

Traits	Dinajpur			Rangpur			Panchagarh			Overall		
	AD	CI	$\bar{x}$	AD	CI	$\bar{x}$	AD	CI	$\bar{x}$	AD	CI	$\bar{x}$
CC	1.97	1.03	44.1	3.74	2.14	47.5	3.65	1.75	42.1	3.45	1.09	44.6
DFF	4.95	2.39	69.5	4.94	2.37	70.6	4.59	2.30	70	4.86	1.35	70
DM	6.80	3.08	110	6.89	3.08	111	7.06	3.14	111	6.94	1.77	111
ETPP	3.19	1.45	18.3	4.57	2.22	15.8	2.50	1.22	20.7	3.94	1.06	18.3
FLL	1.51	0.69	13.2	2.09	0.99	15.5	1.08	0.52	14.7	1.64	0.48	14.4
GWPS	7.44	3.64	41	5.39	2.56	23.7	5.72	2.73	41.4	9.23	2.49	35.4
NGPS	17.9	7.46	43.9	11.7	4.87	36.5	9.71	4.22	47	13.1	3.39	42.5
NSPP	3.19	1.45	18.3	4.57	2.22	15.8	2.50	1.22	20.7	4.73	1.29	23.7
NTPP	3.62	1.64	23.1	7.33	3.15	23.8	3.30	1.62	24.1	8.78	2.50	98.6
PH	5.40	2.65	102	9.31	4.46	90.7	8.55	4.09	104	11.5	3.26	37.5
SDW	6.17	2.80	33.2	14.7	6.14	35.7	13.6	6.61	43.6	1.15	0.30	8.80
SL	1.22	0.56	9.33	1.04	0.47	8.64	0.98	0.52	8.42	5.34	1.54	38.4
TGW	5.44	2.73	38.9	4.96	2.60	37.5	5.44	2.73	38.9	10.8	2.71	27
YPP	11.4	5.04	24.7	9.93	4.31	25.9	10.9	4.61	30.3	45.8	11.7	133

Here, PH= plant height, FLL= flag leaf length, CC= chlorophyll content, SL= spike length, NTPP= number of tillers per plant, ETPP= effective tillers per plant, NGPS= number of grains per spike, GWPS= grain weight per spike, SDW= shoot dry weight, DFF= days to flowering, DM= days to maturity, TGW = thousand grain weight, YPP= yield per plant and YPPLOT= yield per plot.

4.9 Selection across the environment with multi-trait genotype ideotype distance index (MGIDI)

For Rangpur, the MTSI Index provided desired selection differential (SD) for 9 out of 14 studied traits with success frequency of 86.13% in selecting desired traits. The remaining five traits with undesired SD were flag leaf length (SD = −0.89), shoot dry weight (SD = −8.64), days to fifty percent flowering (SD = −4.63), days to maturity (SD= - 5.82) and number of tillers per plant (SD= -1.72). The selection differential (SD) percent for traits ranged from −7.22% (number of tillers per plant) to 32.9% (yield per plant). The traits with high heritability were number of grains per spike, shoot dry weight, grain weight per spike and yield per plot (99%), followed by days to fifty percent flowering, days to maturity and chlorophyll content (98%). The lowest heritability percentage was observed in flag leaf length (92%) (table 17).

For Panchagarh desired selection differential (SD) for 7 out of 14 studied traits with success frequency of 64.68% in selecting desired traits. The remaining seven traits with undesired SD were flag leaf length (SD = −0.56), shoot dry weight (SD = −8.49), days to fifty percent flowering (SD = −4.26), days to maturity (SD= - 5.87), plant height (SD= -4.24), chlorophyll content (SD= - 2.58) and grain weight per spike (SD= -1.72). The selection differential (SD) percent for traits ranged from −19.5% (shoot dry weight) to 35.6% (yield per plant). The traits with high heritability were plant height, shoot dry weight, days to maturity and yield per plot (99%), followed by days to fifty percent flowering, number of grains per spike and yield per plant (98%). The lowest heritability percentage was observed in flag leaf length (71%) (table 18).

For Rangpur desired selection differential (SD) for 6 out of 14 studied traits with success frequency of 82.29% in selecting desired traits. The remaining eight traits with undesired SD were flag leaf length (SD = −0.52), shoot dry weight (SD = −4.61), days to fifty percent flowering (SD = −3.22), days to maturity (SD= - 1.64), plant height (SD= -6.43), spike length (SD= -0.41), chlorophyll content (SD= - 0.60) and grain weight per spike (SD= -5.73). The selection differential (SD) percent for traits ranged from −14.0% (grain weight per spike) to 52.2% (yield per plant). The traits with high heritability were number of grains per spike and yield per plant (99%), followed by days to maturity (98%). The lowest heritability percentage was observed in number of tillers per plant (85%) (table 19).

MTSI index provided selection ideotype index within the environments, and the results revealed that most of the traits under study contributed towards the selection of desired barley genotype including Rangpur, Panchagarh and Dinajpur. Traits, viz. FLL, SDW, NTPP, DM and DFF during Rangpur; FLL, SDW, DFF, DM, PH, CC as well as GWPS for Panchagarh and FLL, SDW, DFF, DM, PH, SL and GWPS were found least contributing in the selection ideotype process. The selection differential percent in all environment was found higher for the yield per plant and lower for the shoot dry weight (52.2 and -24.2) respectively (Table 17-19).

Niranjana *et al.* (2021) found that the MTSI Index showed 80% success in selecting desired traits for 12 out of 15 studied traits, with undesired traits being Days to 50% flowering, Protein, and Ash. The top thirteen genotypes showed desired values for most guar traits, with high heritability being Grain yield, Protein, Gum, Days to 50% flowering, Fat, and Carbohydrates. The MTSI index revealed that traits like grain yield and protein content significantly influence the selection of desired guar genotypes during E1 and E2, with selection differential percent higher.

Ibrahim *et al.* (2023) shown on in the process of choosing qualities with desired gains, the MGIDI performed better. It is capable of handling multicollinearity in addition to the higher degree of computation primary benefit: the breeder outlines the requirements before determining the index (Olivoto *et al*., 2022). The MGIDI showed that, when all examined qualities were taken into account, 16 out of 20 traits had desired gains, and when GY and its 4 related traits were taken into account, 4 out of 5 traits had desired gains. The corresponding rates of increase were 80.53 and 7.18.

Using the MGIDI, Olivoto and Nardino (2021) and Olivoto *et al*. (2022) have shown how to select superior and suitable genotypes in plant experiments that combine all selected traits (MTSI) that satisfy the breeders and achieve their desired goals.

Table 17: Predicted genetic gains for the factor analysis and ideotype-design (FAI-BLUP) index.in Rangpur location

Traits	Xo	Xs	SD	SDperc	h^2	SG	SGperc
FLL	15.5	14.6	-0.89	-5.80	0.92	-0.82	-5.34
NGPS	36.5	48.1	11.7	32.0	0.99	11.6	31.9
SDW	35.7	27.0	-8.64	-24.2	0.99	-8.61	-24.1
DFF	70.6	66.0	-4.63	-6.56	0.98	-4.55	-6.45
DM	111	105	-5.82	-5.24	0.98	-5.74	-5.17
PH	90.7	91.9	1.20	1.33	0.96	1.15	1.27
SL	8.64	9.04	0.39	4.57	0.96	0.38	4.42
NTPP	23.8	22.1	-1.72	-7.22	0.94	-1.62	-6.80
CC	47.5	48.1	0.62	1.31	0.98	0.61	1.29
TGW	37.5	37.9	0.41	1.12	0.90	0.37	1.01
ETPP	15.8	18.3	2.49	15.7	0.93	2.33	14.7
GWPS	23.7	28.8	5.09	21.5	0.99	5.05	21.3
YPP	25.9	34.4	8.52	32.9	0.97	8.34	32.2
YPPLOT	124	156	32.1	25.9	0.99	32.1	25.9

Here, PH= plant height, FLL= flag leaf length, CC= chlorophyll content, SL= spike length, NTPP= number of tillers per plant, ETPP= effective tillers per plant, NGPS= number of grains per spike, GWPS= grain weight per spike, SDW= shoot dry weight, DFF= days to flowering, DM= days to maturity, TGW = thousand grain weight, YPP= yield per plant and YPPLOT= yield per plot; Xo= original value, Xs= selected value, SD= selection differential, SDperc= selection differential percentage, h^2= broad sense heritability, SG= selection gain and SGperc= selection gain percentage.

Table 18: Predicted genetic gains for the factor analysis and ideotype-design (FAI-BLUP) index.in Panchagarh location

Traits	Xo	Xs	SD	SDperc	h^2	SG	SGperc
FLL	14.7	14.1	-0.56	-3.79	0.71	-0.40	-2.72
NGPS	47.0	56.4	9.37	19.9	0.98	9.28	19.7
SDW	43.6	35.1	-8.49	-19.5	0.99	-8.46	-19.4
DFF	70.0	65.7	-4.26	-6.09	0.98	-4.19	-5.99
DM	111	105.	-5.87	-5.28	0.99	-5.82	-5.23
PH	104	99.3	-4.24	-4.10	0.99	-4.23	-4.09
SL	8.42	8.84	0.42	5.02	0.90	0.38	4.53
NTPP	24.1	25.8	1.66	6.88	0.91	1.53	6.32
CC	42.1	39.5	-2.58	-6.13	0.76	-1.97	-4.67
TGW	38.9	39.9	0.96	2.48	0.93	0.90	2.32
ETPP	20.7	22.4	1.67	8.08	0.82	1.38	6.66
GWPS	41.4	41.2	-0.19	-0.47	0.94	-0.19	-0.45
YPP	30.3	41.0	10.8	35.6	0.98	10.6	35.0
YPPLOT	158	210	51.6	32.7	0.99	51.6	32.7

Here, PH= plant height, FLL= flag leaf length, CC= chlorophyll content, SL= spike length, NTPP= number of tillers per plant, ETPP= effective tillers per plant, NGPS= number of grains per spike, GWPS= grain weight per spike, SDW= shoot dry weight, DFF= days to flowering, DM= days to maturity, TGW = thousand grain weight, YPP= yield per plant and YPPLOT= yield per plot; Xo= original value, Xs= selected value, SD= selection differential, SDperc= selection differential percentage, h^2= broad sense heritability SG= selection gain and SGperc= selection gain percentage.

Table 19: Predicted genetic gains for the factor analysis and ideotype-design (FAI-BLUP) index in Dinajpur location

Traits	Xo	Xs	SD	SDperc	h^2	SG	SGperc
FLL	13.2	12.7	-0.52	-3.97	0.92	-0.48	-3.66
NGPS	43.9	55.5	11.7	26.6	0.99	11.7	26.6
SDW	33.2	28.6	-4.61	-13.9	0.96	-4.45	-13.4
DFF	69.5	66.3	-3.22	-4.63	0.97	-3.14	-4.52
DM	110	109.	-1.64	-1.49	0.98	-1.62	-1.47
PH	102	95.2	-6.43	-6.33	0.96	-6.22	-6.12
SL	9.33	8.92	-0.41	-4.34	0.95	-0.38	-4.12
NTPP	23.1	23.2	0.152	0.661	0.85	0.131	0.567
CC	44.1	44.1	-0.06	-0.13	0.94	-0.05	-0.13
TGW	38.9	40.3	1.38	3.55	0.93	1.29	3.32
ETPP	18.3	20.2	1.87	10.2	0.87	1.63	8.93
GWPS	41.0	35.3	-5.73	-14.0	0.97	-5.56	-13.6
YPP	24.7	37.6	12.9	52.2	0.99	12.8	51.7
YPPLOT	118	165	46.6	39.5	0.96	45.2	38.2

Here, PH= plant height, FLL= flag leaf length, CC= chlorophyll content, SL= spike length, NTPP= number of tillers per plant, ETPP= effective tillers per plant, NGPS= number of grains per spike, GWPS= grain weight per spike, SDW= shoot dry weight, DFF= days to flowering, DM= days to maturity, TGW = thousand grain weight, YPP= yield per plant and YPPLOT= yield per plot; Xo= original value, Xs= selected value, SD= selection differential, SDperc= selection differential percentage, h^2= broad sense heritability, SG= selection gain and SGperc= selection gain percentage.

4.10 Best linear unbiased prediction (BLUP)

Dariush *et al*. (2023) found significant effects of genotype on traits like RY, WSY, SC, and ECS at 1% probability level. High heritability was observed, with WSY and RY having the highest heritability. Selection accuracy was high for WSY and RY, but average for ECS and SC. The genotypic correlation between environments was low in all traits, with the SC having the lowest correlation between environments. The significance of GEI indicates the different response of genotypes and the superiority and weakness of them in different environments. Therefore, in such a situation, using the BLUP method can bring better and more reliable results (Olivoto *et al*., 2019). Estimating traits heritability plays an important role in the breeding programs to identify and recommend genotypes (Olivoto *et al*., 2019, Benakanahalli *et al*., 2021).

Estimating traits heritability plays an important role in the breeding programs to identify and recommend genotypes. Results showed high heritability for days to maturity (97%) followed by days to fifty percent flowering (96%). Among studied traits, CC (2%) followed by ETPP (14%) had the lowest heritability (Table 20). The correlation coefficient was low in all traits, especially the SL. Selection accuracy for DFF (0.99), DM (0.99) and TGW (0.99) were high, however for SL (0.98) was in average (Table 20). This parameter shows the correlation between observed and predicted values. The average and high selection accuracy values of the traits indicated the reliability of the model in selecting superior genotypes. If low correlation exists, accurate information and details are needed to select superior genotypes (Koundinya *et al*., 2021).

Table 20: Estimation of variance components from linear mixed model for studied traits in barley genotypes.

Traits	Phenotypic variance	Heritability	h^2mg	Accuracy	CVg	CVr	CVratio	R^2gei	rge
PH	110	0.38	0.67	0.82	6.57	2.78	2.37	0.55	0.89
FLL	4.35	0.35	0.68	0.83	8.47	7.28	1.16	0.40	0.61
NGPS	246	0.76	0.91	0.95	32.4	3.42	9.47	0.22	0.96
SDW	226	0.67	0.87	0.93	32.9	5.51	5.97	0.31	0.94
DFF	42.1	0.96	0.99	0.99	9.06	1.93	4.69	0.37	0.47
DM	73	0.97	0.99	0.99	7.60	1.29	5.89	0.31	0.41
CC	10.2	0.02	0.08	0.28	1.05	4.33	0.24	0.79	0.79
SL	2.02	0.79	0.96	0.98	14.4	6.45	2.22	0.05	0.24
NTPP	37.4	0.17	0.42	0.65	10.6	11.5	0.93	0.63	0.76
TGW	54.6	0.84	0.98	0.99	17.6	7.60	2.32	0.13	0.14
ETPP	21.2	0.14	0.38	0.61	8.9	10.3	0.86	0.59	0.60
GWPS	68.3	0.34	0.63	0.79	13.7	6.76	2.02	0.58	0.87
YPP	164	0.68	0.88	0.94	39.3	9.90	3.97	0.27	0.86
YPPLOT	2.86e+3	0.60	0.83	0.91	31.1	8.38	3.72	0.35	0.89

Here, PH= plant height, FLL= flag leaf length, CC= chlorophyll content, SL= spike length, NTPP= number of tillers per plant, ETPP= effective tillers per plant, NGPS= number of grains per spike, GWPS= grain weight per spike, SDW= shoot dry weight, DFF= days to flowering, DM= days to maturity, TGW = thousand grain weight, YPP= yield per plant and YPPLOT= yield per plot.

The mean values of yield and other measured traits at the three test locations are shown in Table 17. PH ranged from 92.9 cm to106 cm. FLL ranged from 13.4 to 16.1 cm, while CC ranged from 42.9 g to 47.2. SL showed a low index of variation and ranged from10.5 to 6.71 cm. NTPP and ETPP showed a low index of variation and ranged from 21.7 to 26.3 and 16.1 to 19.3 while DM and DFF showed a high index of variation and ranged from 102 to 126 days and 62.9 to 82.8 days respectively.

In response of number of grains per spike, the highest number 59.7 for BARI_8 and lowest number 25.4 was observed in EEB_409. GWPS varied from 29.4 to 42.1. While the highest value was in EEB_114 and the lowest value for EEB_91. The variability of the traits found significant differences among the nine genotypes. SDW ranged from 25.5 (EEB_91) to 57.1 (EEB_114). TGW varied from 28 to 52g while lowest value in EEB_152 and highest value for EEB_409. Yield per plant showed a low index of variation and ranged from 14.8 (EEB_18) to 40.4g (BARI_8) while yield per plot showed a high index of variation and ranged from 93 (EEB_409) to 182g (BARI_9), respectively (table 21).

Among the genotypes tested, genotypes EEB_450, EEB_91, BARI_7 and BARI_9 with the highest values were identified as the most stable genotypes compared to other genotypes. The relative performance describes the point of adaptation. Indeed, this indicator is valuable for identifying the specific adaptability of genotypes, and can take advantage of the response of genotypes to improved growing conditions. In other words, this index simultaneously shows the adaptability and stability of genotypes.

Alireza *et al.* (2023) examined BLUP-based adaptability and stability indices, average grain yield, and tested genotypes. The harmonic mean of genotypic values (HMGV) index was used to select stable genotypes, with the top 10 ranked genotypes being G52, G6, G24, G57, G11, G29, G38, G48, G18, and G22. The study also analyzed grain yield, PLH, TKW, DHE, and DMA at four test locations.

Table 21: Overall mean value of Best linear unbiased prediction (BLUP)

Genotype	PH	FLL	CC	SL	NTPP	NGPS	SDW	DFF	DM	ETPP	GWPS	TGW	YPP	YPPLOT
BARI_7	95.6	13.8	44.1	8.42	22.8	57	27	63.5	106	19.3	36.5	37	34.5	166
BARI_8	94.1	13.8	44.6	9.20	22.5	59.7	26.2	62.9	105	18.5	35.3	35.1	40.4	172
BARI_9	92.9	13.4	46.8	9.14	22.4	55.2	30.5	64.5	107	18.9	38	41.7	37.3	182
EEB_114	106	16.1	42.9	8.73	24.1	38.4	57.1	82.8	126	16.6	42.1	37.2	21.9	107
EEB_152	105	13.4	43	10.5	25	33.2	42.5	74.8	112	16.1	32.8	28	17.3	101
EEB_18	98.1	15.9	42.9	7.10	26.3	29.2	51.8	70.7	121	14.7	37.9	33.5	14.8	101
EEB_409	94.8	14.1	44.8	9.53	21.7	25.4	40.2	70	103	13.1	31.4	52	17.5	93
EEB_450	105	15	45.3	9.85	25.7	50.4	36.5	68.5	102	18.3	34.8	39.1	35.6	171
EEB_91	95.7	14.4	47.2	6.71	22.6	33.7	25.5	72.8	115	18.1	29.4	42.4	23.4	107

Here, PH= plant height, FLL= flag leaf length, CC= chlorophyll content, SL= spike length, NTPP= number of tillers per plant, ETPP= effective tillers per plant, NGPS= number of grains per spike, GWPS= grain weight per spike, SDW= shoot dry weight, DFF= days to flowering, DM= days to maturity, TGW = thousand grain weight, YPP= yield per plant and YPPLOT= yield per plot.

4.11 Multi - trait genotype- ideotype distance index (MGIDI)

Multi - trait genotype- ideotype distance index (MGIDI) was computed based on quantitative and qualitative traits. To select potentiality high- yielding varieties, superior genotypes must be selected with accuracy (Tebra *et al*., 2023). Thus, to account for the multicollinearity problems leading to false conclusions DeLacy *et al*., 1996; Chandra *et al*., 2020; Al-Otayk *et al*., 2019 and Olivotoet *et al*., 2021) proposed the FAI-BLUPS, which is a multi-trait selection index based on factor analysis associated with the best liner unbiased prediction. The selection index FAI-BLUPS (Rocha *et al*., 2019 and Chandra *et al*., 2020) helped to identify genotypes with traits closer to the desired phenotype, which are a short phenology period (decrease) coupled with a high yield and superior morphological traits (increase) (Tebra *et al*., 2023).

The experimental genotypes are ranked from the highest to the lowest value of the MGIDI so that the genotype with the highest value of MGIDI is in the center and the genotype with the lowest value of MGIDI is located in the outer most circle. The genotypes determined in red color dots were selected based on their MGIDI values at 20% selection intensity (Dariush *et al*, 2023).

In fig 16, according to30% selection intensity (SI) BARI_7 was the first rank followed by EEB_450 and BARI_9 as the most ideal stable genotypes in Rangpur location which was same as Panchagarh location. By using same SI index, EEB_91 was the first rank followed by BARI_8 and BARI_9 as the most ideal stable genotypes in Dinajpur location which were further used to compute selection differentials. The selection differential is the difference in mean phenotype between individuals selected and the population mean (Niranjana *et al*., 2021).

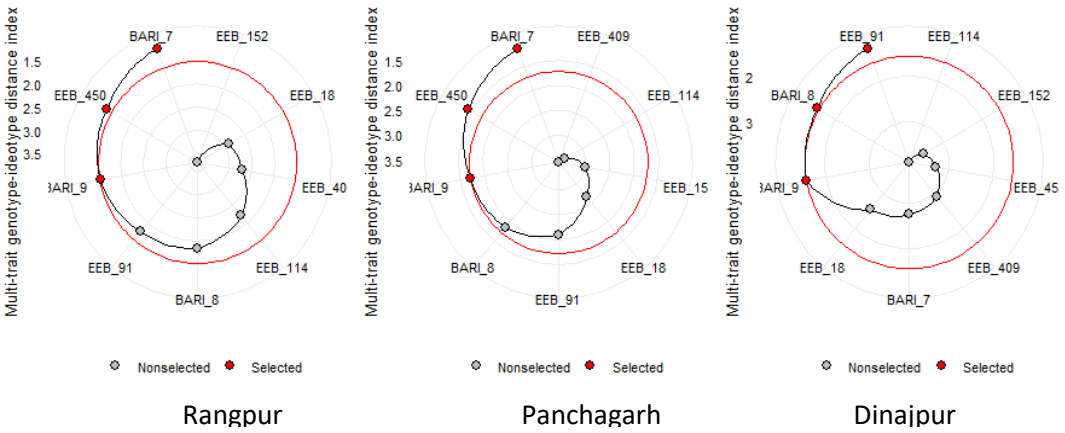
Sharif *et al*. (2021) and Koundinya *et al*. (2021) reported that MTSI would be very useful to the plant breeders for the selection of superior genotypes for multiple traits based on multi-environment data. Zufo *et al*. (2020) used MTIS to identify stable soybean genotypes under drought and salinity stress conditions. Based on the MTSI results, Rajabi, *et al*. (2022) introduced five sugar beet genotypes as stable genotypes under field condition infected with rhizomania disease. These results were consistent with the findings obtained from this study regarding the efficiency of MTSI in identifying superior genotypes.

Since grain yield is a quantitative trait, it is usually influenced by genotype, environment, and other growth traits; hence, selecting better genotypes through indirect selection using other traits can

increase genetic progress (Tucak *et al*., 2020). Previously, most breeders applied classic stability models to identify a stable genotype (Araus *et al*., 2002). These models are often determined only by grain yield data, and ignore other agronomic traits. To solve this challenge, several multi-trait-based selection indices have been proposed. One of these indices is the multiple trait selection index (MTSI) (Abdolshahi *et al*., 2015).

The MGIDI index is a unique and easy-to-interpret selection procedure that has many practical applications to obtain long-term genetic gain in primary traits (such as grain yield) without jeopardizing gains of secondary traits (such as plant height) (Ashok *et al*., 2023).

In order to identify the barley genotypes that were most stable and suitable in three locations, a Venn diagram was utilized. Figure 17 represents the Venn diagram of the most stable genotypes. A total of 3 genotypes were considered from the most stable group in Panchagarh, Rangpur and Dinajpur, respectively. The genotypes that exhibited overlap in both locations were identified. Among three locations, 1 genotype was found to be common across the year that was BARI_9. Among them, 3 genotypes showed overlap in both locations Rangpur and Panchagarh, including lines EEB_450, BARI_7 and BARI_9. In Dinajpur only found BARI_8 and EEB_91 genotypes. Alireza *et al.* (2023) showed that each selection index identified different genotypes. However, the four genotypes G18, G24, G29, and G57 were highlighted as desirable genotypes in terms of grain yield and other agronomic traits for barley. Similarly, Pour-Aboughadareh and Poczai (2021) Selami *et al*. (2021) Costa *et al*. (2023), Hussain (2021) and Zali *et al.* (2023) confirmed the effectiveness of these selection indices in various crops such as wheat, lentil, mango, chickpea, and barley, respectively.



Selection Index= 30%

Figure 16: MGIDI selected genotype in three locations

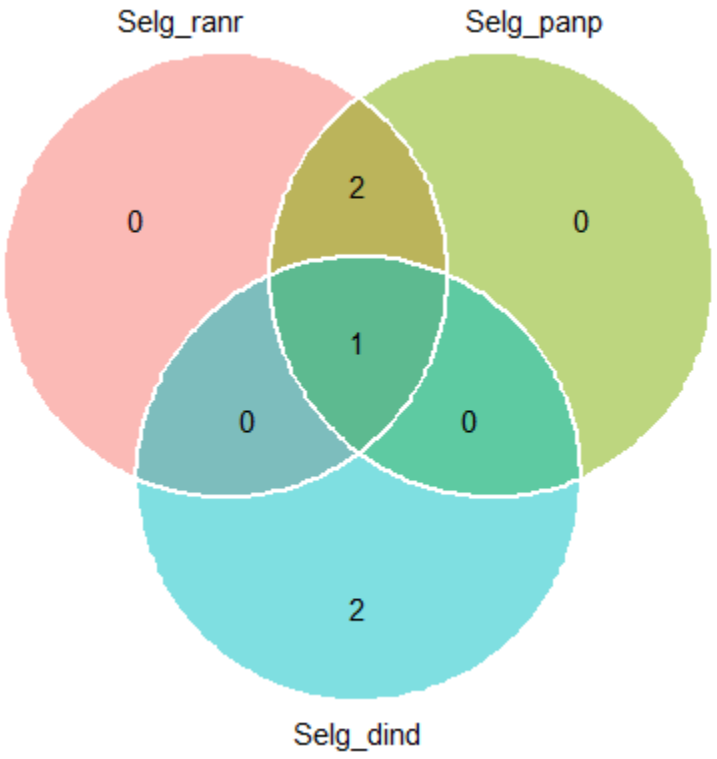


Figure 17: Venn diagram

4.12 Interrelationship among all stability analyses

The stability analysis of barley refers to studying how barley varieties perform across different environments or conditions. This analysis is crucial for understanding the adaptability and consistency of barley varieties across various locations, climates, and management practices. In the study we use several stability analyses such as parametric and non-parametric stability statistics, AMMI, GGE and MGIDI of barley, and they are interconnected in multiple ways.

According to stability soft analysis EEB_450 is the most superior stable genotype between all studied genotypes. In case of AMMI analysis, we found superior environment and stable genotypes. So, we found EEB_450, BARI_8 and BARI_9 is more superior and Panchagarh is more suitable environment for barley cultivation. For GGE analysis for three different traits, we found EEB_450, BARI_7, BARI_8 and BARI_9 that perform well across a range of environmental conditions are considered more stable and better perform Panchagarh environment.

According to MGIDI selection process, we found similar result. EEB_450, EEB_91, BARI_7, BARI_8 and BARI_9 is often more stable across different environments compared to those with all phenotypic backgrounds.

The ultimate goal of stability analysis is to identify barley varieties that combine high yield potential with stability across diverse environments. Breeding programs aim to develop varieties that exhibit both high mean performance and low sensitivity to environmental fluctuations. Different methods are analyzed to know how robust is our selection process because, further its help to develop a barley variety. So, we claimed that EEB_450 is more stable like BARI released variety.

The stability analysis of barley is a multidimensional process that considers the interactions between genetic factors, environmental conditions, management practices, statistical methods, breeding objectives, and market demands. Understanding these interrelations is essential for developing and selecting barley varieties that can thrive in various agricultural settings and meet the needs of farmers and consumers.

Experiment No. 2: Assessment of genetic variation in 9 barley genotypes through molecular study

In this study, seven (7) microsatellite markers were used genotypes. The molecular research was undertaken in the Molecular Breeding Laboratory of the Department of Genetics and Plant Breeding, HSTU. The experiment's outcomes are reported under the subsequent subheadings.

4.13 Analysis of DNA fingerprinting based on SSR markers

In the beginning, DNA was taken from young leaves of 3-week-old seedlings of 9 genotypes of barley. The CTAB technique was modified to extract the DNA. Prior to PCR amplification, the quality of the extracted DNA samples was evaluated through quantification using a Thermo Scientific NanoDropTM1000 Spectrophotometer. Nine different genotypes of barley had DNA quantities ranging from 55.4 to 397.3. Dido *et al*. (2022) studied 384 Ethiopian barley genotypes collected from different barley growing regions of Ethiopia using 49 simple sequence repeat (SSR) markers. The 3 ng/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 plus DNA ladder.

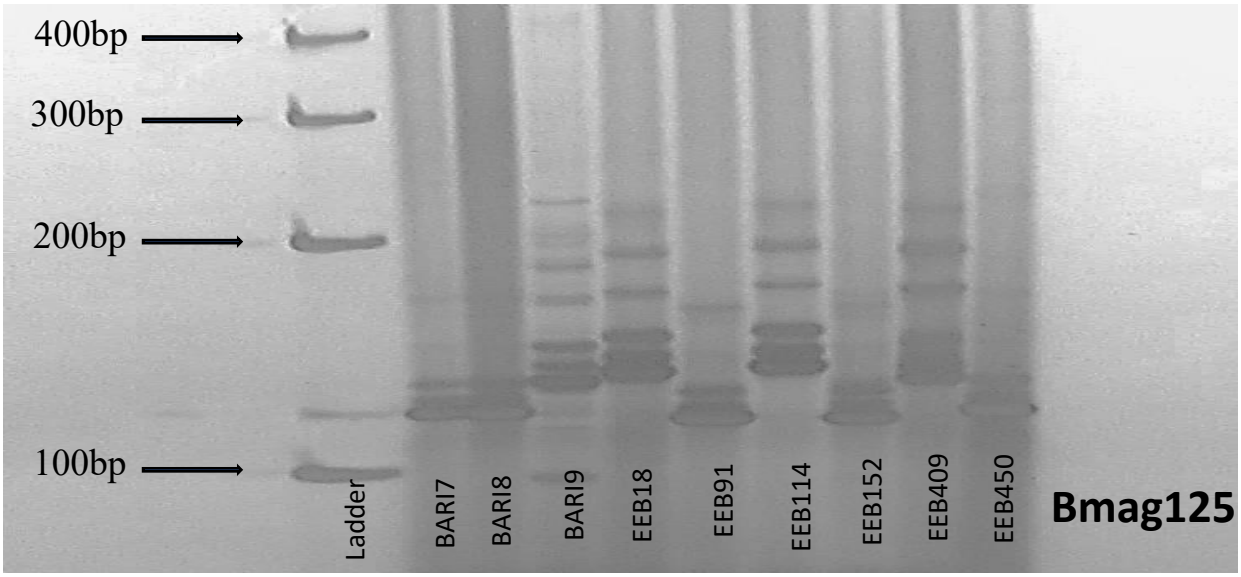
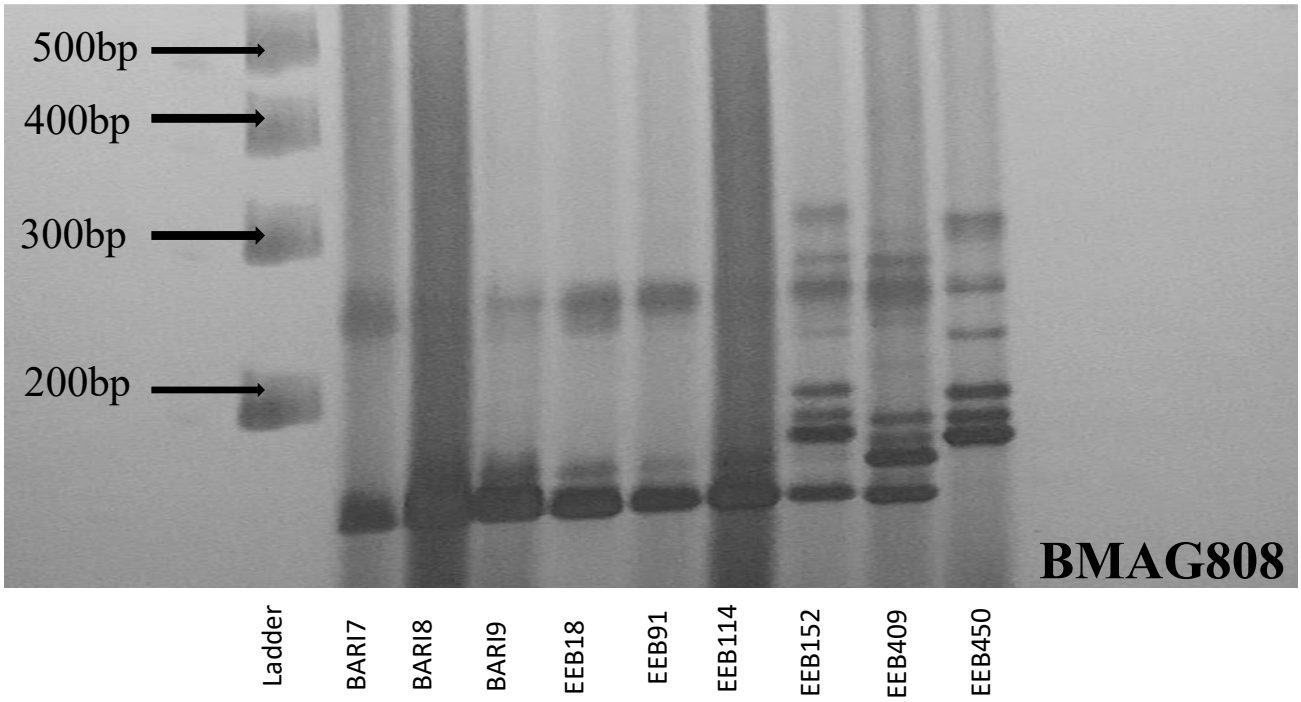


Figure 18: Gel picture of microsatellite markers obtained from polyacrylamide gel electrophoresis using 100 bp plus ladder



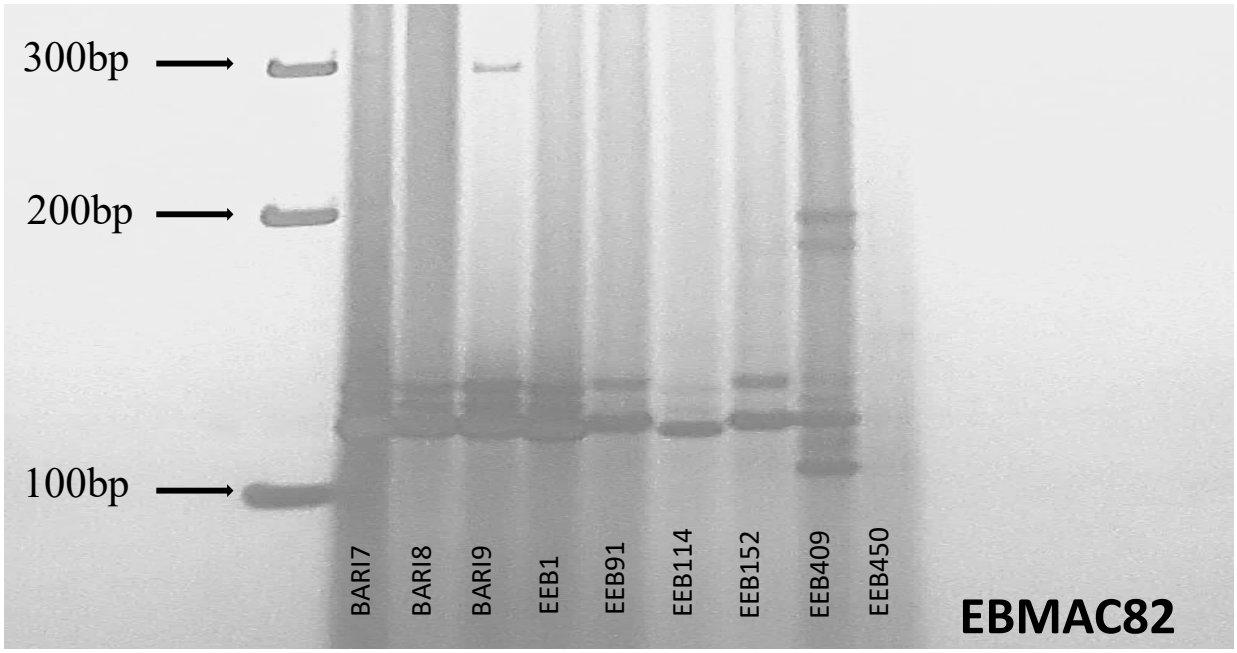


Figure 18: Gel picture of microsatellite markers obtained from polyacrylamide gel electrophoresis using 100 bp plus ladder (continued)

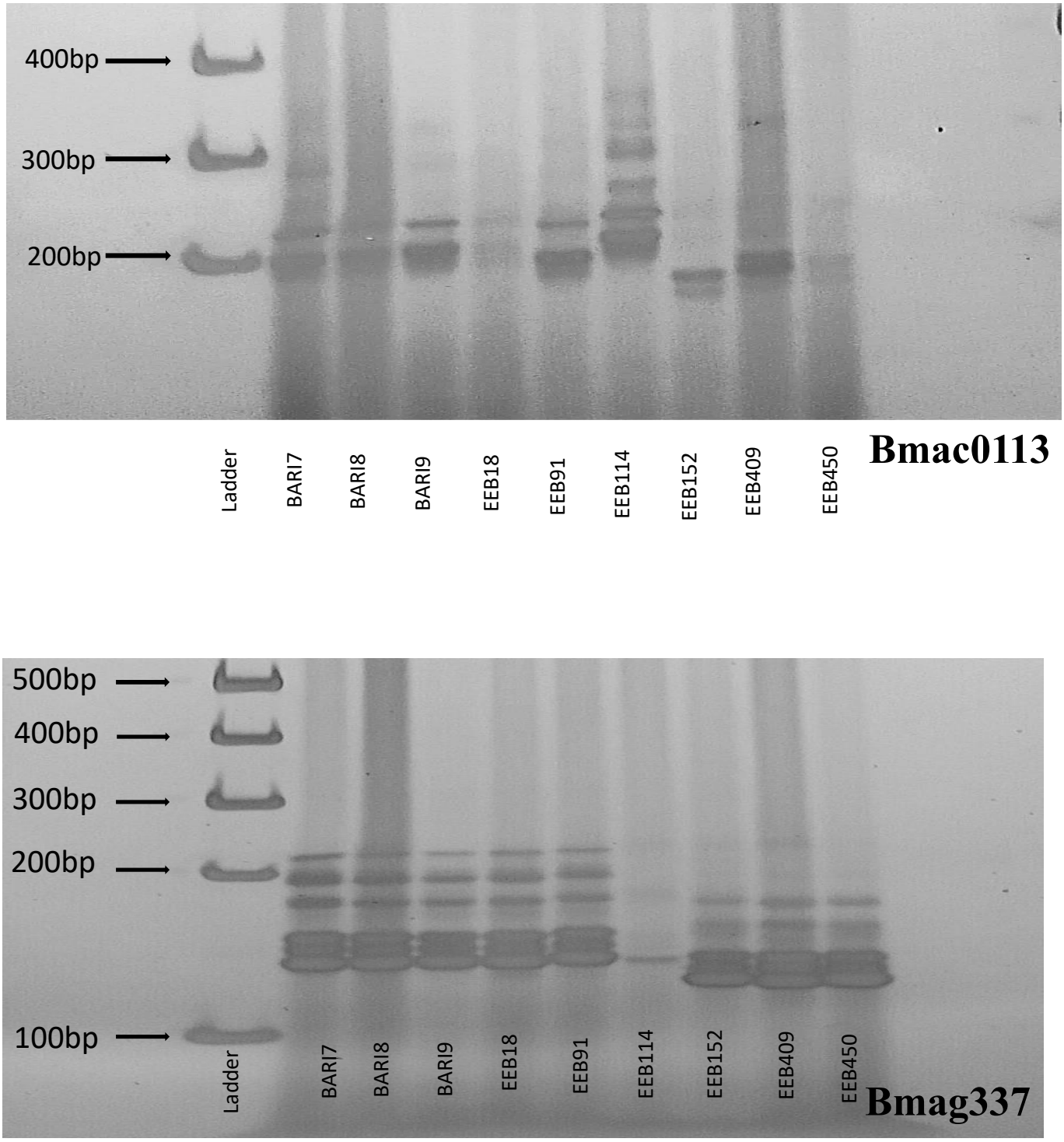
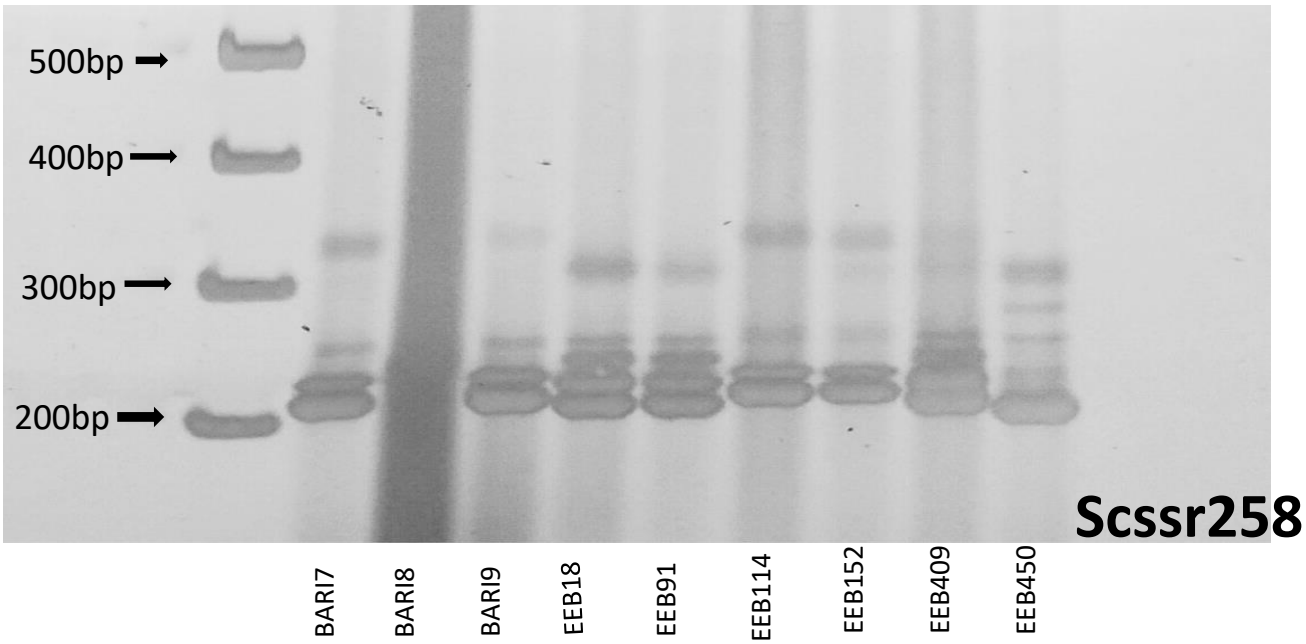


Figure 18: Gel picture of microsatellite markers obtained from polyacrylamide gel electrophoresis using 100 bp plus ladder (continued)



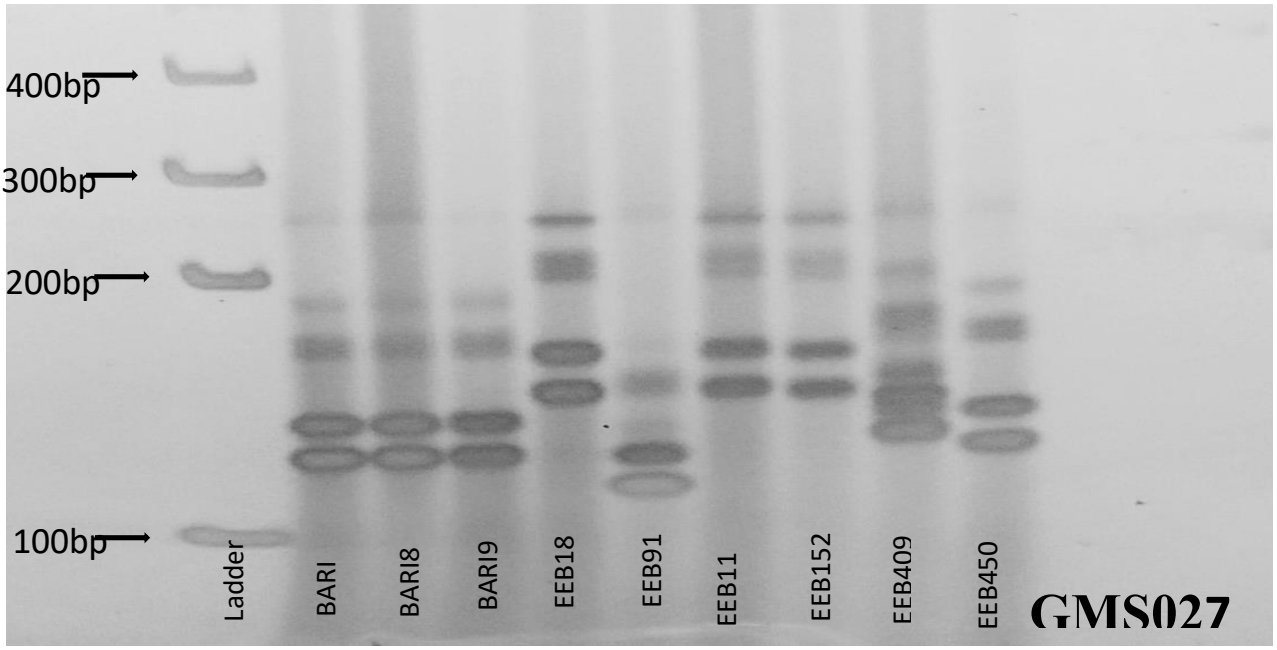


Figure 18: Gel picture of microsatellite markers obtained from polyacrylamide gel electrophoresis using 100 bp plus ladder

4.14 Assessment of polymorphism from SSR Profiles

A total of 36 alleles were identified with 7 SSR markers over 9 barley genotypes (Table 22). According to the Table 2, maximum range of band sizes was found by Bmag125 (100- 325bp) which was followed by BMAG808 (100- 310bp). The number of alleles per marker ranged from 3 to 6, with an average of 5.14 alleles across the 36 allele. The marker Bmag125 produced the highest number of polymorphic alleles of six (6) including Bmac0113 and Bmag337. BMAG808, EBMAC624 and GMS027 produced five (5) alleles followed by Scssr25691 produced the least number of polymorphic bands per locus of three (3) respectively (Table 22). The PIC (Polymorphism Information Content) values of SSRs ranged from 0.63 to 0.80 with an average of 5.19. The highest PIC value (0.80) was recorded for Bmag125, and Bmag337 followed by GMS027 (0.78) while the lowest value was Scssr25691 (0.63). 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 22: Allele number, size range and polymorphism information content (PIC) found among 9 barley genotypes for 7 microsatellite markers

Sl. No.	Markers	Allele No.	Size range (bp)	PIC
1	BMAG808	5	100-310	0.70
2	Bmag125	6	100- 325	0.80
3	Bmag006	5	100-200	0.71
4	Scssr25691	3	100-280	0.63
5	Bmac0113	6	100-290	0.77
6	GMS027	5	100-250	0.78
7	Bmag337	6	100-210	0.80
	Total	36		5.19
	Average	5.14		0.74

4.15 Model-based population structure

9 barley genotypes were evaluated for estimation of population structure based on 7 markers using Structure software. The estimated membership fractions of the 9 genotypes for different k values, ranged from 1 to 10 (Figure 19). The population structure analysis declared the log-likelihood value (ΔK) maximized to the highest value of at K= 2 (Figure 20), demonstrating a sharp peak expressing the classification of entire genotypes into three specific sub-groups, here denoted as Population I Population II respectively (Figure 20).

The Population I consisted 66 % of genotypes, Population II consisted 44% of genotypes. Here the membership fractions were used to classify the populations either pure or an admixture type. Here, in population I comprised of 6 genotypes, where 2 genotypes were pure and 4 were admixed (Figure 20). Population II comprised of total 3 genotypes those found pure. Mohammadi *et al.* (2020) also revealed that K=3 population structure with SSR markers in barley.

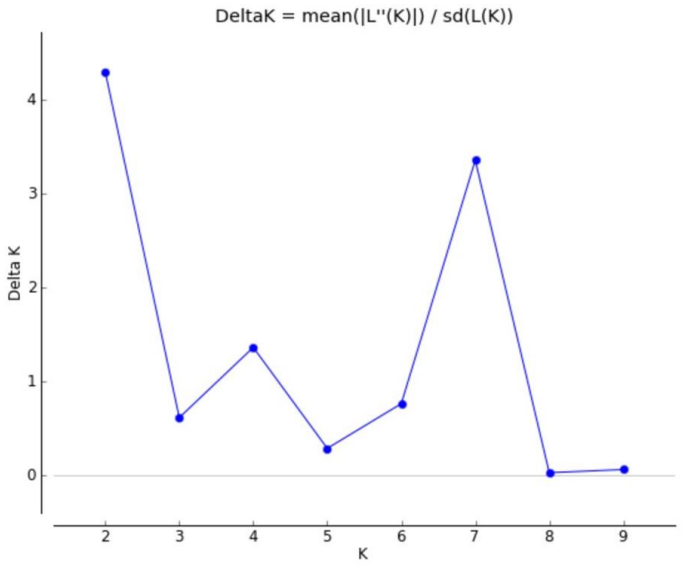


Figure 19: Representation of population structure dividing the landrace in two subgroups based on K value

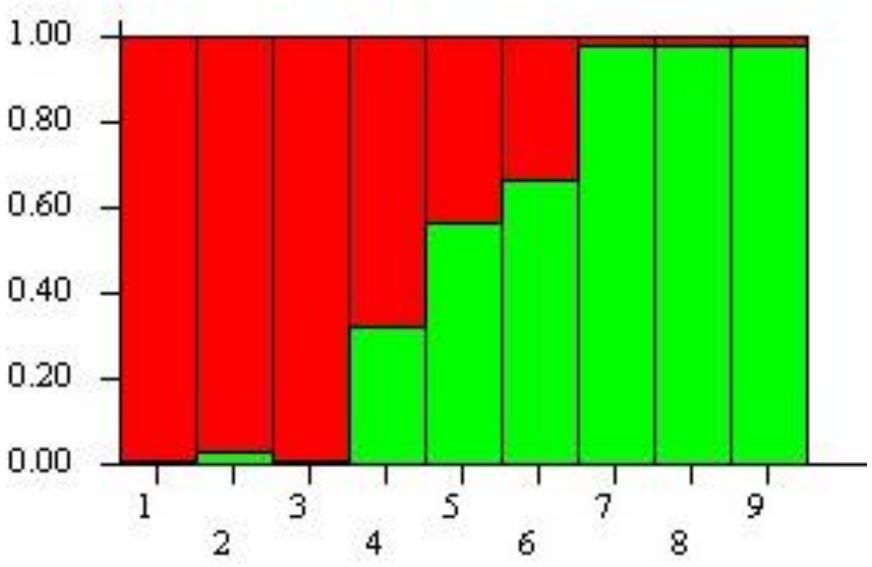


Figure 20: Population structure at K=2 of 9 barley genotypes based on genotypic data using 7 microsatellite markers. Color codes are – Population I= Red and Population II = Green. Here, 1= BARI_Barley 7, 2= BARI_Barley 8, 3= BARI_Barley 9, 4= EEB_18, 5= EEB_91, 6= EEB_114, 7= EEB_152, 8= EEB_409 and 9= EEB_450.

4.16 UPGMA dendrogram

The genetic distance-based results in the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis revealed three major clusters (Cluster I, and Cluster II) in 9 barley genotypes with a dissimilarity coefficient.

Cluster I consisted of 6 genotypes namely-EEB_18, EEB_91, EEB_114, BARI_7, BARI_8 and BARI_9(figure 21). Cluster I consisted of 4 sub group, EEB_18 and EEB_91 was one, BARI_7 and BARI_8, BARI_9, EEB_114 consisted of another sub cluster (figure 21). Among them the highest and lowest similarity found between sub-cluster comprised viz, BARI_7 and BARI_8. Cluster II consisted of 3 genotypes viz., EEB_152, EEB_409 and EEB_450(figure 21). 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).

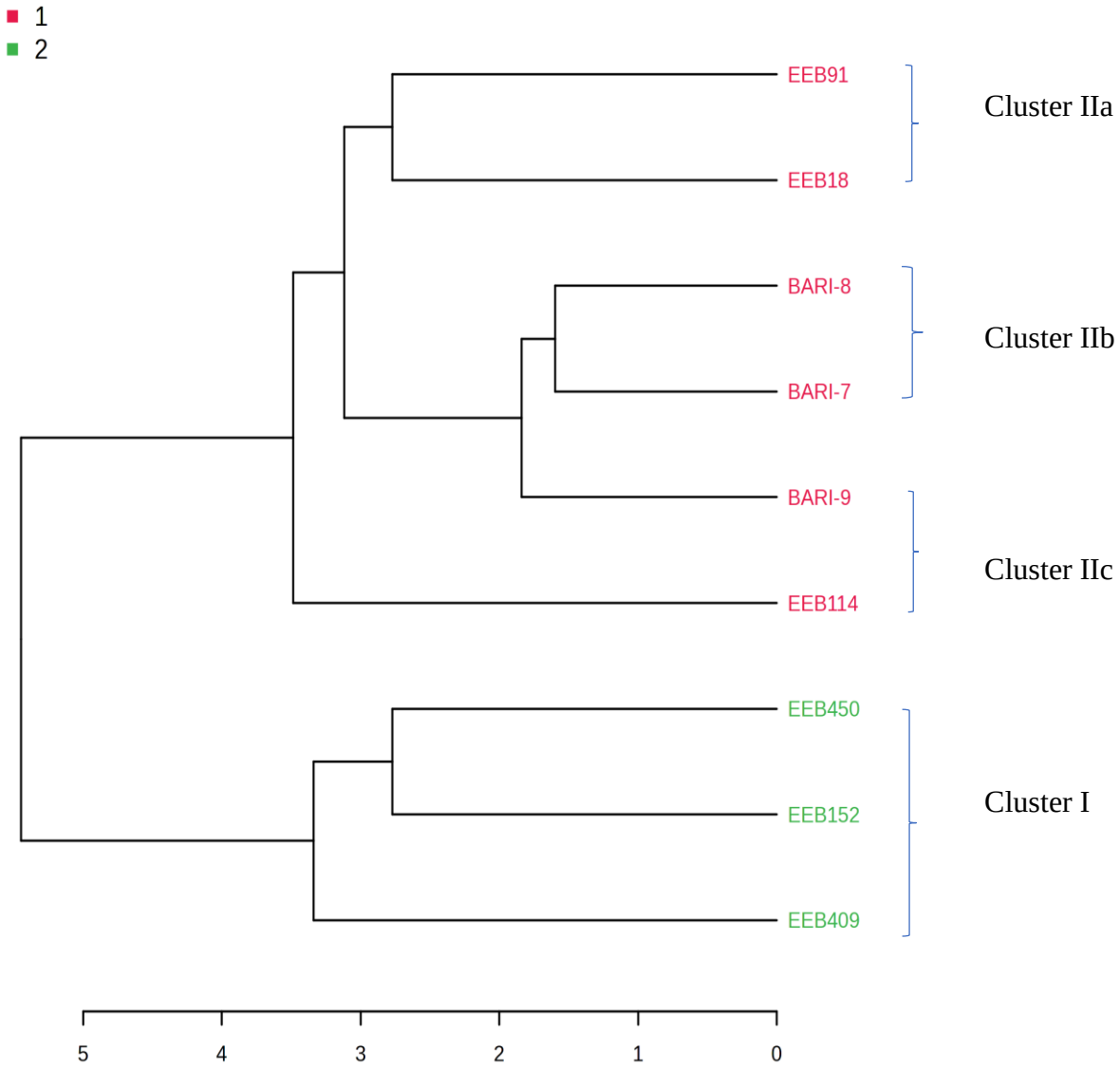


Figure 21: An UPGMA cluster dendrogram showing the genetic relationships between 9 barley landraces based on alleles detected by 7 microsatellite markers

4.17 Principal component analysis

Principal Component Analysis Associations among 9 barley genotypes were computed using PCA method. Here, first principal component (PC1), and the second principal component (PC2) explained 32.4% and 19.5% of total variation, respectively and localization of genotypes in a 2D PCA plot indicates the genetic distances among the barley genotypes (Figure 18). Here, Population I and Population II were not 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 22). In PCA scree plot, the first three eigen values explained 67.5% of the cumulative variation, which were then plotted to identify the diversity of the genotypes (Figure 22). The first three principal components had eigen values of 32.4%, 19.5% and 15.6% and an overall maximum cumulative variation of 67.5% were observed with first three components of principal coordinates (Figure 22). Yadav *et al.* (2020) also revealed that in the two-dimensional plot, with the first two principal components, the close clustering of the varieties among 88 barley germplasm accessions from a common developing centre could be seen.

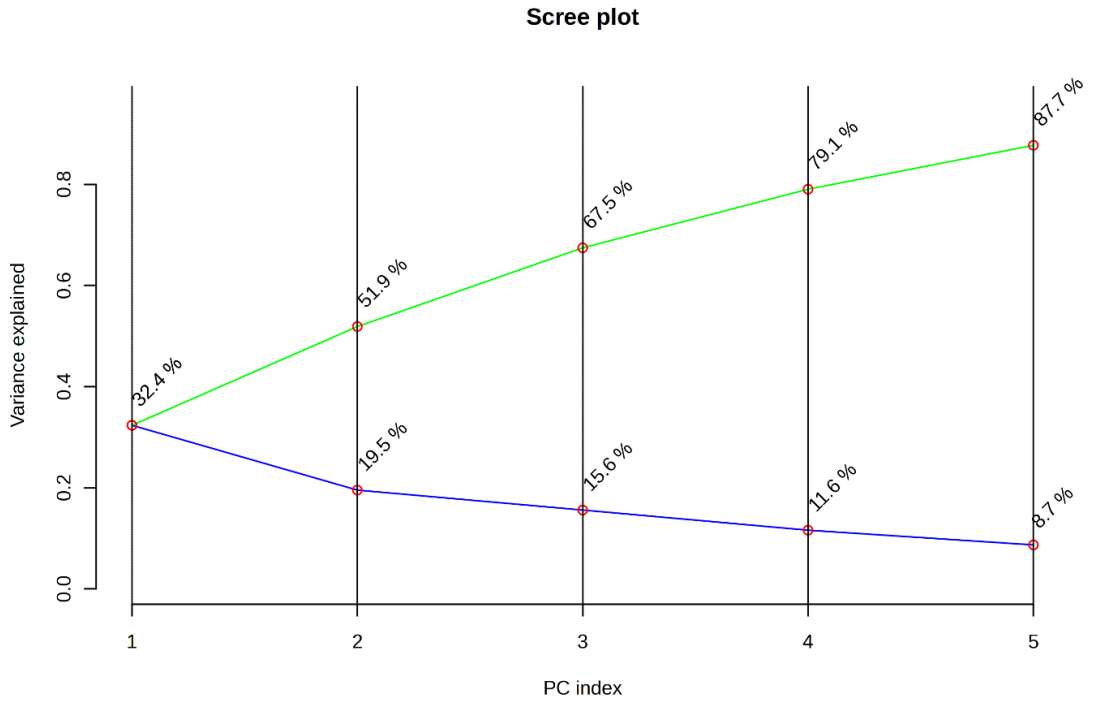


Figure 22: The scree plot displays Principal coordinate analysis of top 5 PCs for 9 barley genotypes based on SSR marker data

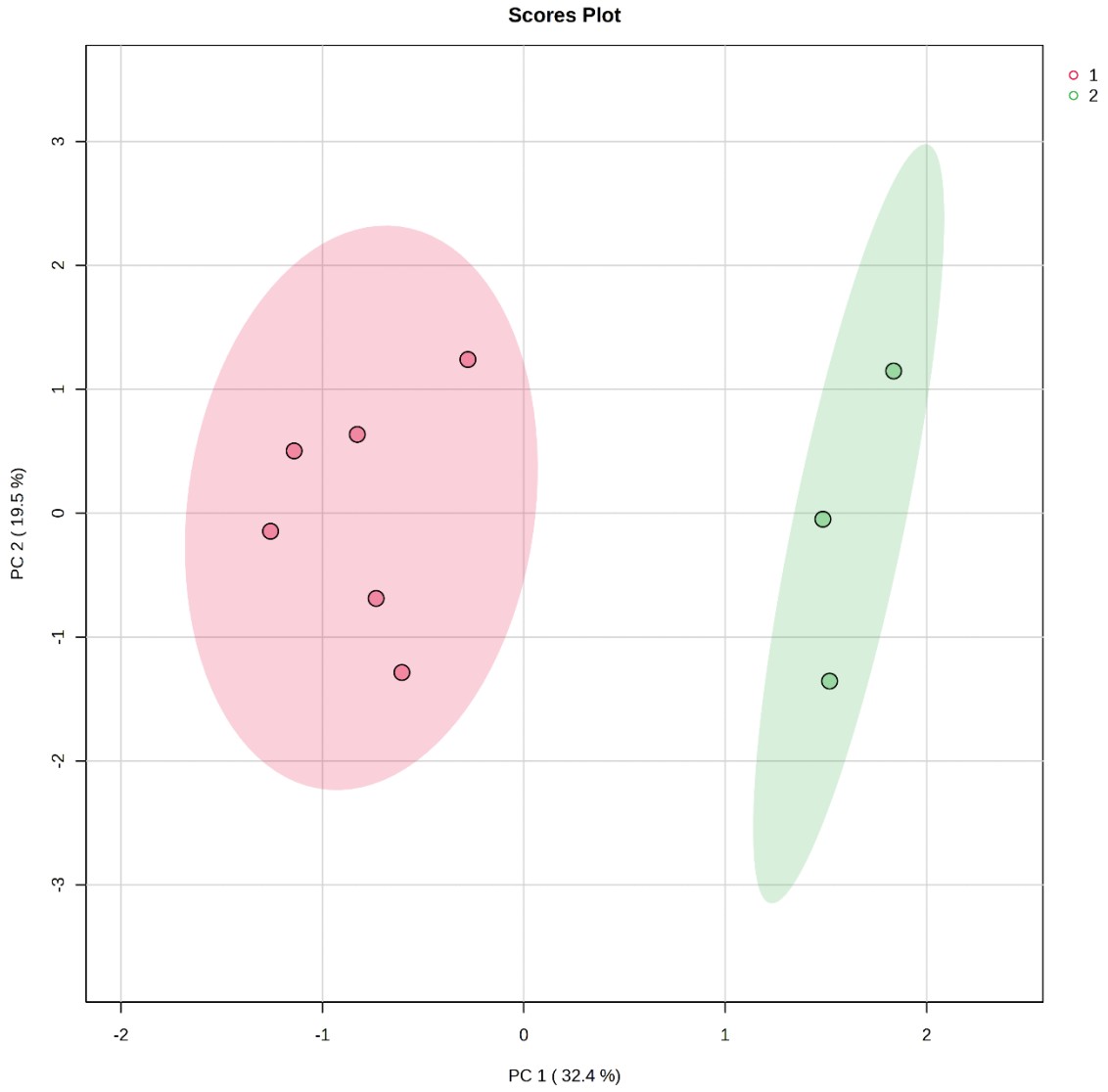


Figure 23: Two- dimensional principal component analysis (PCA) based on SSR polymorphisms in the 9 barley genotypes

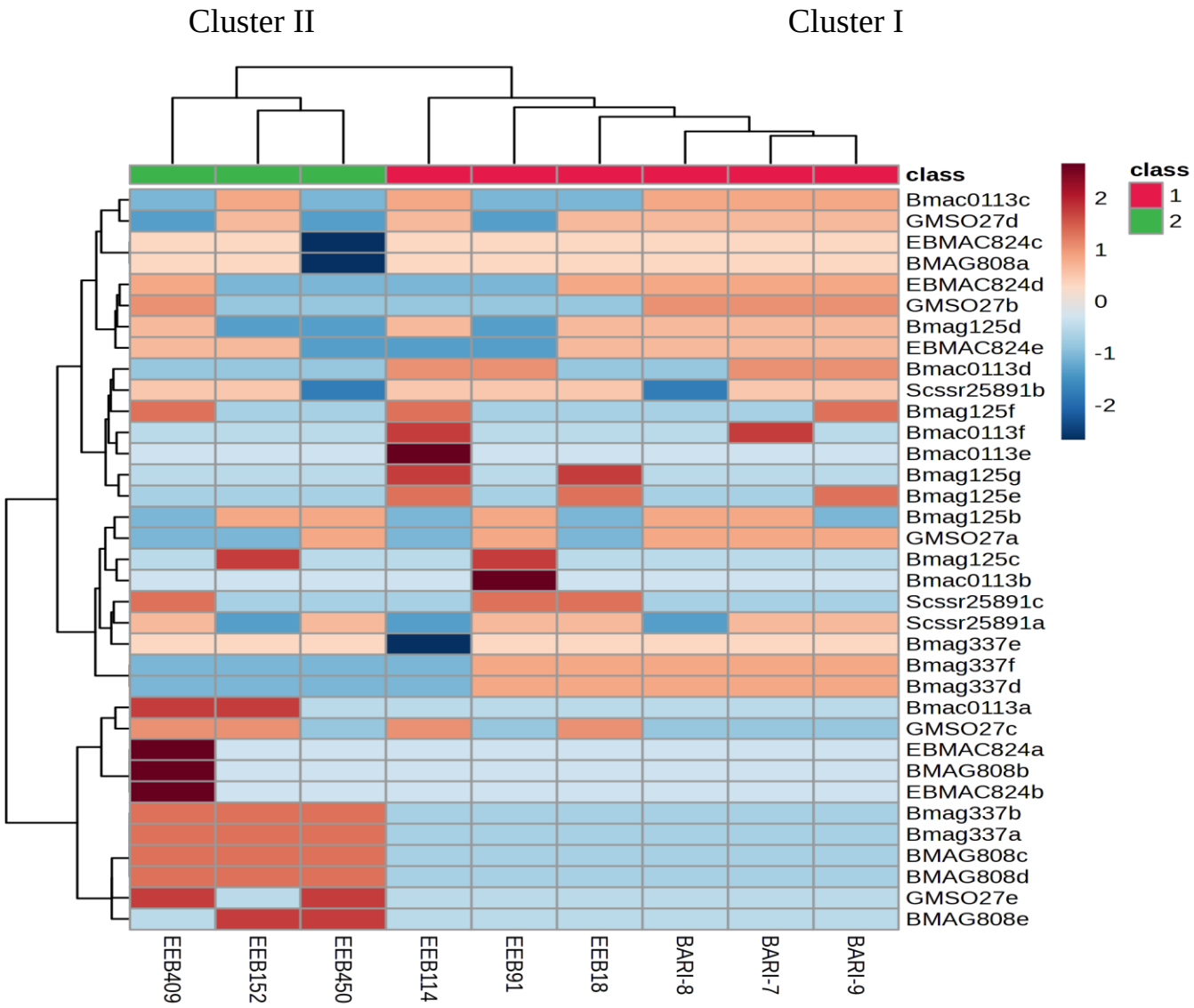


Figure 24: A heat map showing the genetic relationships between 9 barley landraces based on alleles detected by 7 microsatellite markers

4.17 Interrelationship of phenotypic and molecular outcome

All the 9 barley genotypes were evaluated individually through molecular and morphologically in the study. Among 6 European barley genotypes, EEB_450, EEB_409, EEB_152, EEB_114, EEB 114, EEB_91, and EEB_18 and 3 check variety BARI_7, BARI_8 and BARI_9 have dissimilar in EEB_450, EEB_409 and EEB_152 genotypes. The genotype EEB_450 are more stable.

In experiment II, the respected genotypes obtained from previous experiment i.e. EEB_450 located in UPGMA cluster II. On the other hand, the respected check varieties genotypes i.e. BARI_7, BARI_8 and BARI_9 are also located in the same cluster as UPGMA cluster I.

With comparison between both molecular and phenotypic results and according to their both data is confirmed that EEB_450 is different from BARI released variety and it is more stable and high yielding genotype based on multi location trials.

CHAPTER V

SUMMARY AND CONCLUSION

One of the most significant cereal crops in the world is barley. It is a minor crop in Bangladesh, but it has the potential to grow into one of the country's most essential crops. High yielding and stable barley genotype are crucial for characterizing breeding lines and variations and provides the foundation for choosing the right genotypes for creating hybrid varieties. In the current study high yielding and stable barley genotype 9 European barley genotypes were assessed during the rabi season from December to April 2022–2023, in the three multi locations of Dinajpur, Rangpur and Panchagarh.

The analysis of variance revealed a wide range of variation among the genotypes for all the yield and yield contributing characters viz. days to flowering (DFF), plant height (cm) (PH), chlorophyll content (CC), spike length (SL), number of spike per plant (NSPP), number of grains per spike (NGSP), thousand-grain weight (g) (TGW), days to maturity (DM), number of tillers number per plant (NTPP), number of effective tillers per plant (ETPP), grains weight per spike (GWPS), shoot dry weight (SDW), yield per plant (g) (YP) and yield per plot (YPPLOT).

The genotypes studied all were significant at 0.1% level of probability. The CV values which ranged from 1.08% for yield per plot in Panchagarh environment to 15.68% for number of spikes per plant in Rangpur environment.

The study found that the optimal temperature conditions for barley plants in Panchagarh were higher than those in Dinajpur and Rangpur, with higher values for days to maturity, number of spikes per plant, effective tillers per plant, yield per plant, and yield per plot. Chlorophyll content and flag leaf length were higher in Rangpur, while spike length and number of grains per spike were higher in Dinajpur. Panchagarh plant height was higher in Panchagarh.

The study analyzed nine barley genotypes for quantitative traits, revealing significant differences in plant height, flag leaf length, and spike length. The highest plant height was found in EEB_114 at Panchagarh, while the lowest was in EEB_18 at Rangpur. Flag leaf length varied significantly among genotypes, with the highest being 22.46 cm in Rangpur and the lowest being 10.00 cm in Dinajpur. The study also found significant differences in spike length at the mature stage, with the

highest being 11.67 cm in Dinajpur and the lowest being 5.50 cm in Panchagarh. These findings highlight the importance of genetic dissection in cereal design for future production and yield.

Chlorophyll is crucial for photosynthesis, with higher levels indicating more food storage and energy supply. Chlorophyll content varies across three environmental conditions, with the highest content found in genotype EEB_409 in Rangpur and the lowest in genotype BARI_7 in Panchagarh. The efficiency of chlorophyll photosynthetic potential can be assessed using traditional spectrophotometric measurements. Days to 50% flowering range from 61.00 to 85.00, with the 61-day genotype showing superior results. Days to maturity range from 99.0 to 127.0, with the maximum required in Panchagarh EEB_114 genotype. Early maturing types can be selected as early type varieties or used as parents for cultivar development.

The study found that Panchagarh EEB_450 had the highest number of spikes per plant at 29.60, while Rangpur EEB_14 had the second highest at 29.2. The highest number of tillers per plant was 38.00 in EEB_152, while the lowest was 11.20 in EEB_409 in Rangpur. The highest number of effective tillers per plant was 29.60 in Panchagarh EEB_450, while the lowest was 7.60 in Rangpur EEB_18. These results suggest that EEB_450 genotypes produce more effective tillers, ensuring higher yield.

The study found significant differences in grain number per spike, shoot dry weight, and grain weight per spike between genotypes. The maximum number of grains per spike was found in genotype BARI_8, while the minimum was found in genotype EEB_18. Shoot dry weight was highest in genotype EEB_114, while grain weight per spike was highest in genotypes Panchagarh EEB_114 and Dinajpur EEB_18. The mean and LSD values were 1.72 and 35.35 ± 1.27, respectively.

The study found that the genotype EEB_409 had the highest thousand grain weight in Panchagarh, while the lowest was in Rangpur. The highest yield per plant was found in BARI_8 in Rangpur, while the lowest was in EEB_152. The mean yield per plot was highest in BARI_9 in Panchagarh, followed by EEB_18 in Rangpur and EEB_409 in Dinajpur.

The study analyzed the variability among 14 characters using various parameters such as genotypic variance, phenotypic variance, environmental variance, genotypic coefficient of variation, phenotypic coefficient of variation, environmental coefficient of variation, heritability, genetic

advance, and genetic advance as percent of mean. The results showed that phenotypic variances were higher than genotypic variances for all characters. The highest phenotypic and genotypic variances were recorded with yield per plot in Dinajpur and Rangpur, respectively. The phenotypic coefficients of variance ranged from 7.19% for plant height to 72.04% for days to maturity, while genotypic coefficients ranged from 6.40% for flag leaf length to 55.22% for yield per plant in Panchagarh and Dinajpur. The study also found that phenotypic variance and genotypic coefficients of variation were higher than their corresponding genotypic and genotypic coefficients, indicating that the expression of these characters was influenced by the environment. Heritability analysis estimated the relative contributions of genetic and non-genetic factors to the total phenotypic variance in a population, which is important in quantitative genetics, particularly in selective breeding.

The likelihood ratio test showed a significant impact of genotype on all trait categories in a single-environment analysis. All traits were significant for genotypes and planting time interaction, except for physiological traits. The average deviation was higher each season, indicating a larger genotypic response in different environments. Most genotypes showed an increase or decrease while maintaining stable grain weight and days to maturity. Genetic factors significantly contributed to variance in most traits across all three locations.

The MTSI Index was used to select desired traits in plant species like Rangpur, Panchagarh, and Dinajpur, with a success rate of 86.13% for 9 out of 14 studied traits. High heritability traits included number of grains per spike, shoot dry weight, grain weight per spike, and yield per plot. The MGIDI performed better in choosing qualities with desired gains, with 16 out of 20 traits having desired gains.

The WAASB model, a combination of AMMI and BLUP models, helps plant breeders select genotypes based on stability and performance. High WAASB scores are considered less stable. The model quantifies genotype stability by combining stability and productivity in a two-dimensional plot. The most stable genotypes are those with the least AMMI Stability Value (ASV) scores, while unstable genotypes have high ASV scores. The least genotype selection index (GSI) is considered the most stable genotype. EEB_450 was more stable.

Using statistical techniques such as AMMI (Additive Main Effects and Multiplicative Interaction), GGE biplot (Genotype plus Genotype by Environment Interaction), or other stability analysis

methods, stable barley genotypes have been identified. EEB_450 was more stable than other. These genotypes exhibit minimal genotype-by-environment interaction, meaning their performance remains consistent across different locations and growing conditions. This stability is crucial for ensuring reliable yields and mitigating production risks associated with environmental variability.

Molecular markers, such as SSRs (Simple Sequence Repeats) have been employed to characterize the genetic variation among barley genotypes. Molecular approaches facilitate the identification of key genomic regions associated with desirable agronomic traits, including yield, disease resistance, and stress tolerance. Marker-assisted selection (MAS) enables breeders to efficiently screen and select genotypes with desired traits, expediting the breeding process and enhancing the precision of genotype selection. The EEB_450 had variation among 3 check variety such as BARI_7, BARI_8 and BARI_9.

Integrating data from multi-location trials with molecular analyses provides a holistic assessment of genotype performance and genetic attributes. There had many variations among check variety among EEB line by integrating phenotypic data on yield, stability, and other agronomic traits with molecular markers linked to favorable alleles, breeders can prioritize genotypes with superior performance and targeted genetic characteristics. In conclusion, it is easier to find and select stable, high-yielding barley genotypes like EEB_450 with desired agronomic qualities when multi-location trials and molecular methods are combined. This integrated approach enhances the efficiency and precision of barley breeding programs, ultimately contributing to sustainable agricultural productivity, crop diversification, nutritional, and food security.

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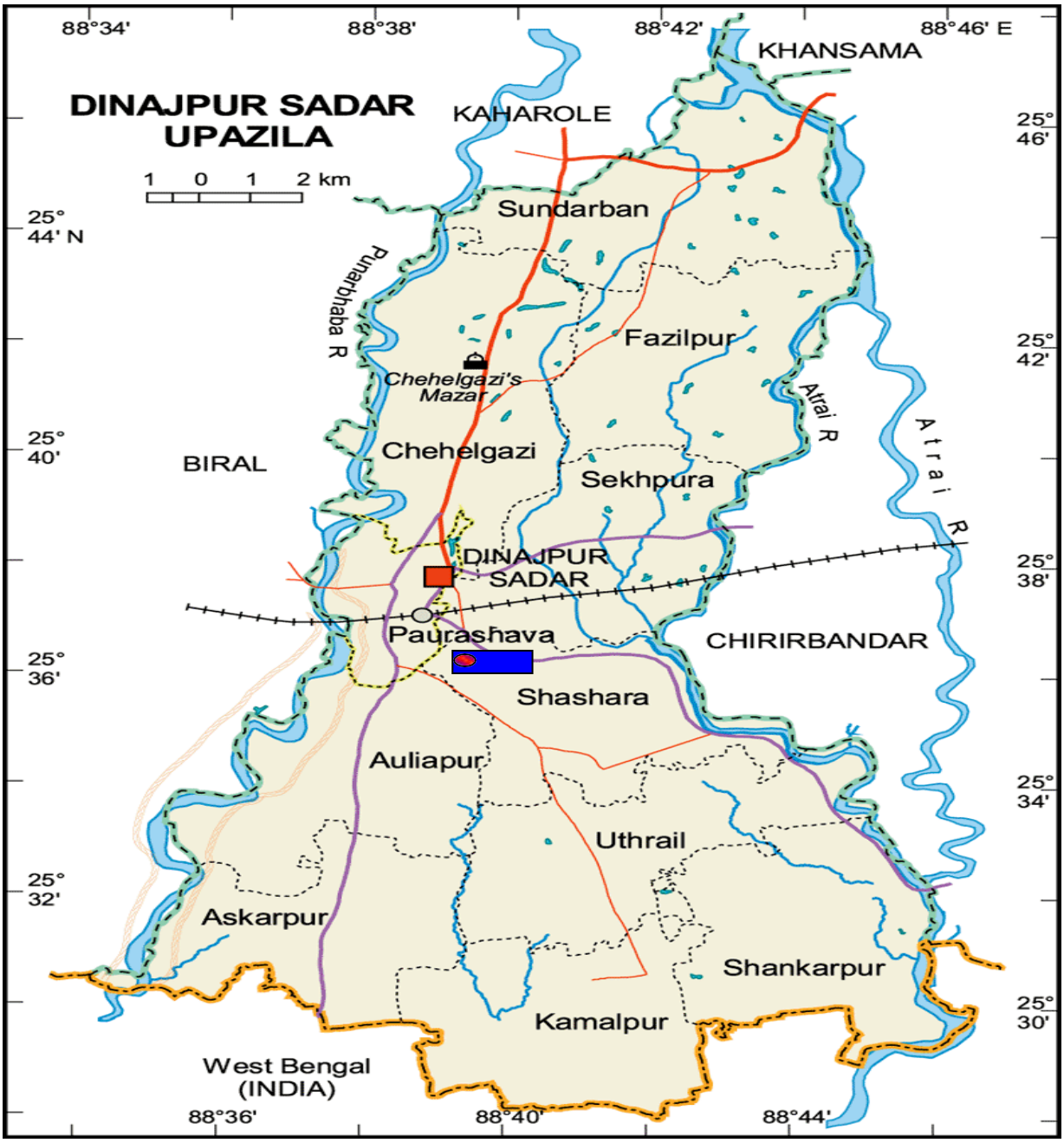
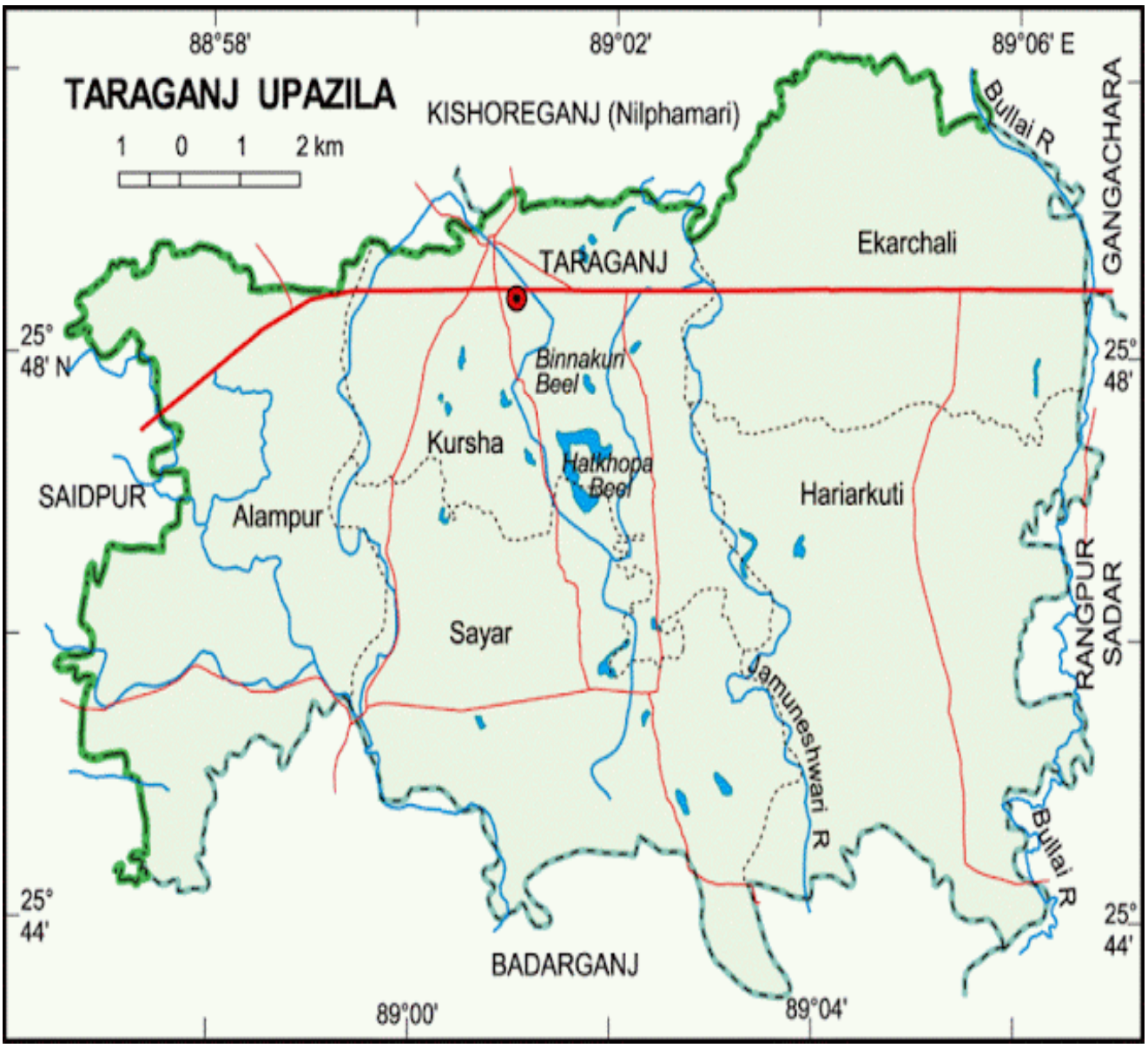
APPENDICES

Appendix I: Map of Dinajpur sadar upazila showing the experimental area



Map Courtesy: Maps of Bangladesh

Appendix II: Map of Rangpur Taraganj upazila showing the experimental area

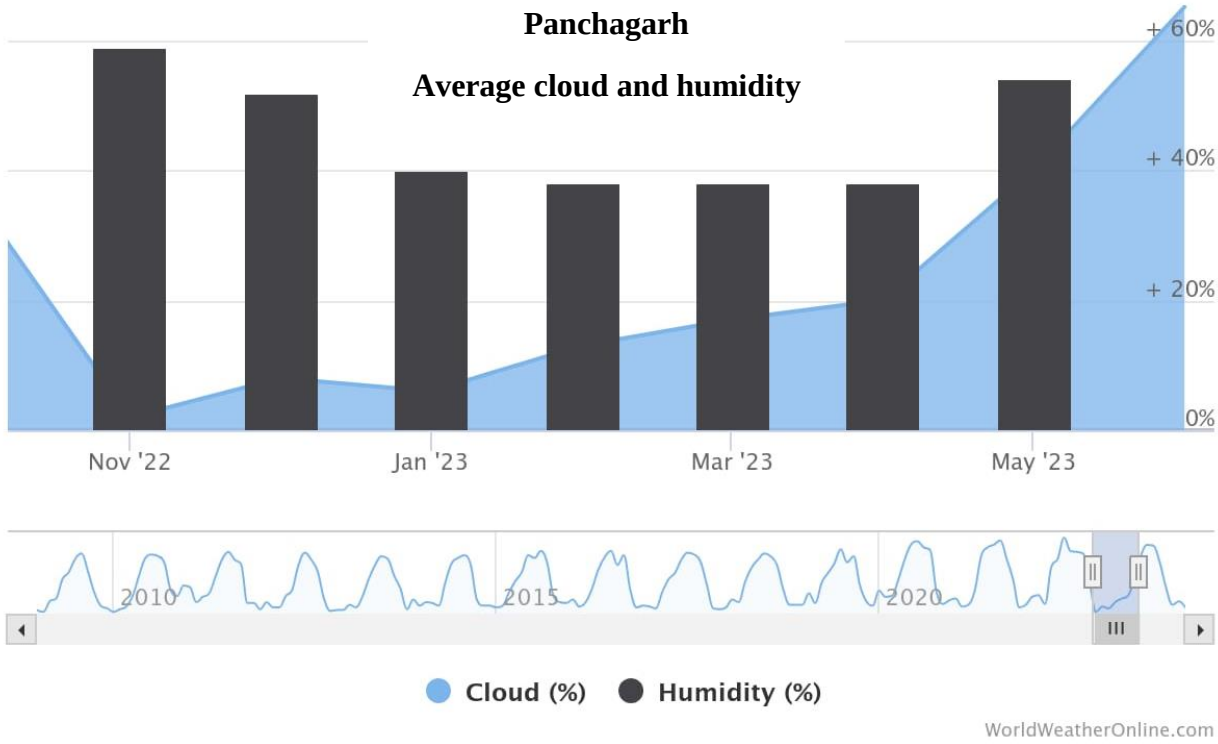


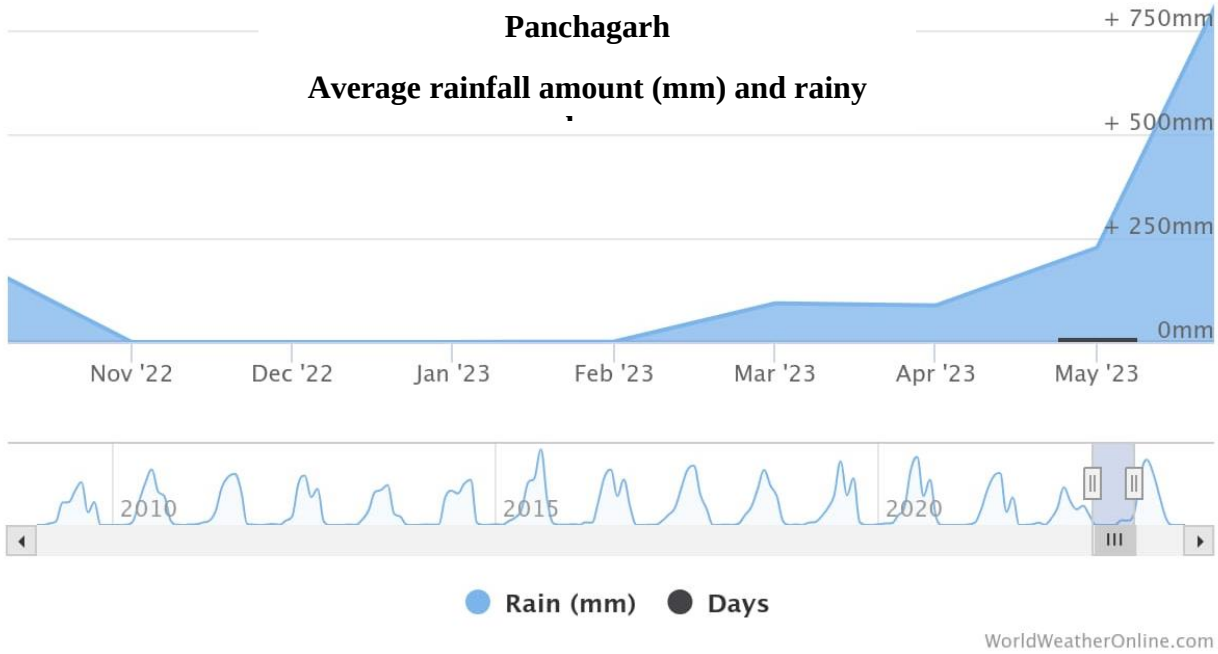
Map Courtesy: Maps of Bangladesh

Appendix III: Map of Panchagarh Debiganj upazila showing the experimental area

Map Courtesy: Maps of Bangladesh

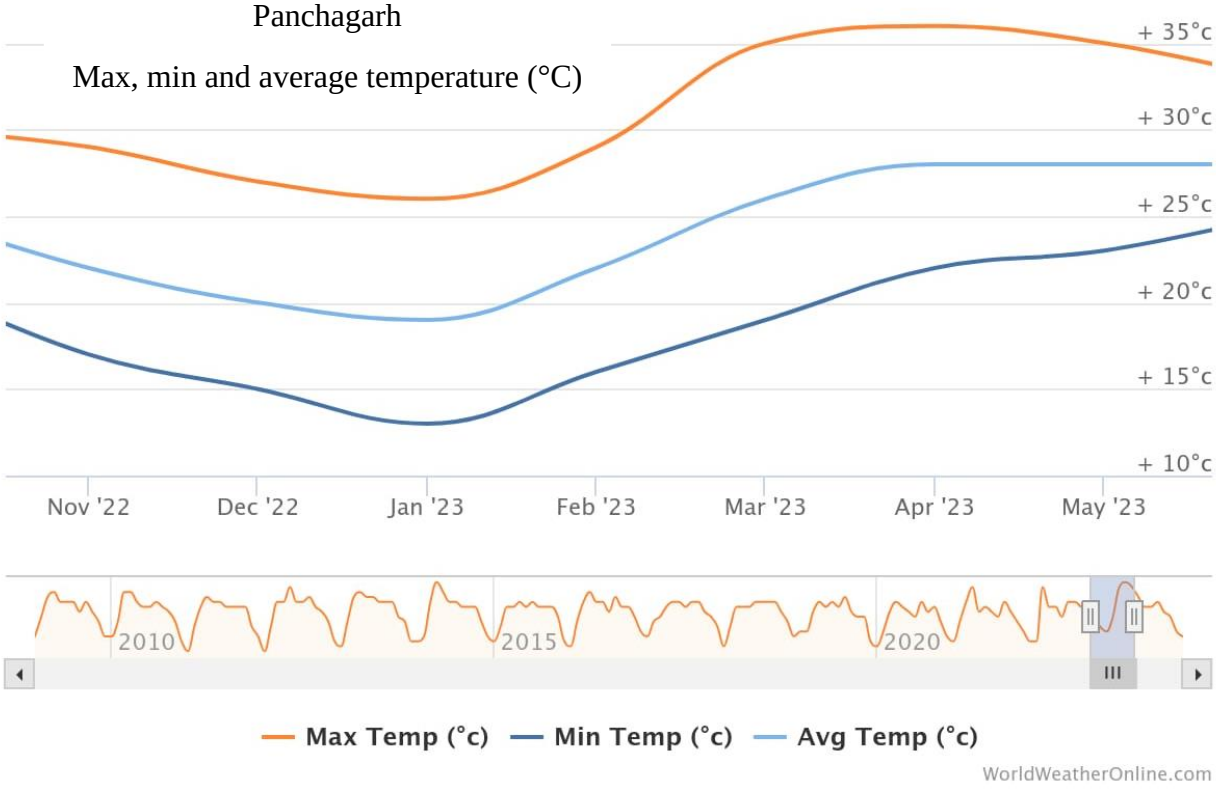
Appendix II: Weather data of the experimental site during the period from November, 2022 to May, 2023 at three environment





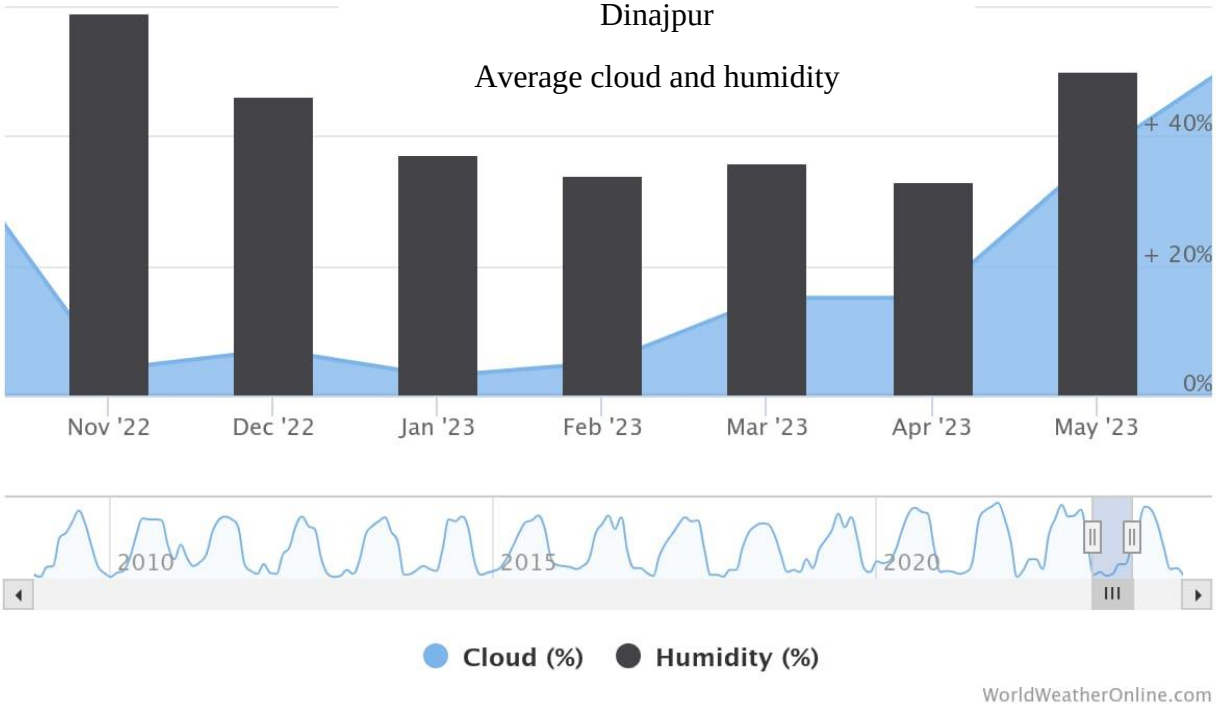
Panchagarh

Max, min and average temperature (°C)



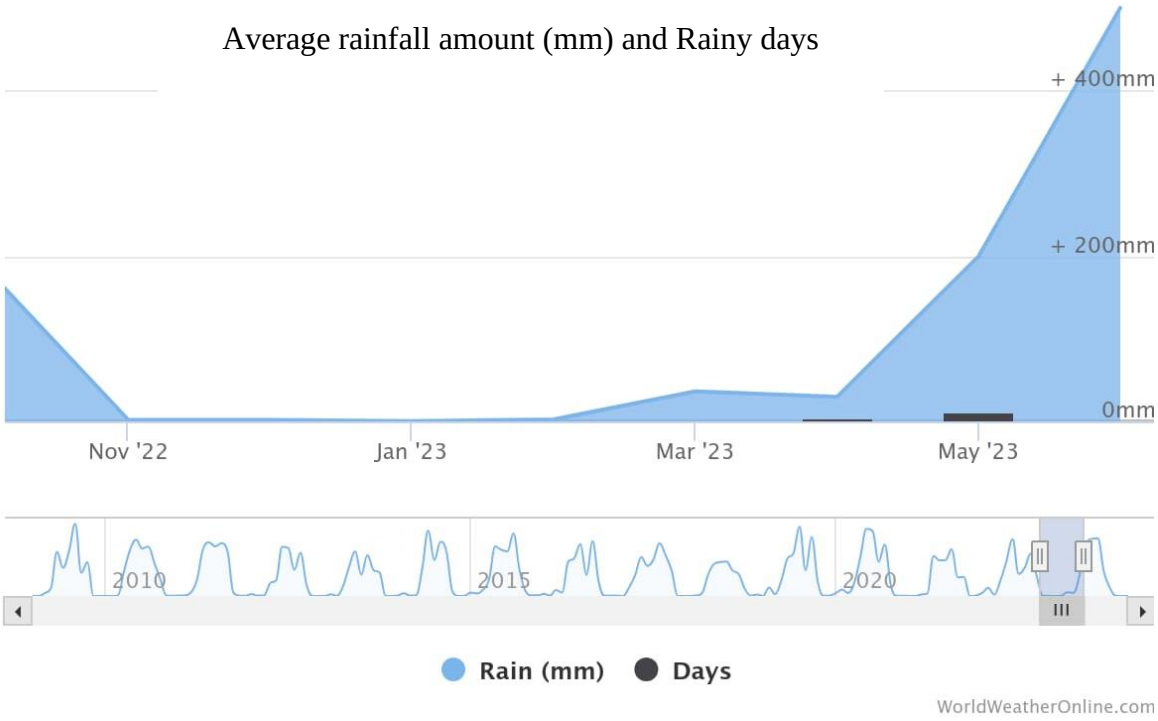
Dinajpur

Average cloud and humidity



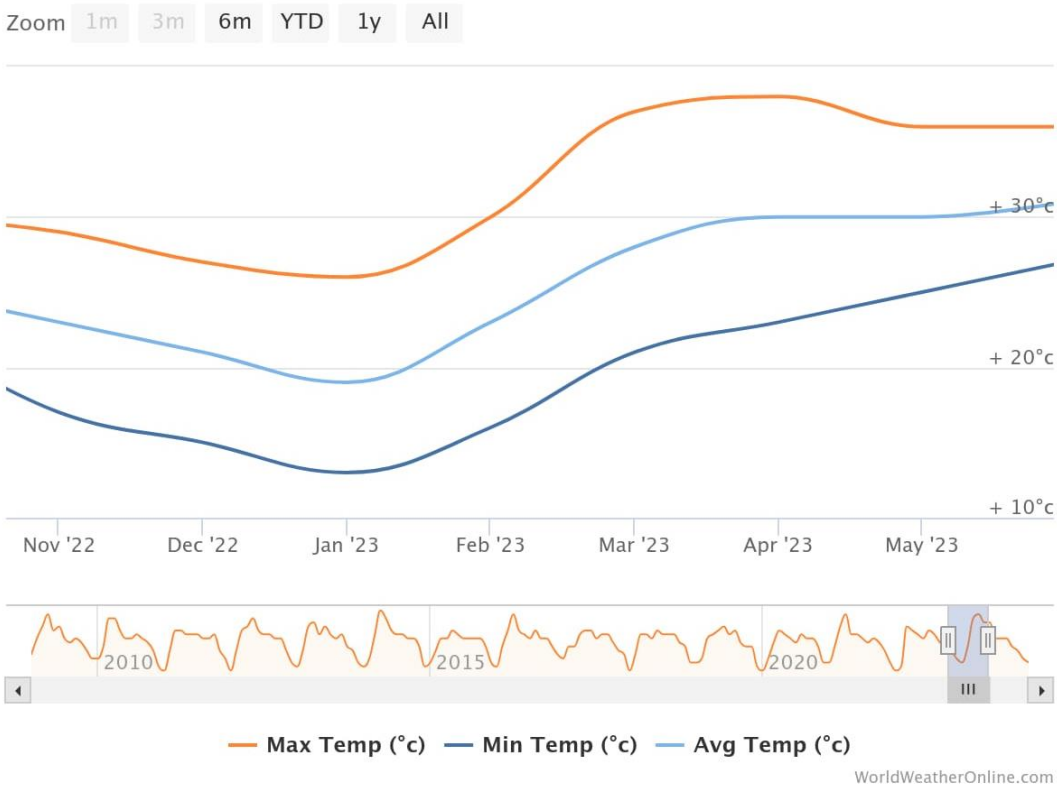
Dinajpur

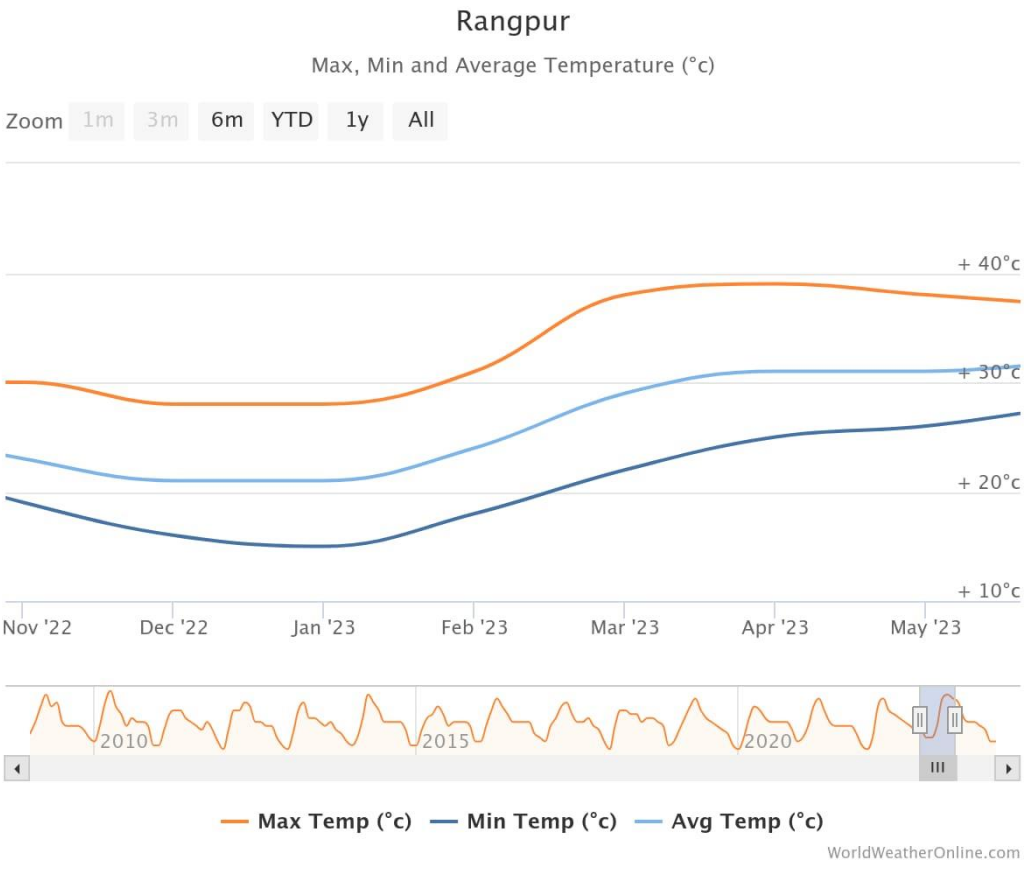
Average rainfall amount (mm) and Rainy days

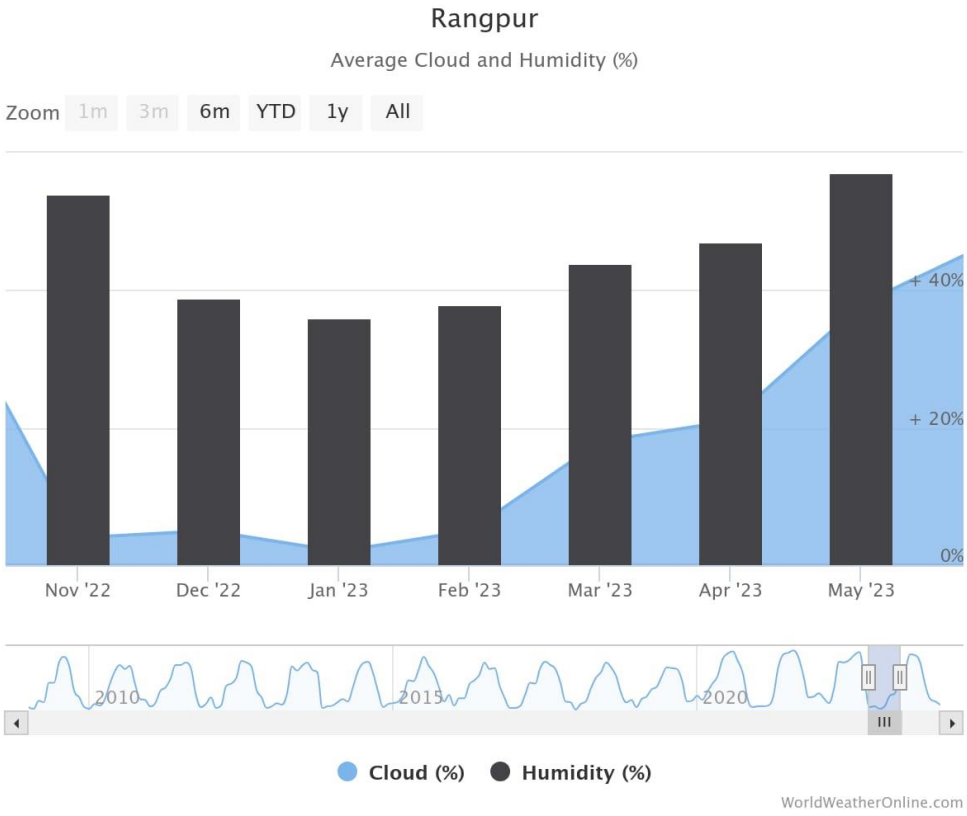


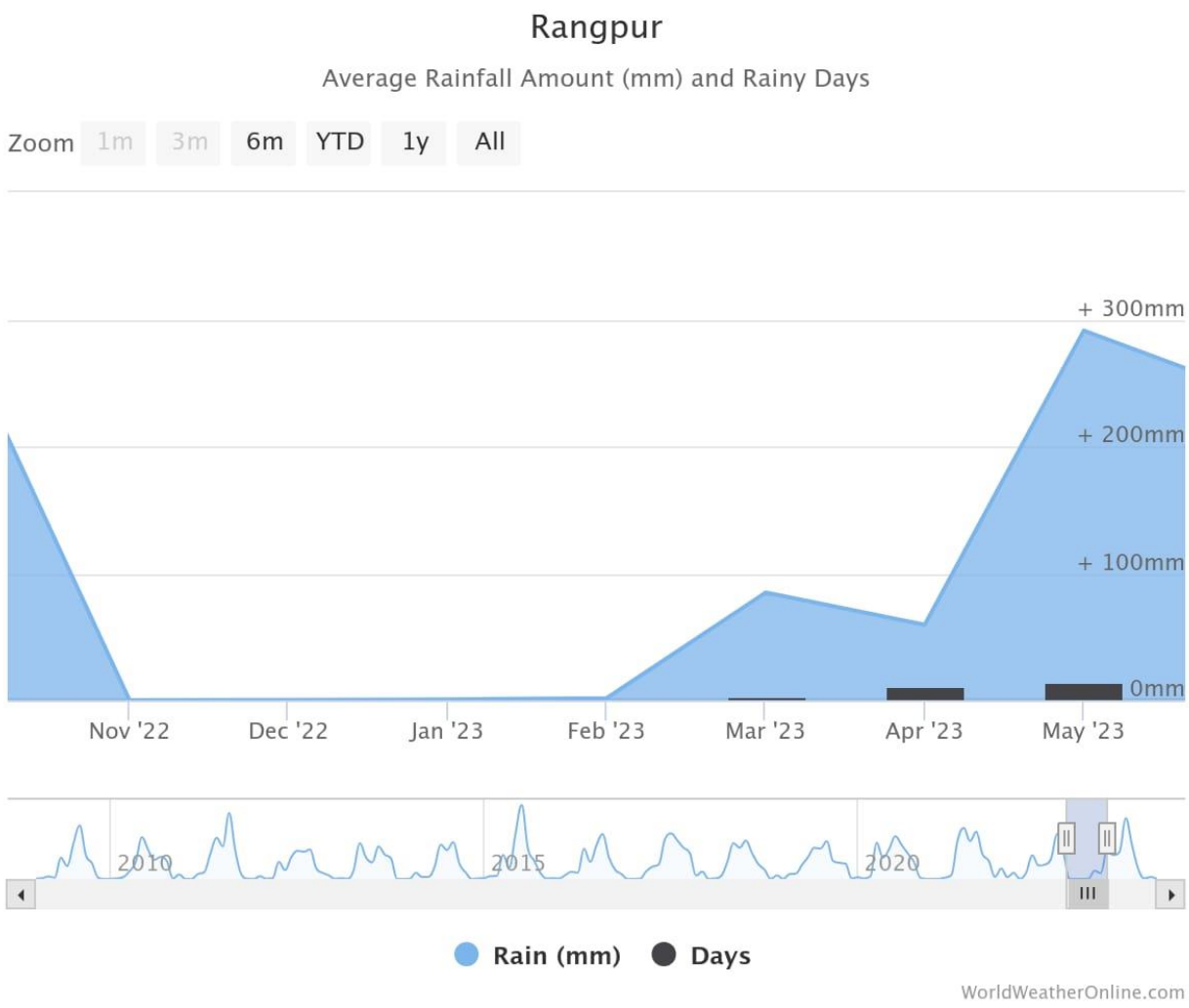
Dinajpur

Max, Min and Average Temperature (°c)




Rangpur
Max, Min and Average Temperature (°c)
Zoom 1m 3m 6m YTD 1y All
+ 40°c
+ 30°c
+ 20°c
+ 10°c
Nov '22
Dec '22
Jan '23
Feb '23
Mar '23
Apr '23
May '23
2010
2015
2020
Max Temp (°c)
Min Temp (°c)
Avg Temp (°c)
WorldWeatherOnline.com


Rangpur
Average Cloud and Humidity (%)
Zoom 1m 3m 6m YTD 1y All
+ 40%
+ 20%
0%
Nov '22
Dec '22
Jan '23
Feb '23
Mar '23
Apr '23
May '23
2010
2015
2020
Cloud (%)
Humidity (%)
WorldWeatherOnline.com


Rangpur
Average Rainfall Amount (mm) and Rainy Days
Zoom 1m 3m 6m YTD 1y All
+ 300mm
+ 200mm
+ 100mm
0mm
Nov '22
Dec '22
Jan '23
Feb '23
Mar '23
Apr '23
May '23
2010
2015
2020
Rain (mm)
Days
WorldWeatherOnline.com

Some Experimental Photographs of my Research