CHARACTERIZATION OF PIGMENTED RICE GERMPLASMS BASED ON MORPHO-NUTRITIONAL TRAITS, ANTIOXIDANT PROPERTIES AND MOLECULAR MARKERS

A THESIS

BY

ZEENA SALWA

STUDENT NO. 1601197

SEMESTER: Jul-Dec, 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 **"CHARACTERIZATION OF PIGMENTED RICE GERMPLASMS BASED ON MORPHO-NUTRITIONAL TRAITS, ANTIOXIDANT PROPERTIES AND MOLECULAR MARKERS"** is a study, prepared by the examinee, bearing Registration No.: 1601197 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, Husband and Honourable Teachers

ACKNOWLEDGEMENTS

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

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

I would like to express my heartiest respect, and immense indebtedness to my respectable teacher and research Co-Supervisor, ***Professor Dr. Md. Hasanuzzaman,*** *Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur for his valuable suggestions, sincere help, scholar instructions and careful corrections of the manuscript of the dissertation.*

I recognize a profound thanks to all the respected teachers, ***Professor Dr. Bhabendra Kumar Biswas, Professor Dr. Md. Abul Kalam Azad, 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 extend my personal gratitude to all the staffs of Dept. of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur for their help and assistance during the period of the study.

I would like to say thanks, to my 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, husband and other relatives whose sacrifices, inspiration and continuous blessings paved the way for my higher studies.

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

December 2023 *The Author*

CHARACTERIZATION OF PIGMENTED RICE GERMPLASMS BASED ON MORPHO-NUTRITIONAL TRAITS, ANTIOXIDANT PROPERTIES AND MOLECULAR MARKERS

Abstract

The experiment conducted during the Kharif season from April 2022 to November 2022 aimed to analyze the morpho-physiological and nutritional traits, genetic parameters, DNA fingerprinting, and molecular genetic diversity of pigmented rice genotypes. The analysis included ten (10) yield and yield-contributing characters, five (5) nutritional characters, and 14 SSR markers. The experiment revealed significant differences among the genotypes for all traits, showing a good opportunity for selecting better parental types to improve grain yield. The mean performance of different yield and yield contributing characters showed wide variations, with traits like plant height (114.43±2.74) cm, productive tiller per plant (28.85±1.11), unproductive tiller per plant (2.82±0.25), and yield per plant (18.82±1.10) g exhibiting notable ranges. Genetic parameters such as genotypic variance, phenotypic variance, heritability, genetic advance, and genetic advance as a percent of the mean were estimated. The highest genotypic and phenotypic variances were recorded with straw weight (13985.59 and 14158.47), DPPH (4216.64 and 4470.27), days to 50% flowering (1367.09 and 1367.56), iron content (938.47 and 947.76), plant height (373.12 and 395.66), respectively. The low values of phenotypic and genotypic variances were recorded with the character unproductive tiller per plant (1.20 and 1.02), spike per panicle (3.11 and 2.82), thousand seed weight (18.21 and 17.73), respectively. the phenotypic coefficient of variances (PCV) ranged from 17.38% for the plant height to 70.99% for the total phenolic content. The genotypic coefficient of variances (GCV) ranged from 16.88% for the plant height to 70.31% for the total phenolic content. The highest PCV and GCV were observed for the total phenolic content (70.99 and 70.31). The heritability estimation varied from 82.28% to 99.99% for total flavonoid content (TFC) and days to 50% flowering respectively. Furthermore, cluster analysis grouped the genotypes into three clusters based on their traits. Principal component analysis (PCA) identified the minimum number of components explaining the maximum variability principal component 1 (PC1) has an eigenvalue of about 5.77 that captures about 38.5% variance, then the eigenvalue falls steadily in component 4 has an eigenvalue of about 0.99 that captures about 6.6% variance, and the biplot analysis revealed correlations between traits and genotypes. The trait productive tiller per plant, total tillers per plant and panicle length denotes positive PC1 score and negative PC2 score and were highly correlated with each other. Here, the genotypes G21 (BRRI dhan 82), G23 (BRRI dhan 48), G33 (Tepiboro 2), G12 (Nara Bet), G28 (BRRI dhan 29) favored these traits. Again, DPPH content, total flavonoid content and thousand seed weight showed positive loading in PC2 but negative score in PC1. The DNA fingerprinting based on SSR markers identified 57 alleles, and the population structure analysis classified the genotypes into four sub-populations, each with distinct characteristics. The analysis of molecular variance (AMOVA) indicated a higher level of genetic variation (90%) within populations than (10%) among them. The study provides comprehensive insights into the variability, heritability, and genetic diversity of pigmented rice genotypes, offering valuable information for future breeding programs and genetic improvement. Overall, the experiment yielded crucial findings on the diversity, genetic parameters, and population structure of pigmented rice genotypes, such as Orabet aus, Malikhori aus and Narabet aus offering significant implications for breeding and conservation strategies in rice cultivation.

CONTENTS (CONT.)

CONTENTS (CONT.)

CONTENTS

LIST OF TABLES

LIST OF FIGURES (CONT.)

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ACRONYMES AND ABBREVIATIONS

%	Percent
^{0}C	Degree Celsius
AEZ	Agro Ecological Zone
ANOVA	Analysis of Variance
DNA	Deoxyribonucleic acid
BARI	Bangladesh Agricultural Research Institute
BRRI	Bangladesh Rice Research Institute
Bp	Base pair
CC	Chlorophyll content
cm	Centimeter
CV	Coefficient of Variation
D^2	Genetic Divergence
DFF	Days to 50% flowering
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
FAO	Food and Agriculture Organization
g	Gram
GA	Genetic Advance
GA%	Genetic Advance as percent
GAM	Genetic Advance as percent of means.
GCV	Genotypic Coefficient of variation
h^2b	Heritability in broad sense
HSTU	Hajee Mohammad Danesh Science and Technology University
IRRI	International Rice Research Institute
Kg	Kilogram
m	Meter
mm	Millimeter
MOP	Muriate of Potash
Ms	Mean Sum of Square
MSE	Mean Square of Error
σ^2g	Genotypic Variance

LIST OF ACRONYMES AND ABBREVIATIONS

σ^2p	Phenotypic Variance
PAGE	Polyacrylamide Gel Electrophoresis Analysis
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
PH	Plant height
PL	Panicle length
R^2	Residual Effect
RCBD	Randomized complete block design
SE	Standard Error
SPAD	Soil Plant Analysis Development
SRDI	Soil Resource Development Institute
SSR	Simple Sequence Repeat
TM	Temperature
TPP	Total tillers per plants per plant
TSP	Triple Super Phosphate
UPGMA	Unweight Pair Group Method Using Arithmetic Mean
USDA	United States Department of Agriculture
Viz	Namely
YPP	Yield per plant

CHAPTER I

INTRODUCTION

Rice (*Oryza sativa L.*) is a staple food for nearly half of the world's population. Bangladesh is the world's fourth largest rice producer and third largest consumer of rice (Shew *et al*., 2019). Rice is a member of the genus *Oryza* and the tribe Oryzae of the Gramineae (Poaceae) family. *Oryza* has 25 identified species, 23 of which are wild and two of which, *O. sativa* and *O. glaberrima*, are cultivated (Morishima *et al*., 1984). The most extensively farmed species is *Oryza sativa*. Between 8000 to 15,000 years ago, it was initially grown in Southeast Asia, somewhere in India, Myanmar, Thailand, North Vietnam or China. People living in the floodplains of the Niger River in Africa are estimated to have domesticated *O. glaberrima* from its wild parent *O. barthii* some 3000 years ago. Today, it is cultivated on every continent except Antarctica (Muthayya *et al.,* 2014). The genome of rice has been extensively studied, and it was the first crop plant to have its complete genome sequenced. An estimated 90.6% of the rice genome is represented by genetically anchored BAC contigs, based on an estimated genome size of ~400 Mb. The integrated physical and genetic map can be accessed with WebFPC (Soderlund *et al*., 2002).

Global rice production in 2022-2023 was 502.97 million tons, a decrease of 9.00 million tons from 511.17 million tons in 2021-2022 (USDA, 2023). Bangladesh ranked the third in the world in rice production, after only China and India. In Bangladesh, overall rice production area is expected to decline slightly to 11.5 million ha for the marketing year 2022-23, while production is expected to decrease to 35.6 million metric tons. Total rice area and output were reduced to 11.6 million hectares and 35.8 million metric tons in 2021-22 (USDA, 2022). Throughout the year, rice is grown in three seasons: Aus, Aman and Boro. Boro rice contributes significantly to Bangladesh's overall rice output.

In Bangladesh, where agriculture forms the backbone of the economy and rice is a dietary cornerstone, the significance of this grain cannot be overstated (Hossain *et al*., 2020). It is no surprise that the agriculture sector dominates the nation's economy, employing a significant portion of the workforce (Hossain *et al*., 2017). Rice, particularly Bangladeshi rice, stands as the primary source of sustenance, nourishing the population and playing a central role in the culture and traditions of the nation (Islam *et al*., 2019). Beyond its cultural relevance, rice has far-reaching economic implications, influencing rural livelihoods, trade, and export earnings (Khaliq *et al*., 2018). Despite its central role, rice

cultivation in Bangladesh faces multifaceted challenges (Mondal *et al*., 2020). Ensuring high yield, addressing the impact of climate change on crop production, and enhancing the nutritional quality of rice are pressing concerns. As the population continues to grow and the climate becomes increasingly unpredictable, it is imperative to seek solutions that not only meet the nation's food needs but also improve the dietary quality of its people (Haque *et al*., 2019). This necessitates exploring alternative rice varieties and cultivation methods that can contribute to the country's food security and nutritional goals.

In recent years, the emergence of pigmented rice as a unique subset of rice varieties has piqued the interest of researchers and nutritionists worldwide (Biswas *et al*., 2020). Pigmented rice distinguishes itself from traditional white rice with its striking colors and exceptional nutritional attributes. In the context of Bangladesh, the exploration of pigmented rice varieties holds immense potential for transforming agriculture, nutrition, and public health. Pigmented rice is known for its vibrant colors, ranging from deep purple to brilliant red and even dark shades of black (Das *et al*., 2019). These colors are a result of the presence of natural pigments, such as anthocyanins and proanthocyanidins, which are largely absent in white rice (Shikha *et al*., 2020).

However, the appeal of pigmented rice extends beyond its aesthetics; it is celebrated for its superior nutritional content, boasting higher levels of essential nutrients, vitamins, minerals, and dietary fibers compared to its white counterpart (Shovon *et al*., 2018). A distinguishing feature of pigmented rice is its rich antioxidant profile (Das *et al*., 2020). These antioxidants, particularly anthocyanins, proanthocyanidins, and carotenoids, have been associated with various health benefits, including the prevention of chronic diseases and the promotion of overall well-being (Das *et al*., 2017). The potential of pigmented rice to offer superior nutritional value and contribute to the nutritional well-being of the Bangladeshi population is a compelling reason to investigate these rice varieties more thoroughly.

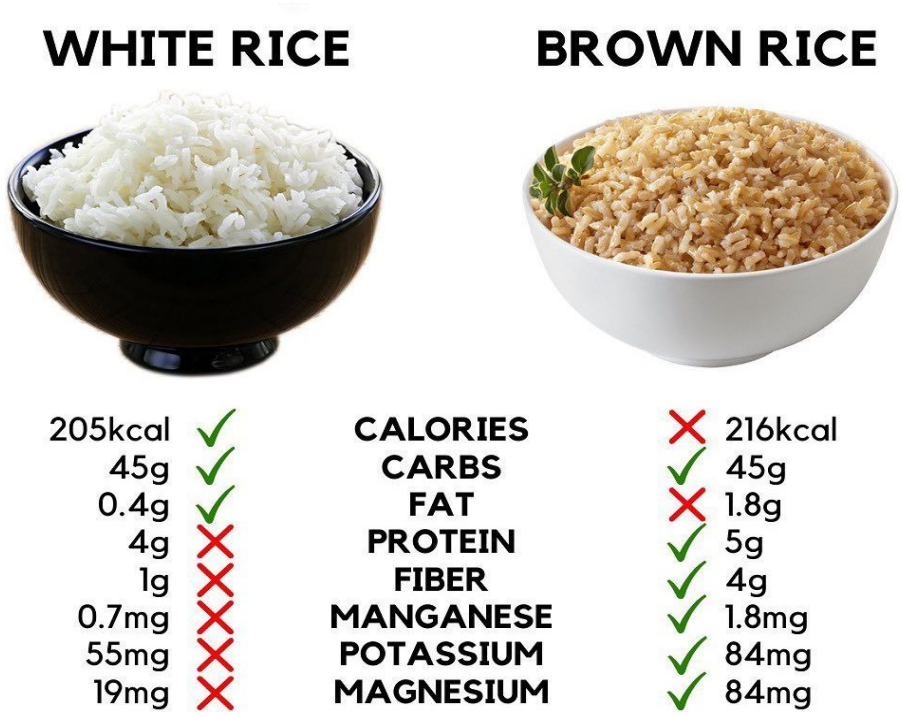

Figure 1. Nutritional differences between white rice and pigmented rice. (pinterest.com)

Variability refers to the presence of differences among the individuals of plant population due to their genetic composition and the environment in which they are raised (Sumanth *et al.,* 2017). Genetic Advance (GA) and Genetic Advance Percentage (GA %) are instrumental in quantifying the potential progress and efficiency of selection in rice breeding programs. They assist researchers and breeders in making informed decisions to enhance specific traits and contribute to the overall improvement of rice varieties. in rice breeding, if researchers observe a high GA for a particular trait, it indicates that selecting individuals based on that trait is likely to result in substantial improvement. Meanwhile, GA% provides insight into the relative efficiency of selection efforts, considering the trait's heritability and the existing variability in the population. In rice breeding, understanding Genotypic Variance (σ^2g) is crucial because it helps identify the heritability of traits, traits with higher Genotypic Variance (σ^2g) are generally more heritable. Phenotypic Variance (σ^2p) reflects the overall variability in traits such as yield, disease resistance, or grain quality among different rice plants. A critical analysis of the genetic variability parameters, namely, genotypic coefficient of variability (GCV), phenotypic coefficient of variability (PCV), heritability, and genetic advance for different traits of economic importance is a major prerequisite for any plant breeder to work with crop improvement programs. Further, information on correlation coefficients between grain yield and its component characters is essential for yield improvement, since

grain yield in rice is a complex entity and is highly influenced by several component characters (Sameera *et al.,* 2015).

Genetic diversity analysis assists breeders in selecting diverse parents to improve a variety of qualities. The primary approach for assessing underlying genetic variation is principal component analysis (PCA). This analysis aids in the discovery of features that aid in the separation of chosen genotypes based on similarities in one or more characters and the classification of genotypes into distinct groups (Sudeepthi *et al*., 2019).

Molecular markers are considered an efficient, powerful tool for the assessment of genetic relationships (Rajesh *et al.,* 2022). For the assessment of genetic diversity molecular markers have been generally superior to morphological, pedigree, heterosis and biochemical data. Molecular marker based genetic diversity analysis also has potential for assessing changes in genetic diversity over time and space (Ravi *et al.,* 2003). Several DNA markers are used for molecular diversity analysis of which simple sequence repeats (SSR) or microsatellites are a class of repetitive DNA element. Microsatellites are PCR based markers that are technically efficient, cost effective, and common in rice (Termnykh *et al.,* 2000). Compared to Restriction Fragment Length Polymorphism (RFLP) microsatellite markers detect a significantly higher degree of polymorphism in rice and are especially suitable for evaluating genetic diversity among closely related rice cultivars (or) accessions (Rajesh *et al.,* 2022).

It is reported that the International Rice Research Institute (IRRI) Gene Bank contains more than 8,000 traditional rice varieties collected from Bangladesh (Hossain, 2013). But now rice diversity is threatened in Bangladesh due to extensive cultivation of modem varieties (MVs) (Ahmed, 2010). On the contrary, we are losing cultivable land every year for infrastructure, garment industries and accommodation of an ever-growing population (Mahmud, 2003). Therefore, it is essential to increase the per hectare rice production. Basically, Aman season has lower yield potential than Boro seasons in spite of covering more production area due to availability of sufficient precipitation compared to Boro season (Williams *et al.*, 2006). The genetic diversity study of boro rice germplasm will offer plant breeders with information to assist them in selecting the parents for hybridization. However, data on the genetic variety of dietary and molecular features is scarce. Bangladesh Rice Research Institute and Bangabandhu Sheikh Mujibur Rahman Agricultural University have created eight biofortified cultivars thus far. However, their

chemical phenomena are still unknown. It is vital to produce biofortified boro rice in order to satisfy nutritional requirements while increasing yields. As a result, the study hypothesis might be identifying high nutritional content and high yield prospective rice genotypes employing land races and high yielding cultivars, as well as identifying diverse parents using phenotypic and molecular data.

Therefore, the specific objectives of the present study were-

i. To study the performances of pigmented rice genotypes based on morpho-nutritional traits,

ii. To estimate genetic diversity based on morpho-nutritional traits and SSR Markers,

iii. To identify parents for future hybridization program.

CHAPTER II
REVIEW OF LITERATURE

2.1 Origin of rice

Gutaker *et al.* (2020) studied the history of rice dispersal in Asia using whole-genome sequences of more than 1,400 landraces, coupled with geographic, environmental, archaeobotanical and paleoclimate data. Originating around 9,000 years ago in the Yangtze Valley, rice diversified into temperate and tropical *japonica* rice during a global cooling event about 4,200 yr ago. Soon after, tropical *japonica* rice reached Southeast Asia, where it rapidly diversified, starting about 2,500 yr BP. The history of *indica* rice dispersal appears more complicated, moving into China around 2,000 yr BP.

Sangeetha *et al.* (2020) studied the domestication of rice began centuries ago, and currently *Oryza glaberrima* and *O. sativa* are the two species grown globally. The history of the domestication of rice can be traced back to 10,000 years ago when the *Oryza* sp. was a mere wild grass and artificial continuous selection by humans has led to the development of a stable amenable cultivar. Several studies show that Asian rice or *O. sativa* has *O. rufipogon* as its progenitor, from which it was domesticated. *O. sativa* is further comprised of five sub-populations, out of which *indica* and *japonica* show distinct genetic variation.

Civáň *et al.* (2019) studied the *aromatic* group of Asian cultivated rice is a distinct population with considerable genetic diversity on the Indian subcontinent and includes the popular Basmati types characterized by pleasant fragrance. Genetic and phenotypic associations with other cultivated groups are ambiguous, obscuring the origin of the *aromatic* population. From analysis of genome-wide diversity among over 1,000 wild and cultivated rice accessions, they show that *aromatic* rice originated in the Indian subcontinent from hybridization between a local wild population and examples of domesticated *japonica* that had spread to the region from their own center of origin in East Asia. Most present-day *aromatic* accessions have inherited their cytoplasm along with 29–47% of their nuclear genome from the local Indian rice. The admixture occurred 4,000–2,400 years ago, soon after *japonica* rice reached the region.

Choi *et al.* (2017) studied the origin of domesticated Asian rice (*Oryza sativa*) has been a contentious topic, with conflicting evidence for either single or multiple domestication of

this key crop species. the evolutionary history of domesticated rice by analyzing de novo assembled genomes from domesticated rice and its wild progenitors were examined. The results indicate multiple origins, where each domesticated rice subpopulation (*japonica, indica,* and *aus*) arose separately from progenitor *O. rufipogon* and/or *O. nivara*. Coalescence-based modeling of demographic parameters estimate that the first domesticated rice population to split off from *O. rufipogon* was *O. sativa* ssp. *japonica*, occurring at ~13.1–24.1 ka, which is an order of magnitude older than the earliest archeological date of domestication. This date is consistent, however, with the expansion of *O. rufipogon* populations after the Last Glacial Maximum ~18 ka and archeological evidence for early wild rice management in China. There is significant gene flow from *japonica* to both *indica* (~17%) and *aus* (~15%), which led to the transfer of domestication alleles from early-domesticated *japonica* to *proto-indica* and *proto-aus* populations.

2.2 Classification of Rice

Mohammadi *et al.* (2019) studied rice grain occurs in a variety of colors, including white, brown, black, purple, and red rices. A third subspecies, which is broad-grained and thrives under tropical conditions, was identified based on morphology and initially called *javanica*, but is now known as *tropical japonica*. Examples of this variety include the medium-grain 'Tinawon' and 'Unoy' cultivars, which are grown in the high-elevation rice terraces of the Cordillera Mountains of northern Luzon, Philippines (CECAP, PhilRice and IIRR. 2000).

Purugganan *et al.* (2009) studied *oryza sativa* contains two major subspecies: the sticky, short-grained *japonica* or *sinica* variety, and the nonsticky, long-grained *indica* rice variety. *Japonica* was domesticated in the Yangtze Valley 9–6,000 years ago, and its varieties can be cultivated in dry fields (it is cultivated mainly submerged in Japan), in temperate East Asia, upland areas of Southeast Asia, and high elevations in South Asia, while *indica* was domesticated around the Ganges 8,500-4,500 years ago and its varieties are mainly lowland rices, grown mostly submerged, throughout tropical Asia.

Garris *et al.* (2004) used simple sequence repeats to sort *O. sativa* into five groups: *temperate japonica, tropical japonica* and *aromatic* comprise the *japonica* varieties, while *indica* and *aus* comprise the *indica* varieties.

Glaszmann *et al*. (1987) studied isozymes to sort *O. sativa* into six groups: *japonica, aromatic, indica, aus, rayada,* and *ashina*.

2.3 Nutritional value of pigmented rice

2.3.1 Overview of nutritional composition

Nafisah *et al.* (2023) studied the pigmented rice which were black, red, and dark purple rice, and all contains a variety of peonidin-3-glucoside, cyanidin-3-glucoside, γ-oryzanol, γ-tocotrienol, proanthocyanidin, cinnamic acid, and anthocyanins that may act as pro-apoptotic, anti-proliferative, and anti-metastasis in breast cancer cells. Therefore, choosing whole-grain red, black, or purple is an excellent choice for health. Plus, these varieties are richer in disease-fighting antioxidants. Rice being a staple food for half of the world can be a source of energy for our generations only if it is accumulated with nutrition.

Kammapana *et al.* (2023) conducted a study to investigate the physical characteristics, phytochemical contents, and antioxidant activity of 10 organic-pigmented rice varieties including Tubtim Chumpae, Niaw Daeng, Nieng Guang, Mali Daeng, Hom Nil, Niaw Dam, Riceberry, Mali Gomain Surin, Malinil Surin, and Pa Ga Am Puen (obtained from Tapthai organic villages, Tha-mo, Prasat, Surin province, Thailand in June, 2021). The anthocyanin content, total phenolic compounds and antioxidant activity of different rice varieties showed a significant difference in the range of 4.38 to 77.96 mg/100 g DW, 42.94 to 341.19 mg GAE/100 g DW and 2.71 to 34.77 mM TE/100 g DW, respectively. Niaw Dam exhibited the highest anthocyanin concentrations. The highest total phenolic content was found in Mali Daeng and Nieng Guang, resulting in high antioxidant activity. Pigmented rice is rich in phytochemicals and antioxidants.

Mendoza-Sarmiento *et al.* (2023) conducted an experiment which shows acute intake of pigmented rice increases antioxidant activity and lowers postprandial glucose and insulin levels. Meta-analysis demonstrated significant reductions in glucose, weight, and diastolic BP following chronic pigmented rice consumption, but no significant effects on TC, LDL, TG, HDL, BMI, or systolic BP. More high quality randomized controlled trials are warranted to further investigate the effect of pigmented rice consumption on cardiometabolic risk factors in adults; additional benefit may be observed in those with established clinical conditions such as dyslipidemia, pre-diabetes/diabetes, and hypertension.

Tiozon *et al.* (2023) studied pigmented rice has attracted considerable attention due to its nutritional value, which is in large conferred by its abundant content of phenolic compounds, considerable micronutrient concentrations, as well as its higher resistant starch and thereby slower digestibility properties. A wide range of phenolic compounds identified in pigmented rice exhibit biological activities such as antioxidant activity, anti-inflammatory, anticancer, and antidiabetic properties.

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Wijaya *et al.* (2021) conducted a study to analyze the nutrients content in red rice milk and its antioxidant activity. Red rice milk contained 98.01% of water, 0.07% of ash, 0.13% of protein, 0.71% of fat, and 1.07% of carbohydrate. Red rice milk inhibited 53.37% of DPPH radical and contained total phenolic about 274.5 ppm.

Poonia *et al.* (2021) tudied Black rice is a type of rice species (*Oryza sativa L.*) and very good source of various nutrients and one of the nutritious varieties of rice. It is a good reservoir of essential amino acids such as lysine, tryptophan, minerals including iron, calcium, phosphorus, zinc and selenium; vitamins such as vitamin B1, vitamin B2 and folic acid.

Jaksomsak *et al.* (2021) studied on grain anthocyanin, zinc, and iron concentrations of eleven purple rice varieties grown under wetland and aerobic conditions. They found that wetland conditions were more favorable than aerobic culture for intense pigmentation in the production of purple rice as well as higher Zn and Fe concentrations.

Ranjkesh *et al.* (2021) conducted a study to evaluate the physical and chemical properties such as plant height, length of panicles, number of effective tillers, total number of grains, number of unfilled and filled grains, weight of 1000 grains, biological and economic yield,

harvest index, chlorophyll index, growth period, total phenol (TP), and total antioxidant capacity based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) in 30 genotypes of elite rice grown in Iran. The highest amount of chlorophyll index was observed in genotype line 101 (G9) with 45.75%. In addition, the range of antioxidant capacity based on DPPH was 9.033–19.067% and genotype 13 (Bijar) had the highest antioxidant capacity. The highest TP content was 1.137 mg gallic acid (GAE) per g. According to the correlation, (0.555) had a positive and significant correlation with the antioxidant capacity based on DPPH.

Bhat *et al.* (2020) conducted a study on the pigmented bran layers, which are rich sources of polyphenols and anthocyanin that are having antioxidant activities and other health-promoting properties. Thus, the extracts of these functional ingredients, particularly those from the phenol groups, have been influential for its medicinal properties and in the treatments of various ailments, including reducing inflammation and oxidative stress as well as atherosclerotic lesions and several human chronic diseases, such as cardiovascular, cancer, obesity, diabetes. Anthocyanins present in the local varieties of red and black rice also leads to the reduction of atherosclerotic plaque formation. Rice bran oil has the ability to decrease cholesterol and minimize the risk of kidney stones. Its high content of vitamin E helps improve neurological functioning and balances the endocrine hormones.

Da Silva *et al.* (2020) conducted an experiment to extract and characterize starch from three varieties of pigmented rice (named white, red and black), preserving the bioactive compounds. The extraction yield was 44.0%, 47.0% and 35.7%, respectively. The scanning electron microscopy showed that the granules of the three varieties presented polygonal and angular format and absence of impurities. The chemical analyzes, showed more than 83.0% of carbohydrates in the three compositions. There was retention of the phenolic compounds from the raw material in the starches. Black rice starch also showed slightly higher crystallinity and thermal stability than white and red rice starches. Gels of red rice starch have higher syneresis in five freeze-thaw cycles, when compared to the others.

Rao *et al.* (2020) studied that the cultivation location had a significant impact on phenolic composition and antioxidant activity of pigmented rice cultivars, and this is important for breeding high-value rice varieties with specific phenolic compositions.

Maulani *et al.* (2019) studied pigmented rice/glutinous rice consisting of black rice (BR) (*Oryza sativa*), black glutinous rice (BGR) (*O. sativa* Var. glutinosa), and brown rice (RR)

(*Oryza nivara*) to determine total flavonoids and total anthocyanins, as well as the antioxidant activity of local variety of BR, BGR, and RR. Rice samples were evaluated for color intensity, the yield of extract, total flavonoids, total anthocyanin, and antioxidant activity used kinetic assays of diphenyl-2-picrylhydrazyl. The result showed that the increase in the color intensity was increase the number of total flavonoids, total anthocyanin, and antioxidant activity. BR and BGR have total flavonoids content 18.68 mg 100 g−1 and 15.45 mg 100 g−1, respectively. The total anthocyanin content of BR and BGR was 39.61 mg 100 g−1 and 28.63 mg 100 g−1, respectively. Based on IC50 values, BR contains the bioactive compounds that are classified as strong antioxidants.

Xiongsiyee *et al.* (2018) studied on grain quality characteristics of 60 upland rice seed samples sharing 49 variety names collected from 6 villages in Luang Prabang in 2015. Most of the samples has non-pigmented pericarp, while red pericarp was found in four samples and purple in five samples. Almost all of the samples were of large grain type, with glutinous endosperm in 70% and non-glutinous endosperm in 30%. The brown (unpolished) rice was found with a wide range of grain nutritional quality, including protein (9.2% ± 0.9%), Fe (15.9 ± 6.9 mg/kg), Zn (19.6 ± 2.1 mg/kg), anthocyanin (0.774 ± 0.880 mg/g), and anti-oxidative capacity (2.071 ± 1.373 mg/g).

Reddy *et al.* (2016) evaluated pigmented rice (Chak-hao Amubi, Chak-hao Poireiton and Chak-hao Angangba) for molecular structure, functional and antioxidant properties. Significant differences were observed in physico-chemical and functional properties ($p \leq 0.05$) of pigmented rice varieties. The amylose content results revealed that Chak-hao Angangba (1.93 %) and Chak-hao Poireiton (1.98 %) are waxy rice, and Chak-hao Amubi (3.16 %) is a very low-amylose rice. The XRD patterns of pigmented rice showed A-type crystalline patterns with peaks at $2\theta = 15.1°$, 17.1°, 18.2° and 23.0°. Pigmented rice showed potent antioxidant activity with a significant difference ($p \leq 0.05$) in DPPH radical scavenging activity, total phenolic and flavonoid content among varieties. The colour, pasting properties, morphology and transition temperatures varied significantly among the rice varieties.

Faiz *et al.* (2015) evaluated 42 pigmented rice genotypes obtained from the International Rice Research Institute (IRRI) were evaluated to determine micronutrient content, antioxidant activity and vitamin E content. The results revealed that the micronutrient content, antioxidant activity and tocochromanol content of all genotypes varied. Iron (Fe)

was the most abundant micronutrient followed by Zn, Mn and Cu. The antioxidant DPPH scavenging effect among all genotypes ranged from 31.85 to 98.45%. The tocotrienol content was higher than tocopherol in grains of selected pigmented rice genotypes. Among rest of genotypes, IR3257-13-56, IR5533-14-1-1 and Khao gam (niaw) contained high micronutrient content and antioxidant properties compared to others.

Jun *et al.* (2012) conducted an experiment to investigate the antioxidant activities and phenolic compounds of pigmented rice (black, red, and green rice) and brown rice brans. Antioxidant activities of 40% acetone extracts of pigmented rice bran, measured in the range of 0 to 1500 μg/mL. At 500 μg/mL concentration, red rice bran, which had the highest total phenolic (259.5 μg/mg) and total flavonoid (187.4 μg/mg) contents, showed the highest antioxidant activity: 83.6%, 71.5%, 1.2%, and 16.4% for DPPH radical assay, ABTS radical cation assay, reducing power, and chelating ability, respectively. Red rice bran showed a lower EC (50) value (112.6 μg/mL) than that of butylated hydroxytoluene (144.5 μg/mL) from the DPPH radical assay. The major phenolic acids of red rice bran were ferulic, vanillic and p-coumaric acids.

Yodmanee *et al.* (2011) studied Eight varieties of pigmented rice grown in southern Thailand, as a dehusked grain for their chemical compositions, antioxidant properties and color parameters. Moisture, protein, lipid, crude fiber and ash contents of all varieties were in the ranges of 5.96-8.19, 6.63-8.46, 1.44-3.47, 0.16-0.35 and 1.35-2.15 g/100 g (db), respectively. The iron and polyphenol contents in these rice samples were in the range of 0.91-1.66 mg/100 g and 58-329 mg GAE/100 g sample, respectively. The antioxidant capacity of dehusked rice grain extract was positively correlated ($p< 0.01$) with polyphenol content ($r= 0.923$). Rice grain color parameters (L*, a* and b*) had negative correlations ($p< 0.01$) with iron content (r=-0.646, -0.654 and-0.791), polyphenol (r=-0.893, -0.851 and-0.928) and antioxidant capacity (r=-0.794, -0.629 and-0.770). The results showed that dark purple grain has higher iron content, polyphenol content and antioxidant capacities than red brown grain.

2.3.2 Comparative analysis with non-pigmented rice varieties

Aalim and Luo *et al.* (2021) studied the effects of roasting and frying on brown and red wholegrain rice. Red rice was characterized by superior phenolic content and the presence of proanthocyanidins which were enhanced by roasting. However, brown rice showed greater phenolic stability compared with red rice. Chromatographic separation showed that

red rice was dominated by protocatechuic acid and (-)-epicatechin, whereas brown rice showed high contents of p-hydroxybenzoic acid, (-)-epicatechin, and syringaldehyde.

Jaksomsak *et al.* (2021) studied the highest grain anthocyanin, Zn and Fe variety was respected to PP1, PP2 and PP4, grown under the wetland condition, but the condition had negative effect on grain yield. The nutritional quality in aerobic grown rice was lower than wetland grown among the varieties with higher levels of anthocyanin, Zn and Fe, with smaller effects of water among varieties in the lower quality ranges.

Mbanjo *et al*. (2020) studied on the improvement of the nutritional quality of rice grains through modulation of bioactive compounds and micronutrients represents an efficient means of addressing nutritional security in societies which depend heavily on rice as a staple food. White rice makes a major contribution to the calorific intake of Asian and African populations, but its nutritional quality is poor compared to that of pigmented (black, purple, red orange, or brown) variants.

Hurtada *et al.* (2018) determined iron, zinc contents of dehulled and well-milled pigmented and non-pigmented rice varieties. Pigmented rice had the highest mineral content compared to the non-pigmented rice, regardless of variety. However, non-pigmented had higher phosphorus content. Among the non-pigmented rice varieties, Jasmin rice contains the highest zinc content. Sinandomeng contains the highest iron content. On the other hand, upon comparing the pigmented rice varieties, Dinorado contains the highest zinc and phosphorus content, Perurutong contains the highest iron, and Malagaya Tapul contains the highest manganese content. The dehulled varieties of pigmented rice contain high mineral content compared to well-milled rice. However, Ballatinao and Malagaya Tapul contain high levels of iron and phosphorus in dehulled rice.

Rahman *et al.* (2017) studied fourteen pigmented hill rice cultivars along with a non-pigmented one for ascertaining the extent of their nutritional and genetic diversity. Moisture contents of the fourteen pigmented hill rice cultivars ranged from 7.49 to 10.10% along with the contents (% dry weight) of nutrients *viz.*, crude fat (4.37–5.27) and crude protein (9.27–11.42). The corresponding range of values for the contents (mg/100g dry weight) of microminerals Zn and Fe were from 3.42 to 4.28 and from 3.21 to 4.18 respectively. The pigmented germplasm had anthocyanin content (mg cyanidine 3-*O* glucoside/100g) ranging from 3.58 to 7.86, total phenolic content (mg GAE/100 g) from

67.89 to 89.43, flavonoid content (mg QE/100g) from 57.75 to 78.74 and antioxidant activity from 19.56 to 29.29%.

2.3.3 Health implications and dietary benefits

Bassolino *et al.* (2022) studied on the impact of antioxidants-rich cereal and *Solanaceae* derived foods on human health by analyzing natural biodiversity and biotechnological strategies aiming at increasing the antioxidant level of grains and fruits, the impact of agronomic practices and food processing on antioxidant properties combined with a focus on the current state of pre-clinical and clinical studies. Despite the strong evidence in in vitro and animal studies supporting the beneficial effects of antioxidants-rich diets in preventing diseases, clinical studies are still not sufficient to prove the impact of antioxidant rich cereal and *Solanaceae* derived foods on human.

Malabadi *et al.* (2022) evaluated the dietary importance, arsenic toxicity, and nutritional value of brown, white, basmati and pigmented rice varieties. Rice is one of the most widely consumed cereals in the world rich in dietary fiber, bioactive compounds such as Melatonin, and Gamma - aminobutyric acid (GABA). However, the edible brown rice is rarely consumed as the most human populations prefer the white polished rice for reasons connected to appearance, taste, palatability, ease of cooking, tradition, safety, and shorter shelf of brown rice which limits the market potential. Rice generally contains more arsenic than any other grains because of its anaerobic growing environment and unique physiology. Brown rice accumulates more arsenic than white rice because arsenic accumulates in the outer hard bran layer of the grain which is removed to make the white rice.

Jaksomsak *et al.* (2021) studied on grain anthocyanin, zinc, and iron concentrations of eleven purple rice varieties grown under wetland and aerobic conditions. They found that wetland conditions were more favorable than aerobic culture for intense pigmentation in the production of purple rice as well as higher Zn and Fe concentrations.

Khanom *et al.* (2021) studied four advanced brown rice genotypes: IZSD-10, IZSD-26, IZSD-44 and IZSD-45 along with Binadhan-20 as check variety were analyzed for grain Fe and Zn concentration using energy Dispersive X-ray Fluorescence Spectrophotometer (ED-XRF). Advanced yield trial was conducted in three different locations of Bangladesh during Aman season of 2020 in a randomized complete block design (RCBD) with three

replications in each location. The Fe concentration varied from 9 to 15 mg kg-1 and 1 to 4 mg kg-1 whereas Zn concentration ranged from 45 to 59 mg kg-1 and 29 to 40 mg kg-1 in unpolished and polished rice, respectively. Almost higher Fe loss (~60 to 94 %) was observed compared to Zn (~18 to 42%) at 10% polishing throughout the grain shape that was responsible due to loss of embryo, pericarp and aleurone layer.

Shin *et al.* (2020) studied the association between dietary patterns and multi-grain rice intake and the risk of breast cancer was also studied in a large-scale prospective cohort study in Korean women. Their study also suggests that a multi grain rice diet may be associated with lower risk of breast cancer in Korean women.

Melini and Acquistucci *et al.* (2017) studied on the anthocyanin content in raw and cooked Khao Nim Thai black rice and Jasmin Thai red rice. Cooking involved water absorption. For the black rice they observed a significant increase in the cyanidin-3-O-glucoside possibly because of an increased pigment extraction, whereas they observed a significant decrease in peonidin-3-O-glucoside possibly due to a degradation of pigments during cooking or to a low sensitivity in the methods used.

Farvid *et al.* (2016) evaluated individual grain-containing foods and whole and refined grain intake during adolescence, early adulthood, and premenopausal years in relation to breast cancer risk in the Nurses' Health Study II in the USA which included 90,516 premenopausal women aged between 27 and 44 years. They found that brown rice consumed by adults was associated with lower risk of overall and premenopausal breast cancer and they concluded that high wholegrain food intake may be associated with lower breast cancer risk before menopause.

Zaupa *et al.* (2015) studied the total antioxidant capacity (TAC) and polyphenolic compounds of differently pigmented rice varieties and their changes during domestic cooking, found that the content of these compounds and the TAC decreased after cooking in all the three studied varieties but to a lesser extent after the risotto method which allows a complete absorption of water.

Sung *et al.* (2010) studied the association between the risk of breast cancer and total carbohydrate intake, glycemic load, and glycemic index and different types of rice consumption was studied in a hospital-based case-control/clinical study in South Korea.

They found that a higher consumption of mixed brown rice may be associated with a reduced risk of breast cancer, especially in overweight postmenopausal women.

2.4 Morphological and nutritional parameter studies in rice

2.4.1 Analysis of variance (ANOVA)

Salgotra *et al.* (2023) conducted a study on the effects of heat and drought stresses on the physiological traits of rice. We focused on different approaches to managing high-temperature and drought stresses, such as an adjustment in cultural practices, genetic improvement through molecular breeding, and the development of transgenics and chemical spray from an agricultural practice perspective.

Gupta *et al*. (2022) evaluated six popular boro rice (*Oryza sativa L.*) varieties using yield and growth characteristics to determine which varieties were the top performers. Plant height (PH), days to flowering (DTF), days to maturity (DTM), number of total tillers per plants per hill (NTH), number of effective tillers per hill (ETH), number of non-effective tillers per hill (NETH), panicle length (PL), number of filled grains per plant (FG), number of unfilled grains per plant (UFG), thousand grain-weight (TGW), grain yield (GY), dry straw weight (DSW), and % harvest index (HI) revealed the significant differences among the varieties. Maximum GY was noted in Binadhan-10 (6.72 t/ha) followed by Binadhan-12 (6.64 t/ha), BRRI dhan29 (6.55 t/ha) and these three varieties were significantly identical.

Tyagi *et al.* (2022) conducted a study to evaluate the antioxidant, total flavonoid, total phenolic, anthocyanin content and individual phenolic compound quantification of nine Korean-grown rice varieties using spectrophotometric, HPLC-FLD-MS/MS and UHPLC Q-TOF-MS/MS methods. The free fractions of DM29 (red rice) had the highest free radical scavenging ability of ABTS and DPPH. In contrast, the highest ferric reducing antioxidant power was observed in the 01708 brown rice variety.

Khan *et al.* (2021) experimented to find out the high yielding potential genotypes and considering these genotypes to develop pure lines for commercial cultivation in Malaysia. Considering the 14 qualitative and 27 quantitative traits of fifteen landraces the variation and genetic parameters namely, variability, heritability, genetic advance, characters association, and cluster matrix were determined. ANOVA revealed significant variation for all the agronomic traits (except plant height). Among the accessions, highly significant

differences ($P \leq 0.01$) were found for almost all the traits excluding fifty percent flowering date, seed length, seed width. The 16 traits out of the 27 quantitative traits had a coefficient of variation (CV) $\geq$ 20%. The trait dry seed weight per plant (g) had the highest GCV = 59.91% and PCV = 59.57% whereas the trait fresh pod weight (99.55%), dry seed weight (98.86%), and yield (98.10%) were highly heritable. The genetic advance recorded the highest for dry seed weight (122.01%) and lowest (3.97%) for plant height.

Sridevi *et al.* (2021) studied Twenty-six Colored rice (Oryza sativa L) genotypes for 8 yield components and 12 physio-chemical, nutritional and Anti-oxidative properties. Among the non-pigmented varieties, BPT 5204, BPT 2270, BPT 2595, BPT 2782 and BPT 2776 recorded the desirable quality traits with excellent cooking quality. The pigmented rice genotypes recorded high number of total phenols, antioxidant activity and flavonoid contents than non-pigmented varieties. When compared with light brown pericarp colored rice genotypes, red and black rice genotypes possess more protein content, high Zn and Fe content also. Variability studies showing that, high GCV and PCV coupled with high heritability and high genetic advance as percent of mean were recorded for test weight and total number of grains per panicle among yield components.

Parimala *et al.* (2020) conducted an experiment to study the genetic variability in seventy-seven rice genotypes for grain yield and its component characters. Analysis of variance revealed the existence of sufficient amount of variability in the material under study. The characters such as number of filled grains per panicle and 1000 seed weight exhibited higher estimates of phenotypic and genotypic coefficient of variation indicates that these characters could be improved through direct selection. High heritability coupled with high genetic advance was observed for 1000 seed weight, reveals the role of additive gene action controlling this trait.

Sabri *et al.* (2020) conducted a multi-environmental yield trial. The growth performance and phenotypic variability of these genotypes are the combination of environment, genotype and genotype by environment (G×E) interaction factors. Analysis of variance revealed that all traits were significantly different for genotypes except days to maturity, number of filled grains and total number of grains. Meanwhile, all the traits differed significantly for genotype × environment (G×E) except number of tillers per hill and number of panicles per hill. Low heritability (<30%) was found for all the traits. Similarly,

low genetic advance was also observed for all the traits except for number of tillers per hill and number of panicles per hill.

Devi *et al.* (2017) studied the analysis of variance revealed highly significant differences among 27 genotypes for all the yield components. The magnitude of difference between PCV and GCV was less for the traits indicating little influence of environment. The higher estimates of PCV and GCV were observed for yield per plant (42.42; 42.04) and filled seeds per panicle (34.67; 33.19) indicates possibility of genetic improvement through direct selection for these traits, while hulling percent, milling percent, kernel elongation ratio, day to 50% flowering, panicle length, kernel length and kernel width showed low PCV and GCV values indicating the need for creation of variability by hybridization or mutation followed by selection.

Bitew *et al.* (2016) conducted a study to assess the extent of genetic variability for yield and yield related traits and to estimate heritability and genetic advance in rainfed upland rice genotypes. The higher phenotypic and genotypic variance were obtained from number of filled grains, plant height, days for 50% heading, days for 85% maturity and biomass yield indicating high influence of the environment on the traits. Heritability estimates were moderately high for days to maturity (66.01%), and thousand-grain weight (66.80%). High genetic advance as percent of means was observed for biomass yield per plot, unfilled grains per panicle, grain yield per plot, and fertile tiller per plant. Thousand-grain weight showed moderately high heritability coupled with high genetic advance as percent of mean.

2.4.2 Mean performance of rice genotypes

Hashim *et al.* (2021) experimented on 40 advanced fragrant rice accessions in different environments to identify genotypes with high grain yield and high stability using multivariate (GGE biplot) and univariate analysis (regression slope, deviation from regression, Shukla's stability variance, Wricke's ecovalence, and Kang's stability statistic). The field experiment trials were laid in a randomized complete block design in three replications. The analysis of variance showed highly significant differences among genotypes, locations, seasons, and the interactions between genotype, locations, and seasons. The environment significantly explained about 43.32% (37.01 and 6.31% for locations and seasons) of the total sum of squares.

Shrestha *et al.* (2021) conducted an experiment in Khumaltar, Lalitpur, Nepal during rainy seasons of 2018 and 2019. Forty rice genotypes were planted in Alpha Lattice Design with two replications to determine the genetic diversity among them. The rice genotypes were grouped into 7 clusters based on growth and yield traits. The traits namely plant height, panicle length, number of tillers/plant and grain yield were found highly significant ($p < 0.01$). Rice genotypes NR 10676-B-1-3-3-3 produced the highest yield (5.65 t/ha), followed by NR10410-89-3-2-1-1 (5.54 t/ha).

Jaksomsak *et al.* (2021) evaluated grain anthocyanin, zinc (Zn) and iron (Fe) concentrations, and grain yield of eleven purple rice varieties (PP1–PP11) grown under wetland (W+) and aerobic conditions (W0) in 2 years. There was a significant variation in the concentrations of anthocyanin, Zn and Fe, by water regime and year among the varieties with a wide range of anthocyanin (1–117 mg 100 g^{-1}) and narrower ranges of Zn (19–41 mg kg^{-1}) and Fe (6–19 mg kg^{-1}) concentrations.

Huang *et al.* (2021) conducted a study on multiple environment trials to evaluate grain yield (GY) and four yield-component traits: panicle length, panicle number, spikelet number per panicle, and thousand-grain weight. Eighty-nine rice varieties were cultivated in temperate, subtropical, and tropical regions for two years. The effects of both GEI (12.4–19.6%) and environment (23.6–69.6%) significantly contributed to the variation of all yield-component traits. In addition, 37.1% of GY variation was explained by GEI, indicating that GY performance was strongly affected by the different environmental conditions.

Singh *et al.* (2020) conducted an experiment with 33 colored and white rice genotypes to study the variability and genetic parameters. The study involved seven red pericarp, eight black pericarp and 17 white rice genotypes, in addition to the check, BPT 5204. The results revealed high genotypic and phenotypic coefficient of variability, heritability and genetic advance as per cent mean for grains per panicle, grain yield per plant, total phenol content, zinc and iron content indicating the effectiveness of selection for these traits.

Sabri *et al.* (2020) studied on total of 19 newly developed genotypes under four varied environments in Peninsular Malaysia. The experiments were carried out using randomized complete block design (RCBD) with three replications at each environment. Data were collected on the vegetative, yield and yield component traits. Genotypic and phenotypic

coefficients, phenotypic variance component, heritability and genetic advance were also determined. Analysis of variance revealed that all traits were significantly different for genotypes except days to maturity, number of filled grains and total number of grains. Meanwhile, all the traits differed significantly for genotype × environment (G×E) except number of tillers per hill and number of panicles per hill. Low heritability (<30%) was found for all the traits. Similarly, low genetic advance was also observed for all the traits except for number of tillers per hill and number of panicles per hill.

Shrivastava *et al.* (2015) conducted an experiment with the aim of evaluating genetic parameters in 125 parental lines of hybrid rice. High genotypic and phenotypic coefficient of variation exhibited by unfilled spikelets panicle^{-1}, filled spikelets panicle^{-1}, seed yield plant^{-1}, panicle weight plant^{-1}, total spikelets panicle^{-1}, effective tillers plant^{-1}, tillers plant^{-1}. High heritability accompanied with high genetic advance showed by tillers plant^{-1}, plant height, culm length, flag leaf length, flag leaf width, biological yield plant^{-1}, panicle weight plant^{-1}, seed yield plant^{-1}.

Roy *et al.* (2014) estimated nutritional values of mineral contents of iron (Fe) and zinc (Zn) in all cultivars by Atomic Absorption Spectrophotometic method. Iron concentration varies from 0.25 µg/g to 34.8 µg/g and zinc from 0.85 µg/g to 195.3 µg/g in the landraces. Highest iron containing rice was Swetonunia with 34.8 µg/g and highest amount of Zn was found in Nepali Kalam which was 195.3 µg/g.

Dutta *et al.* (2013) studied 68 genotypes for twelve agronomical important characters to estimate variability and genetic parameters. Considering genetic parameters, high heritability (broad sense) and genetic advance as percentage of mean were shown by eight characters viz., tillers per plant, days to flowering, harvest index, spikelet per panicle, spikelet density, grain yield per panicle, and spikelet yield.

2.4.3 Genetic parameters (variability, heritability, GA, PCV, and GCV) studies in rice

Rahman *et al.* (2023) studied forty rice genotypes, evaluating their genetic variability, heritability, clustering patterns, trait associations, principal component analysis and path analysis for yield-contributing characteristics. The experiment followed a randomized complete block design (RCBD) with three replications. The phenotypic coefficient of variation (PCV) slightly exceeded the genotypic coefficient of variation (GCV) for all

traits, implying that environmental factors had negligible influence on trait expression. The GCV ranged from 2.67% for days to 80% maturity to 8.29% for grain yield. High heritability (>60%) and moderate genetic advance as a percentage of the mean (>10%) were observed for plant height (14.55%), yield (14.75%), and panicle length (10.13%), while low genetic advance as a percentage of mean was observed for the number of panicles per hill (6.48), days to 50% flowering (5.96), and days to 80% maturity (5.1).

Behera *et al.* (2022) evaluated Total 21 high zinc rice genotypes under five different locations for 14 different yield attributing traits, including grain yield/plant (gm) to determine the pattern of variation, the relationship among the individuals and their characteristics through Principal Component Analysis (PCA) during the *Kharif*-2017. PCA was done for all the locations individually as well as pooled analysis for all locations using R software. Out of the 14 PCs, the initial four PCs contributed more to the total variability. The highest cumulative variability of the first four PCs found at Bhikaripur (81.11%).

Sarwar *et al.* (2022) conducted a study to assess the extent and pattern of genetic variability of forty linseed genotypes based on diverse agro-morphological and yield attributes. The field experiment was conducted following a Randomized Complete Block Design with three replications. Linseed germplasm showed a wide range of phenotypic expression, genetic variability and heritability for 30 studied traits. A low to high phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were observed. Based on principal component analysis (PCA), few characters such as YPP, days to first flowering and plant heights at different growth stages revealed important and effective traits for consideration in the selection of linseed breeding programs.

Akshay *et al.* (2022) conducted a study with forty-four rice genotypes to analyze the variability and genetic parameters for yield and its components, quality and nutritional traits. The traits plant height, number of productive tiller per plants per plant, panicle length, number of grains per panicle, 1000 grain weight, grain yield per plant, head rice recovery percentage, grain length, grain width, length/breadth ratio, protein content, iron and zinc content all showed moderate to high variability, high heritability coupled with high genetic advance as per cent of mean revealing the role of additive gene effect and simple selection procedures may be effective for improving these traits. Low PCV and GCV were recorded for the traits viz., days to 50% flowering. High heritability coupled

with moderate genetic advance as per cent of mean was observed for days to 50% flowering, hulling percentage and milling percentage indicating that role of both additive and non-additive gene effects in the inheritance of these traits.

Acharjee *et al.* (2021) studied genetic variability and principal component analysis was carried out based on different morphological traits of 60 landraces of northeast India belonging to upland rice. The sensitivity of the landraces under drought stress conditions was confirmed by the first-order statistics of genetic variation as well as Principal component analysis (PCA) since comparatively more numbers (6) of principal components were found under drought stress over the irrigated conditions with 74.12 per cent of cumulative variability.

Lakshmi *et al.* (2021) conducted an experiment with 60 rice genotypes to study the genetic. High phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability and genetic advance as per cent of mean was observed for total tillers per plants per plant, ear bearing tillers per plant, filled and ill-filled grains per panicle in addition to grain yield per plant, indicating the effectiveness of direct selection for improvement of these traits.

Sudeepthi *et al.* (2020) conducted a study with 107 elite rice genotypes to evaluate the variability, heritability and genetic advance as per cent of mean for yield and yield component traits. High PCV and GCV were recorded for ear bearing tillers per plant, while high heritability was recorded for all the traits studied. Further, the high genetic advance as per cent of mean was recorded for plant height, the number of ears bearing tillers per plant, the total number of grains per panicle, test weight and grain yield per plant. Among these, ear bearing tillers per plant had recorded a high variability, heritability and genetic advance as percent of mean indicating its effectiveness as important selection criterion for the yield improvement.

Sarif *et al.* (2020) conducted a study to evaluate genetic variability and diversity among 32 coloured rice accessions using agro-morphological characteristics and simple sequence repeats (SSR) markers. Quantitative traits (morphological, grain quality and antioxidant properties) and 34 SSR molecular markers were used as tools for determining cultivar identities and genetic diversity. Most of the quantitative traits showed significant differences ($p \leq 0.01$) among all rice accessions. Clustering analysis from quantitative

traits categorised the accessions into four groups. Similarly, the 32 accessions were grouped into 4 cluster groups based on the analysis of 34 SSR markers. The accessions YTM15, Pulut Merah 3, Padi Randau, Ringan Bawang, DNJ128 and DV 107 can be potentially selected for development of new varieties for local cultivation. Finally, these accessions can be used as parents in further breeding programmes.

Saha *et al.* (2019) conducted an experiment to estimate genetic parameters of thirteen yield and yield attributing traits in 40 landraces of rice with a view to select better yield attributes in rice. The higher value of phenotypic co-efficient of variation (PCV) compared to the corresponding genotypic coefficient of variation (GCV) for all the studied traits indicated that there was an influence of the environment. Number of unfilled grains per panicle exhibited high estimates of PCV and GCV followed by number of filled grains per panicle, number of grains per panicle, flag leaf area.

Asante *et al.* (2019) conducted an experiment with one hundred rice genotypes in a 10 × 10 lattice design in three replications under field conditions. The GCV ranged from 4.3% for panicle length to 17.9% for grain yield. Grain yield (GY) had the highest PCV (37.3%), while kernel length had the lowest PCV (7.0%). High heritabilities and moderate genetic advances were observed for days to flowering (DF), plant height (PH), kernel length (KL), and kernel length-to-width ratio (KLW).

Pratap *et al.* (2018) conducted an experiment with thirty-eight rice germplasms to assess their genetic variability, heritability, and genetic advance. The high estimates of GCV and PCV were observed for traits like grain yield per plant, filled grains panicle-^{1}, effective tillers plant-^{1} indicating their importance in selection for improving the rice yield. High heritability coupled with high expected genetic advance as percent of mean was observed for the traits field grain panicle-^{1}, spikelet fertility percentage and days to maturity.

Bitew *et al.* (2016) conducted a study to assess the extent of genetic variability for yield and yield related traits and to estimate heritability and genetic advance in rainfed upland rice genotypes. The higher phenotypic and genotypic variance were obtained from number of filled grains, plant height, days for 50% heading, days for 85% maturity and biomass yield indicating high influence of the environment on the traits. Heritability estimates were moderately high for days to maturity (66.01%), and thousand-grain weight (66.80%). High genetic advance as percent of means was observed for biomass yield per plot, unfilled

grains per panicle, grain yield per plot, and fertile tiller per plant. Thousand-grain weight showed moderately high heritability coupled with high genetic advance as percent of mean.

2.5 Molecular characterization

2.5.1 Genetic diversity analysis

Basavaraj *et al.* (2023) studied substantial diversity in terms of yield and other agronomic traits, influenced by genetic, environmental, and management factors. Variability parameters, including genotypic and phenotypic coefficients of variation, heritability, correlation, and path analysis provide crucial insights into the extent of trait variation and their potential for improvement through breeding programs. Furthermore, correlation studies unveil the interdependencies between different traits, shedding light on potential connections influencing yield. This analysis uncovers important trait associations, guiding breeders and researchers in the pursuit of crop improvement strategies.

Kimwemwe *et al.* (2023) conducted an experiment to assess the genetic diversity and population structure of 94 rice (*Oryza sativa L.*) genotypes from the Democratic Republic of Congo using a set of 8389 high-quality DArTseq-based single nucleotide polymorphism (SNP) markers. The average polymorphic information content (PIC) of the markers was 0.25. About 42.4% of the SNPs had a PIC value between 0.25 and 0.5, which were moderately informative. The ADMIXTURE program was used for structure analysis, which revealed five sub-populations (K = 5), with admixtures. In principal component analysis (PCA), the first three principal components accounted for 36.3% of the total variation. Analysis of molecular variance revealed significant variation between sub-populations (36.09%) and within genotypes (34.04%).

Dwiningsih *et al.* (2022) conducted a study on the aspects of phenotypic variation of grain elemental concentration in the diverse rice genotypes, relationship of environmental conditions and rice grain elemental accumulation, correlation between rice grain elemental content and others agronomic traits, and also genetic basis of grain elemental concentration in rice. All of these aspects are important to develop rice varieties with a balanced elemental nutrients and lower toxic heavy metal elements. Enhancing the concentration of essential mineral elements and reducing the accumulation of toxic elements in the rice grain are important to improve the rice quality for human health in addressing mineral

deficiency and toxicity that could be accomplished by using plant breeding, agronomic, and genetic engineering approaches.

Andarini *et al.* (2022) conducted a study to analyze the genetic diversity of 15 accessions of local pigmented rice collected from Eastern Indonesia (Sulawesi and East Nusa Tenggara) in IAARD-ICABIOGRAD Gene Bank based on 33 morpho-agronomical characters and 24 SSR markers. Based on the characteristic of SSR markers, the average allele number, major allele frequency, gene diversity, heterozygosity, and the PIC were 4.88, 0.47, 0.66, 0.02, and 0.62, respectively. The similarity coefficient between accessions based on morpho-agronomic characters and SSR markers was still quite high (80%). PCoA and phylogenetic analyses based on the results of combined SSR and morpho-agronomic data formed three different groups at a cophenetic distance of 0.22.

Bekis *et al.* (2021) conducted an experiment with 30 lowland rice genotypes to determine the magnitude of genetic distance through cluster analysis, which helps to identify parental lines for hybridization programs. Data were collected for 17 agronomic characters and analysis of variance revealed significant differences among the genotypes for all characters. Cluster analysis showed the existence of five divergent groups and the maximum inter-cluster distance was between clusters II and III (D^2=6758); while the minimum inter-cluster distance was between clusters III and V (D^2=2432). It is suggested to cross genotypes from cluster II and III, I and III to get genotypes/varieties with high grain yield and early maturing genotypes. For future breeding program that employ hybridization, parental material selection should be carried out between clusters rather than within clusters.

Nithya *et al.* (2021) conducted an experiment to resolve the population structure and genetic diversity in bold type rice varieties of southern India, we used a total of 81 rice genotypes by 100 simple sequence repeat markers composed of 36 improved varieties and 45 landraces, which are representative and important for bold type grain rice breeding. All the genotypes were clustered into mainly 5 clusters. Principle component analysis revealed that the first principal component revealed 42.87% variation, while the second component showed 14.01% variation. Among the eight morpho-physiological and plant production traits studied, the relative water content and spikelet fertility percentage contributed towards maximum diversity.

Karimah *et al.* (2021) evaluated 43 accessions at the agro-morphological and genetic levels. Clustering based on the agro-morphological resulted in four sub-groups. Analysis at the genetic level was conducted using 22 microsatellites, which revealed a total number of alleles to be 203, with a range per allele between 2 and 17 and an average of 9.2 alleles per locus. The highest and lowest Polymorphic Information Content (PIC) values were found in RM431 and RM11, which were 0.95 and 0.67, respectively. The genetic diversity value ranged from 0.71 to 0.95. The genetic similarity among accessions ranged from 0.00 to 0.90.

Aesomnuk *et al.* (2021) conducted a study on the genetic diversity and population structure of 365 accessions of lowland and upland landraces from four populations from different geographical regions of Thailand were investigated using 75 SNP markers. Clustering analyses using maximum likelihood, Principal Coordinate Analysis (PCoA), and Discriminant Analysis of Principal Components (DAPC) clustered these landraces into two main groups, corresponding to *indica* and *japonica* groups. The *indica* group was further clustered into two subgroups according to the DAPC and STRUCTURE analyses (K = 3). The analysis of molecular variance (AMOVA) analysis results revealed that 91% of the variation was distributed among individuals, suggesting a high degree of genetic differentiation among rice accessions within the populations.

Al-Shammari *et al.* (2021) studied the genetic diversity and relationships among 24 tomato lines were evaluated by simple sequence repeat (SSR) markers. A total of 65 bands were generated with 15 SSR primers, of which 64 bands were polymorphic. The mean polymorphic information content was 0.356. There was a high degree of polymorphism between tomato cultivars. The mean marker index and heterozygosity were 0.045 and 0.454, respectively.

Singh *et al.* (2020) conducted a study on twenty-two genotypes of rice during Kharif, 2018 in RBD with three replications in five different locations. The 22 rice genotypes were characterized based on 16 quantitative traits viz., days to first flowering, days to 50 per cent flowering, days to maturity, the total number of effective tillers per plant, plant height (cm), panicle length (cm), the number of spikelets per panicle, the number of grains per panicle, spikelet fertility percentage, grain weight per panicle (g), grain yield per plant (g) , 1000-grain weight (g), grain yield per plot (kg), grain yield per hectare (kg), grain L/B Ratio, grain zinc content (ppm or mg /kg) using mahalanobis D2 statistic. D2 analysis

distributed the 22 genotypes into six clusters, of which cluster I was the largest with 15 genotypes. Cluster II had maximum intra-cluster values of 5.16 and the maximum inter-cluster distance was observed between the clusters III and V (40.51) followed by cluster IV and V (28.39) indicating the importance of the genotypes present in these clusters for exploiting heterosis for the desirable traits of these clusters.

Dhakal *et al.* (2020) conducted a study which was carried out with 30 rice landraces at the Institute of Agriculture and Animal Science, Lamjung Campus, during June–November 2018 to determine relation among individuals, estimate the relative contribution of various traits of rice using principal component analysis, and identify the potential parents for hybridization using Mahalanobis distance (D^2). The principal component analysis revealed that five among the thirteen principal components were significant (eigenvalue >1) and contributed to 29.96%, 20.26%, 13.56%, 11.68%, and 9.22% of the total variance, respectively. PC1 included the traits that were related mostly to the yield, yield attributing, and grain characteristics. Landraces from Anadi group, Jetho Budo, Jarneli, and Rato Masino performed well in PC1 while landraces such as Mansara, Pakhe Sali, and Aanga performed well in PC2.

Feng *et al.* (2020) studied fourteen expressed sequence tag-derived simple sequence repeat (EST-SSR) and seven sequence-related amplified polymorphism (SRAP) markers to evaluate the genetic diversity in fifty *Polygonatum* Mill. accessions. The EST-SSRs and SRAPs produced 173 (90.58%) and 113 (93.39%) polymorphic bands, respectively. Unweighted Pair-Group Method Analysis (UPGMA) based on the combined data matrices of EST-SSRs and SRAPs divided the fifty *Polygonatum* Mill. accessions into fourteen groups. In addition, accessions of *P. cyrtonema* Hua obtained from Anhui and Zhejiang provinces were clustered according to their geographic origin. Furthermore, some accessions were gathered together based on species, such as *P. kingianum* Coll. et Hemsl, *P. punctatum* Royle ex Kunth, *P. odoratum* (Mill.) Druce, and *P. sibiricum* Red., and bootstrap analysis for clustering fully supported the grouping of the accessions.

Suvi *et al.* (2020) conducted a study to assess the genetic diversity and population structure of 54 rice accessions using 14 polymorphic simple sequence repeats (SSR) markers to select unique parents for breeding. Data analysis was based on marker and population genetic parameters. The mean polymorphic information content (PIC) was 0.61 suggesting high polymorphisms for the selected SSR markers among the rice accessions. The

population structure revealed a narrow genetic base with only two major sub-populations. Analysis of molecular variance revealed that only 30% of the variation was attributed to population differences while 47% and 23% were due to variation among individuals within populations and within individual variation, respectively. The genetic distance and identity among genotypes varied from 0.083 to 1.834 and 0.159 to 0.921, respectively.

Bhandari *et al.* (2017) conducted a study on the preference for the development of new crop varieties shifts over a period with environmental changes. Plant breeders develop climate-resilient varieties possessing all the desirable traits, including resistance to various biotic and abiotic stresses. Genetic diversity in the form of mutant lines, wild species, breeding stocks, etc., is used for the improvement and development of modern crop varieties

Gour *et al.* (2017) conducted an experiment with total 83 rice genotypes comprising traditional landraces and released varieties from Jawaharlal Nehru Krishi Vishwavidhyalay Krishi Nagar, Madhya Pradesh, India. The rice genotypes were evaluated for 33 quantitative and quality traits by principal component analysis for determining the pattern of variation, relationship among individuals and their characteristics. Principal component analysis was utilized to examine the variation and to estimate the relative contribution of various traits for total variability. The PC1 showed 18.683%, while, PC2, PC3, PC4, and PC5 exhibited 15.404%, 13.401%, 9.433%, 8.037%, and 5.232% variability. Rotated component matrix revealed that PC3 accounts for yield & yield attributing traits. PC1 was also dominated by yield related traits. The PC2, PC4 & PC5 was dominated by quality traits.

2.5.2 Application of molecular markers for cultivars improvement

Zulfiqar *et al.* (2023) conducted a study with fourteen polished rice genotypes to assess genetic diversity. Ten SSR makers were utilized, with eight of them yielding polymorphic data. Monomorphic results were found for RM211 and RM331. Two markers, RM430 and RM437, were found to have a P-value of less than 0.05. RM430 had a P-value of 0.033 for zinc, whereas RM437 had a P-value of 0.046 for iron and 0.001 for zinc, with R2 values of 46.07, 23.46, and 45.07, respectively.

Kumar *et al.* (2022) conducted a study to use 25 SSR markers for the assessment of genetic diversity and relatedness among 31 rice landraces and all of them were found polymorphic. Twenty-five SSR markers revealed 50 alleles in the 31 land races, the number of alleles per locus with an average of 2 per locus. Major allele frequency ranged from 0.58 (RM 25) to 0.94 (RM 229) with an average of 0.74 per marker. The PIC (polymorphism information content) value ranged from 0.12 (RM 229) to 0.66 (RM 481 and RM 25) with an average of 0.36 per marker. The PIC value of each marker, which was evaluated on the basis of its alleles, varied greatly all tested SSR loci-from 0.58 to 0.94 with an average of 0.74. The highest PIC value 0.49 was obtained for RM481 and RM25, followed by RM443 (0.47), RM224 (0.46) RM 159, RM 1054, RM226 and RM24 (0.44).

Hasan *et al.* (2021) discussed the positive aspects of molecular marker-assisted selection and its precise applications in plant breeding programmes. Molecular marker-assisted selection has considerably shortened the time for new crop varieties to be brought to the market. To explore the information about DNA markers, many reviews have been published in the last few decades; all these reviews were intended by plant breeders to obtain information on molecular genetics. The progress made in molecular plant breeding, plant genetics, genomics selection, and editing of genome contributed to the comprehensive understanding of DNA markers and provides several proofs on the genetic diversity available in crop plants and greatly complemented plant breeding devices.

Embate *et al.* (2021) used 64 SSR markers to estimate genetic diversity in 43 pigmented traditional rice varieties (TRVs). The PIC values revealed that RM26550, RM28166, RM10665, RM27492 and RM23251 could be the best markers for genetic diversity estimation of these TRVs. The diversity at gene level showed average of 3.48 alleles ranging from 2 to 11 per locus. Mean gene diversity (H) value for all SSR loci was at 0.34, indicates moderate genetic diversity of TRVs used in the study.

Mehmood *et al.* (2021) conducted an experiment with 8 exotic and 7 local rice genotypes, using 5 different SSR markers, i.e., RM3, RM259, RM341, RM520, and RM11943. A set of 5 simple sequence repeat primers, covering four chromosomes, amplified a total of 14 alleles and showed 100% polymorphism with an average PIC value ranging from 0.39 to 0.91. The UPGMA cluster analysis separated the 15 rice genotypes into 3 main groups based on 32.5% similarities and the highest genetic distance (45.1%) was observed

between two genotypes (Fakher-e-malakand and Musa), having different geographical origins.

Shivani *et al.* (2021) conducted a study to analyze the genetic diversity among 77 germplasm lines at molecular level employing 36 randomly chosen microsatellite markers. A total of 30 markers were found to be polymorphic among the genotypes with a total of 70 alleles. The number of alleles per locus ranged from 2 to 3. The polymorphic information content (PIC) value ranged from 0.072 to 0.640 with an average of 0.32. Based on the principle of Unweighted Pair Wise Method using Arithmetic Average (UPGMA) constructed by Jaccard's similarity Coefficient, the cluster analysis distinguished these accessions into eight clusters. Cluster I had maximum number of genotypes (59) followed by cluster II (8), clusters III (3), whereas the clusters IV and VII have two genotypes each and cluster V, VI and VIII had only one genotype.

Tarang *et al.* (2020) studied 60 microsatellite markers were used in 63 rice genotypes of Central and West Asia to group rice cultivars. Based on data from 60 markers, it was observed that a total of 252 polymorphic alleles were amplified with an average of 4.2 alleles per primer. The mean number of effective alleles was 3.78 which RM490 and RM5423 markers had the lowest and the RM225 and RM246 markers had the highest value for this index. Nei gene diversity and amount of polymorphic information content showed that RM23 and RM212 markers had the highest value and the RM3 marker had the lowest value for these two indices. Genetic similarity and distance between populations revealed that the genetic distance between studied populations ranged from 0.147 to 0.54, indicating high variation among genotypes of these populations.

Tripathi *et al.* (2020) evaluated to assess the genetic diversity among 27 rice cultivars with 12 SSR markers. The results revealed a total of 40 alleles were detected across 27 rice cultivars tested. PIC values varied widely among SSR loci tested and it ranged from 0.38 to 0.65, with an average of 0.56 per marker. The 27 rice cultivars were grouped into two major clusters i.e., cluster I and II with similarity coefficient 0.13. Cluster I was sub divided into two minor sub-groups IA and IB having 5 and 8 genotypes respectively. These subgroups were further subdivided into minor groups. In similar way, the second main cluster i.e., Cluster II was also sub divided into two minor sub-groups that is IIA and IIB having 5 and 9 genotypes respectively.

Sarif *et al.* (2020) conducted a study to evaluate genetic variability and diversity among 32 colored rice accessions using simple sequence repeats (SSR) markers. Quantitative traits (morphological, grain quality and antioxidant properties) and 34 SSR molecular markers were used as tools for determining cultivar identities and genetic diversity. Most of the quantitative traits showed significant differences ($p \leq 0.01$) among all rice accessions. Clustering analysis from quantitative traits categorized the accessions into four groups. Similarly, the 32 accessions were grouped into 4 cluster groups based on the analysis of 34 SSR markers. The accessions YTM15, Pulut Merah 3, Padi Randau, Ringan Bawang, DNJ128 and DV 107 can be potentially selected for development of new varieties for local cultivation.

CHAPTER III

MATERIALS AND METHODS

The experiment was conducted at the experimental farm and also at the laboratory of the Department of Genetics and Plant Breeding, Hajee Mohammad Danesh Science and Technology University, Dinajpur. The next subsections in this chapter provide the presentation of the study's materials and methodology.

3.1 Location and duration of the experiment

The experimental field was placed at 25°41'50.2" North latitude and 88°39'00.5" East longitude, under the Dinajpur sadar upazila, and 37.5 meters above mean sea level. The area is part of the Old Himalayan Piedmont plain's agro-ecological zone (AEZ-1). The trial ran from April to November of 2022.

3.2 Climate

The subtropical climate of the experimental field experiences substantial rainfall during the Kharif season (March to August) and sparse rainfall during the Rabi season (October to February). The air temperature rose as the Kharif season progressed under rainfed conditions during this crop's growth phase.

3.3 Soil

The experimental field was a medium-high area of the Old Himalayan piedmont plain's non-calcareous dark gray floodplain soil, which is located beneath the agro-ecological zone (AEZ-1). The soil is sandy loam under the order Inceptisol. The experimental field had well organized irrigation and drainage system.

3.4 Design and arrangement for experimentation

Three replications and a Randomized Complete Block Design (RCBD) were used to set up the experiment. The land area measured 13.50 m by 14.0 m in total.

3.5 Material for experiments

A total of thirty land races and six BRRI-released varieties comprising genotypes of aus and boro rice were employed as experimental materials in the study; these genotypes are listed in Table 1.

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

Code No.	Genotypes	Accession No.	Source of the genotypes
G1	Chenri Aus	808	Genetic Resource and Seed Division, BRRI, Joydebpur
G2	Balam Aus	809	
G3	Lohar gura Aus	812	
G4	Gungur Murali Aus	813	
G5	Gungur Bali Aus	814	
G6	Begun Bichi Aus	1202	
G7	Kala manik Aus	1203	
G8	Gori Matir Aus	1206	
G9	Noroi-4 Aus	1210	
G10	Kasmiri Lota Aus	1211	
G11	Surjamoni Aus	1283	
G12	Nara Bet	1285	
G13	Mali Khori Aus	1288	
G14	Ora Bet Aus	1290	
G15	Aus Baku	1318	
G16	Jamrishaity Aus	1317	
G17	Batulshi Aus	1320	
G18	Baktulshi Aus	1321	
G19	Manik madhu 2	1322	
G20	Manik madhu	1323	
G21	BRRI Dhan-82	NA	
G22	Ratul Aus	NA	
G23	BRRI Dhan-48	NA	
G24	BRRI Dhan-85	NA	
G25	Purple-2	NA	
G26	BRRI Dhan-28	NA	
G27	BRRI Dhan-72	NA	
G28	BRRI Dhan-29	NA	
G29	Kali boro-1	260	
G30	Kaikka boro	262	
G31	Bawoi boro	1708	
G32	Baran boro	259	
G33	Tepi boro-2	930	
G34	Jamir boro	1706	
G35	Jagli boro-1	255	
G36	Sada boro	1714	

Here, BRRI= Bangladesh Rice Research Institute

3.6 Processing of seeds

A 1% mercuric chloride ($HgCl_2$) solution was used to disinfect the seeds of different genotypes of rice for ten minutes. To get rid of the materials that were sticking to them, they were then repeatedly rinsed with distilled water.

3.7 Procedure for germination of seeds

Following disinfection, each beaker of seeds was submerged in water for a full day. The following day, the seeds were removed and placed in gunny sacks to grow over the course of 48 hours in the dark. When the sprouts turned white and emerged to be a few millimeters long, the main field was sowed. When sprouts were come out in few millimeters long becoming white in color, then it was sown the main field. Sprouted seeds were sown on single.

Figure 2: Germination of seeds

3.8 Preparing the land and planting seeds

The following sub-headings provide details on seed sowing and experimental site preparation.

3.8.1 Setting up the experimental area

Two ploughings and one cross-ploughing with a power tiller were used to prepare the field. The area was further ploughed and cross-ploughed with a country plough after a few days, and then laddering was done to create a nice condition for puddles. Weeds and stubbles

were removed from the field prior to the sowing of seeds and transplanting of seedlings. Irrigation channels were constructed around each block and manures and fertilizers were applied in accordance with recommended dosages.

3.8.2 Applying fertilizers and manure

Table 2 contains information on fertilizer dosage and application technique. The field was treated with half of the urea, triple super phosphate (TSP), murate of potash (MoP), and the entire amount of well-decomposed cow dung at the time of soil preparation. The remaining urea was applied in two equal portions as part of the ring insertion procedure. After transplanting, the first installment was applied 15 days later. One week prior to the start of the panicle, the second installment was applied.

Table 2. Fertilizer dosages and application techniques in rice fields

Name of the fertilizer/ manure	Application rate (kg/ha)
Cowdung	10000
Urea	140
Triple Super Phosphate (T.S.P.)	65
Murate of Potash (MoP)	75
Zinc sulphate	3
Gypsum	13

Source: Bangladesh Rice Research Institute (BRRI), Joydebpur, Gazipur,
Field Survey, 2021-22

3.8.3 Seed-planting

On April, the main field was sown when the sprouts appeared, measuring 1-3 millimeters and turning white in color. With a spacing of 25 cm between plants and 30 cm between rows, sprouted seeds were sowed singly for every genotype.

3.9 Intercultural operation

Intercultural activities were carried out for the plants' superior growth and development during the cropping period. These are explained in the section below.

3.9.1 Weeding

The first two top treatments of urea were used for weeding. In order to reduce fertilizer loss through de-nitrification and leaching and, ultimately, assure better plant growth and development, weeds were removed from the plots to allow for easy aeration of the soil, break the soil crust, and facilitate the incorporation of urea fertilizer.

3.9.2 Watering

A minimum of five irrigations were administered to maintain a water depth of 5-7 cm during the milking to hard dough stage of the rice crop. Appropriate precautions were also made to minimize the invasion of pests and diseases.

3.9.3 Harvesting

Crop maturity was defined as the point at which 80% of the rice seeds reached physiological maturity. Each plant was collected independently. While other plants were normally harvested for yield, the seeds were collected separately for future study. After being properly marked and packaged, the collected crop was carried to the threshing floor. The yield of grain was recorded after thoroughly drying in the sun.

3.9.4 Processing

Because rice grains had the ability to absorb moisture from the air, they were harvested and stored in dry conditions. The spikes were then placed in an oven to finish drying. The spikes were removed from the oven to be threshed. To find out how much grain each plant produced, grains were gathered.

3.10 Data collection

The data on 10 morpho-physiological and bio-fortified traits recorded considering each plant of the plots.

3.10.1 Morpho-physiological trait measurement

Following germination, five (5) plants from each row were randomly tagged. For ten morpho-physiological characters—days to 50% flowering, plant height (cm), productive and unproductive tillers per plant, total tillers per plant, panicle length (cm), spike per panicle, straw weight (g), thousand seed weight (g), and yield per plant (g)—data were recorded on the selected plants per genotypes in three replications.

Ten (10) morpho-physiological data recording procedures are briefly described below:

1. **Days to 50% flowering**

   The day roughly fifty percent of the rice plants' blossoms bloomed was when this characteristic was observed. It is calculated by adding the date of the recording to the day of sowing. In order to determine days to 50% flowering of the spike (estimated by sight) emerged in each plot, the number of days from the date of sowing was counted.

2. **Plant height (cm)**

   Using a measuring scale, plant height was determined from its base to the tip of its tallest leaf, and the result was expressed in centimeters (cm).

3. **Number of productive tillers per plant**

   Considered to be effective tillers were those who had at least one grain in each panicle. From each sample plant, the total count of tillers bearing ears was determined. Every genotype's data for this experiment came from three different plants.

4. **Number of unproductive tillers per plant**

   Tillers deemed ineffective were those that produced no grain in a panicle. For every plant in the sample, the number of unproductive tillers was determined. For each genotype, data were gathered from three plants in this experiment.

5. **Number of total tillers per plant**

   Total number of effective and non-effective tillers per plant were counted.

6. **Panicle length (cm)**

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

7. **Spikes per panicle**

   Spikelet containing any food material or not were counted as total spikes per panicle.

8. **Straw weight (g)**

One of the byproducts of harvesting paddy is rice straw. Using an electrical scale and three randomly chosen plants from each replication, the straw weight was determined.

9. **Thousand seed weight (g)**

Using an electronic balance, one thousand clean dry grains were weighed and tallied from the seed stock that was taken from three randomly chosen hills in each plot.

10. **Yield per plant (g)**

Each plant's grains were carefully weighed after being sun-dried. To calculate the grain yield per plant at maturity and quantify it in grams, the dry weight of the grains was measured.

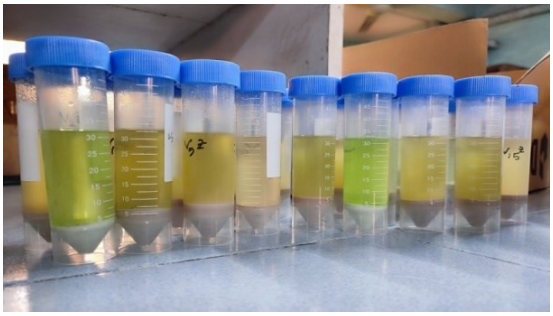
3.10.2 Evaluation of nutritional characteristics

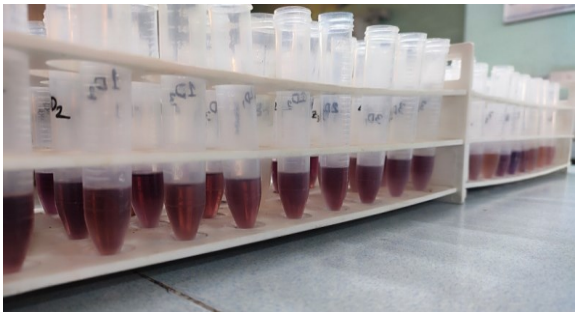
In the labs of the Soil Resource Development Institute and the Food Process and Engineering Lab, a total of five bio-fortified traits were measured: DPPH scavenging activity, iron content, zinc content, total phenolic content, and total flavonoid content. This is a quick description of the data collection process for these.

1. Zinc content (mg/kg)
2. Iron content (mg/kg)
3. Total flavonoid content (mg/g)
4. Total phenolic content (mg/g)
5. 2,2-diphenyl-1-picrylhydrazyl (DPPH) content (millimole/100g sample)

Getting the extracts ready

With a few minor adjustments, the Sengul *et al*. (2014) method for polyphenol extraction was used. A mortar and pestle are used to paste the rice sample. Methanol and powder samples were combined in a sample-to-solvent ratio of 1:10 and vortexed for 10 minutes. Whatman No. 1 filter paper was used to filter the suspension. The completed solutions served as a stock solution for additional examination.



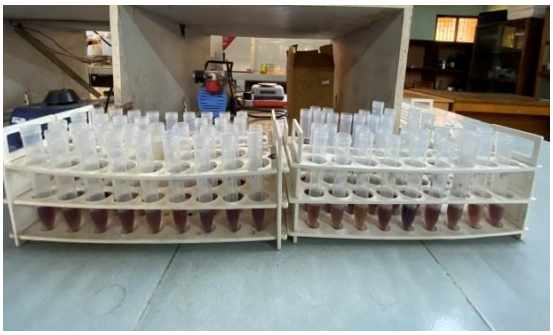


A **B**

Figure 3 (A-B): Preparation of extraction solution and measurement of total phenolic content of rice grain.

A **B**

Figure 4 (A-B): Measurement of total flavonoid content of aus and boro rice



A **B**

Figure 5 (A-B): Measurement of DPPH of aus and boro rice using UV-Vis spectrophotometer.

1. Total phenolic content (TPC)

The total content of phenol was calculated by Singleton and Rossi, 1965 and Saikia *et al.* 2012. Following the reference procedure, 0.5 mL of filtered sample along with 0.5 mL of Folin Ciocalteu's reagent was taken in a 25 mL falcon tube and mixed thoroughly. The solution was allowed to react with 1 mL of 7.5% saturated sodium carbonate (Na_2CO_3) to the falcon tube for neutralization and then vortexed for 30 seconds. After the mixture was allowed, it was left in a dark place for 35 minutes at room temperature and centrifuged at 4000 g for 10 minutes. The absorption of the sample was read by a visible spectrometer (UV/VIS, UV-1800) at 725 nm. Gallic acid was used to execute a standard (calibration) curve. The findings have been shown to be equal mg/100 g of Gallic acid per 100 g of rice sample.

2. Total flavonoid content (TFC)

A colorimetric method described by Kim *et al.* (2003) was used to evaluate the total flavonoid content in the juice. 1 mL of extract along with 4 mL of water and 5% $NaNO_2$ with 0.3 mL distilled water poured into a 20 mL of falcon tube for 5 minutes. Afterwards, the 0.3 mL $AlCl_3$ (10%) was applied to the reaction mixture and allowed to stand for 1 min, followed by 2 mL of 1 M NaOH and centrifuged at 4000 g for 5 minutes. After that, the tubes were incubated at room temperature for 15 minutes while being measured as mg/100 g using a spectrophotometer (UV/VIS, UV-1800) set at 510 nm.

3. DPPH radical scavenging capacity

The free radical scavenging of pigmented rice samples was estimated using the protocol given by Madhujith and Shahidi (2006). Briefly, an aliquot of 0.1 mL of supernatant was taken in a falcon tube. Subsequently, 1.9 mL of 0.3 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenger in methanol was added to the sample. The mixture was allowed to rest for 30 minutes. DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) was calculated using a spectrophotometer (UV/VIS, UV-1800) with 517 nm and the results were expressed as mmol/100 g rice grain extracts.

4. Iron and zinc content

The determination of micro and macro minerals was carried out according to modified method of Rybicka 2017. All of the samples were mineralized in a 2:1 ratio of nitric acid and perchloric acid. 0.5g samples were mixed with 15 mL nitric acid and perchloric acid solution. In order to fully digest the material, wet mineralization was done in a mineralization block (Model Q-439, BUSHI) at 205°C. Samples were cooled, then filtered into 100 mL flasks and refilled with demineralized, filtered water. Prepared samples were stored for up to two weeks at 4°C in the dark, pending mineral assays. The levels of Fe and Zn (SpectrAA-7000, Shimadzu, Kyoto, Japan) at the SRDI (Soil Resource Development Institute), Dinajpur lab were estimated using atomic absorption spectroscopy in the flame (F-AAS).

3.11 Statistical analysis

The data of each character against each entry were furnished into a Microsoft Excel spread sheet. The data were analyzed of randomized complete block design model by Cochran and Cox (1950).

3.11.1 Analysis of variance

Analysis of variance of 15 morphophysiological and nutritional traits was performed using the statistical program (RStatistics of version 4.3.2, 2023) with the following model:

$$y_{ij} = g_i + r_j + \epsilon_{ij}$$

here,

Y_{ij} = Observation of genotype i in replication j

g_i = Effects of genotype i

r_j = Effects of replication j

$\mathcal{E}_{ij}$ = Residual error of genotype i in replication j

The significance of difference among the means of genotypes was evaluated by Tukey's test for interpretation of results.

3.11.2 Calculation of genotypic and phenotypic variances

Genotypic and Phenotypic variances:

Genotypic and phenotypic variances were estimated according to the formula given by Johnson *et al.* (1955).

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

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

Where, MS_g = Mean sum of squares for genotypes;

MS_e = Mean sum of squares for error, and

r = Number of replications

σ^2_e = Error mean square

3.11.3 Estimation of genotypic and phenotypic co-efficient of variations

Genotypic and phenotypic co-efficient of variations were calculated according to the formula given by Burton (1952), and Singh and Chaudhary, (1985).

$$\text{Genotypic co} - \text{efficient of variation (GCV)} = \frac{\sqrt{\sigma^2 g}}{\bar{x}} \times 100$$

Where, $\sqrt{\sigma^2 g}$ = Genotypic variance; and

x = Population mean

$$\text{Phenotypic co} - \text{efficient of variation (PCV)} = \frac{\sqrt{\sigma^2 p}}{\bar{x}} \times 100$$

Where, $\sqrt{\sigma^2 p}$ = Phenotypic variance; and

x = Population mean

3.11.4 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, $\sigma^2 g$ = Genotypic variance; and

$\sigma^2 P$ = Phenotypic variance.

Heritability is classified as low (below 30%), medium (30% - 60%) and high (above 60%).

3.11.5 Estimation of genetic advance

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

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

Where, h^2_b = Heritability in broad sense;

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

σ_p = Phenotypic standard deviation

3.11.6 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

$$GA(\%) = \frac{GA}{\bar{x}} \times 100$$

Where, GA = Genetic advance; and

x = Population mean

Genetic advance in percentage of mean is classified as low (<10%), moderate (10% - 20%) and high (>20%).

3.11.7 Cluster analysis

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

3.11.8 Principal component analysis (PCA)

The technique was used to examine the interrelationships among seventeen quantitative characters. The principal components were computed from the correlation matrix obtained from the sum of squares product matrix of the characters and genotype score obtained from the first component and the succeeding component with latent roots greater than unity. The latent roots are called Eigen values. The component has the property of accounting for maximum variances. The PCA displays most of the original variability in a smaller number of dimensions, since it finds linear combinations of a set of varieties that maximize the variation contained within them. Contributions of the different characters towards divergence are discussed from the latent vectors of the first two principal components.

3.11.9 Biplot analysis

A PCA biplot is a two-dimensional chart that shows both PC scores of samples and loading of variables. By using the relative value of the trait, a genotype × trait biplot was constructed from a two-way matrix of seventeen traits and pigmented rice genotypes using factoextraFactoMineR packages of R software version 4.2.2 (R Core Team 2023).

3.12 SSR marker-based molecular characterization and diversity analysis of rice

3.12.1 Isolation of genomic DNA

Young leaves were cut into pieces (2-4) and placed in an Eppendorf tube. They were then dried in silica gel for 6-7 days. Using a mortar and pestle, the samples were ground. Next, 800 µl of CTAB buffer that had been heated to 65°C was added to each tube, and it was completely vortexed. Samples were incubated in water bath at 65°C for 45 minutes and at every 10 minutes they were mixed gently by inversion (400 µl of 2% β- Mercaptoethanol was added to 200 ml of extraction buffer prior to warming). The tubes were taken out of the water bath and left at room temperature for 5 minutes. 600 µl of chloroform isoamyl alcohol (24:1) was added. To combine the two layers, the samples were gently inverted 100 times in a period of around two minutes. After that, the materials were centrifuged for 20 minutes at room temperature at 4000 rpm. Using a large diameter pipette, the aqua phase was eliminated. The nucleic acids were precipitated by gently mixing two-thirds of an ethanol volume with the aqua phase, which had been transferred to a clean 1.5 ml tube. At this stage the samples were stored in 4°C for overnight. After that the samples were centrifuged at 10000 rpm for 20 minutes and the supernatant was discarded. Then the DNA was dried so that there was no ethanol. 500 µl of washing buffer was added in each tube and the DNA was washed gently inversion. Once more, the samples were centrifuged for 20 minutes at 10,000 rpm, after which the supernatant was drained and the tubes were left on the bench to dry. After adding 100 µl of double-distilled water, the samples were allowed to dissolve. Lastly, the samples were temporarily kept at 4°C.

A

B

C

D

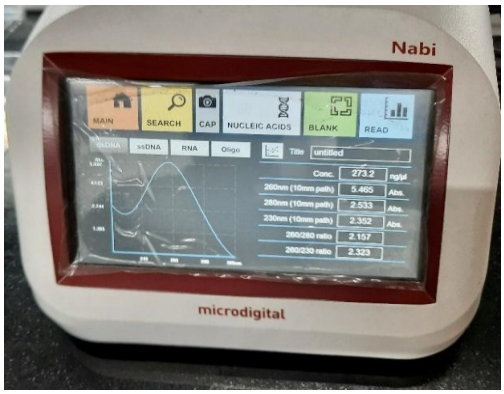
Figure 6 (A-D): Genomic DNA isolation from rice genotypes with the application of a modified CTAB technique

3.12.2 DNA quantification

A Thermo Scientific NanoDrop^TM1000 Spectrophotometer (Thermo Fisher Scientific, USA) was used to quantify the extracted DNA samples and assess their quality before PCR amplification.

3.12.3 PCR amplification and separation by electrophoresis

Using a Techne Prime Thermal Cycler (USA), PCR amplifications were carried out in 10 µL tubes. Each tube held two microliters of template DNA, eight microliters of reaction mixture (0.5 microliters each of the forward and reverse primers, 2 microliters of nuclease-free water, and 5 microliters of G2 Green Master Mix). One cycle of 94°C for 5 minutes was followed by 35 cycles of 95°C for 0.5 minutes, 53 to 58°C (depending on the particular primers) for 0.5 minutes, and an extension for 0.5 minutes. Finally, a final extension at 72°C for 5 minutes concluded the PCR amplification. Reaction products were mixed with one fifth volume of loading buffer (100 mM/L EDTA pH 8.0,10 mM/L Tris-HCl pH 7.5, 5% Ficoll 400; 0.05% bromophenol, 0.05% xylenecyanol) and 8 µL were loaded vertically, 8% denaturing polyacrylamide gels in 1 × TBE (90 mM/L Tris borate pH 8.3, 2 mM/L EDTA) at 50 mA for two to three hours is necessary for electrophoresis (Wang *et al*., 2007). After silver staining the gels, an x-ray viewer was used to take pictures.

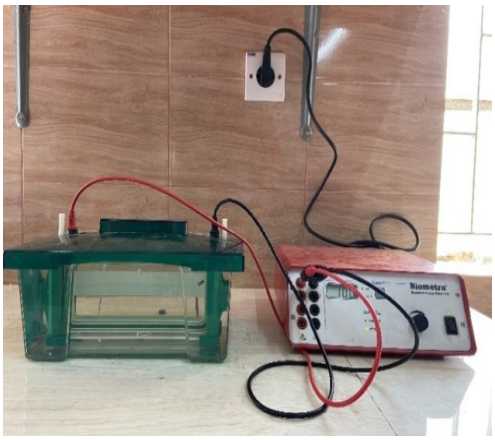


A

B

C

D



E

F

Figure 7: (A-F) Genomic DNA quantification, polymerase chain reaction amplification, polyacrylamide gel electrophoresis and gel washing.

3.13 Markers for microsatellite/simple sequences repeat (SSR)

To analyze the genetic diversity of the rice genotypes, a total of 14 microsatellite (SSR) markers encompassing all 24 chromosomes were used, as indicated in the Table by Kumar *et al*. (2016). The final Polymerase chain reaction (PCR) amplification was conducted using these SSR primers, each of which has a unique chromosome number. Table 3 displays the chromosomal location, annealing temperature, and primer sequences for SSR marker.

Table 3: SSR markers are employed to analyze rice germplasm diversity

SSR Loci	Forward primer (5′–3′)	Reverse primer (5′–3′)	Annealing temperature (degree centigrade)
RM1	GCGAAAACACAATGCAAAAA	GCGTTGGTTGGAACCTGAC	55
RM27	TTTCCTTCTCACCACTTCA	TCTTTGACAAGAGGAAAGAGGC	55
RM452	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG	57
RM338	CACAGGAGCAGGAGAAGAGC	GGCAAACCGATCACTCAGTC	58
RM249	GGCGTAAAGGTTTTGCATG	ATGATGCCATGAAGGGTCAGC	56
RM585	CAGTETTGCTCCGTTTGTTG	CTGTGACTGACTTGGTCATAGG	56
RM162	GCCAGCAAAACCAGGGATCCGG	CAAGGTCTTGTGCGGCTTGCGG	58
RM234	ACAGTATCCAAGGCCCTGG	AACGTGAGACAAGGACGGAG	56
RM107	AGATCGAAGCATCGCGCCCGAG	ACTGCGTCCTCTGGGTTCCCGG	57
RM316	CTAGTTGAGCATACGATGGC	ACGCTTATATGTTACGTCAAC	58
RM171	AACGCGAGGACACGTACTTAC	ACGAGATACGTACGCCTTTG	55
RM19	CAAAAACAGAGCAGATGAC	CTCAAGATGGACGCCAAGA	56
RM20	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG	58
RM481	TAGCTAGCCGATTGAATGGC	CTCCACCTCCTAGTTGTTC	56

3.14 Analysis of molecular statistical data

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.

PIC and gene diversity for every locus were computed using Excel (Liu and Muse, 2005). The AMOVA results indicated that genetic variation was more prevalent within populations than between them. Singode and Prasanna (2010) and Da Silva *et al*. (2015) reported similar outcomes. The hierarchical analyses of molecular variance (AMOVA) were performed using GenAlEx version 6.5 (Peakall and Smouse, 2012).

3.15 Analysis of molecular diversity and population structure

Principal component analysis and STRUCTURE analysis were used to account for population structure. The population structure was analyzed using 14 SSR markers. The population structure of the thirty aromatic rice genotypes was estimated using the software STRUCTURE V2.3.4 (Pritchard *et al*., 2000) in order to produce a population structure matrix (Q) that would be utilized as a covariate. To determine the optimal number of sub populations, an admixture ancestry model was used with a burnin of 100,000 followed by 10,000 Monte Carlo Markov Chain (MCMC) replications for k=2 to k=12. STRUCTURE HARVESTER (Earl and von Holdt, 2012) was used to identify the optimal number of sub populations using the Δk method (Evanno *et al.,* 2005). If the membership probability was more than 0.8, a person was considered to be a member of the population (Richards *et al*., 2017). Those who did not receive a score of 0.8 were considered to be of mixed heritage.

Each band was regarded as a distinct location. The bivariate 1-0 data matrix was generated by taking into account all of the scoreable loci. Then, using the Unweighted Pair Group of Arithmetic Means (UPGMA) method as outlined by Nei & Jin (1989), genetic distances (GD) between the genotypes were estimated. Finally, a dendrogram was constructed to estimate genetic diversity using the MetaboAnalyst (Online Version) software (Chong and Xia, 2018).

CHAPTER IV

RESULTS AND DISCUSSION

The experiment was carried out from April to November of 2022, during the Kharif season. Rice genotype's variances, mean comparison, variability, heritability, genetic advancement, diversity analysis, and principal component analysis were examined using the experiment's results. The analyses were done on 10 (ten) yield and yield contributing characters viz. plant height (cm), productive tiller per plant, unproductive tiller per plant, total tillers per plant, panicle length (cm), spike per panicle, days to 50% flowering, straw weight (g), thousand seed weight (g), yield per plant (g) and 5 (five) nutritional characteristics viz. zinc content, iron content, total phenolic content, total flavonoid content, DPPH radical scavenging activity. On the other hand, 14 SSRs/microsatellite markers i.e., RM1, RM27, RM452, RM338, RM249, RM585, RM162, RM234, RM107, RM316, RM171, RM19, RM20, and RM481 were used to identify the variation among the genotypes. As a result, the study's findings are covered and discussed in the headings below.

4.1 Morpho-nutritional characterization of pigmented rice germplasms

4.1.1 Variation analysis between nutritional and morpho-physiological characteristics in aus and boro rice

Table 4 displays the results of the analysis of variance for 15 yield and yield contributing variables among pigmented rice genotypes. The sources of variation in table 4 were error, CV%, replication, and genotype. For every trait, including days to 50% flowering, plant height (cm), productive and unproductive tillers per plant, total tillers per plant, panicle length (cm), spike per panicle, straw weight (g), thousand seed weight (g), and yield per plant (g), the analysis of variances showed highly significant differences among the genotypes. Thus, there is a good opportunity to select better parental types to improve the grain yield. Similar results were reported by Asante *et al.* (2019). For every character under study, the mean squares against three replications were determined to be non-significant, with the exception of total phenol content (TPC). All that the coefficient of variation represents is the variable's dispersion measured.

Table 4: Analysis of variance (Mean squares) produced from the RCBD one factor model on 15 morpho physiological and nutritional characteristics of rice

Characters	Mean sum of square					
	Genotype	Replication	Error	CV %	Grand Mean	SE (±)
PH	1141.91***	9.33	22.54	4.15	114.43	2.74
PT	164.96***	1.90	3.69	6.66	28.85	1.11
UT	3.24***	0.00	0.19	15.31	2.82	0.25
TT	193.84***	2.70	2.81	5.28	31.73	0.97
PL	84.76***	1.01	1.40	5.59	21.13	0.68
SPP	8.75***	0.48	0.29	6.18	8.67	0.31
SW	42130.00***	372.00	173.00	6.95	189.17	7.59
TSW	53.67***	0.06	0.48	2.87	24.13	0.40
DFF	4101.73***	0.26	0.47	0.87	78.71	0.40
YPP	458.52***	3.52	3.65	10.14	18.82	1.10
Zn	311.16***	0.19	0.13	5.65	40.28	0.21
Fe	2824.68***	0.23	0.08	5.89	51.78	0.16
TFC	325.30***	9.72	21.78	15.99	29.19	2.69
TPC	128.44***	3.49*	0.83	9.83	9.28	0.53
DPPH	12903.50***	372.60	253.60	14.66	108.64	9.19

Legend, CV (%) = Coefficient of variation (%), SE = Standard error, PH= Plant height, PT=Productive tiller per plant, UT=Unproductive tiller per plant, TT=Total tillers per plant, PL=Panicle length, SPP=Spike per panicle, SW=Straw weight, TSW=Thousand seed weight, DFF= Days to 50% flowering, Zn= Zinc content, Fe= Iron content, YPP= Yield per plant, TFC= total flavonoid content, TPC= total phenolic content, DPPH= 2,2-Diphenyl-1-Picrylhydrazyl.

*, **, *** = Significant at 5%, 1%, 0.1% level of probability and NS= non-significant respectively.

4.1.2 Mean performance of different yield and yield contributing characters

For distinct rice genotypes, notable differences were found in the yield and yield-contributing characteristics as well as the harvested plant materials. The morpho-nutritional characteristics displayed in the studied pigmented rice germplasm.

1.Chenri Aus

2. Balam Aus

3. Lohar gura Aus

4.Gungur Murali Aus

5. Gungur Bali Aus

6. Begun Bichi Aus

7.Kala manik Aus

8. Gori Matir Aus

9. Noroi-4 Aus

10.Kasmiri Lota Aus

11. Surjamoni Aus

12. Nara Bet

Figure 8: Grain characteristics of the genotypes used in the experiment (Cont.)

13. Malikhori Aus

14. Ora Bet Aus

15. Aus Baku

16. Jamrishaity Aus

17. Batulshi Aus

18. Baktulshi Aus

19.Manik madhu 2

20.Manik madhu

21. BRRI Dhan-82

22. Ratul Aus

23. BRRI Dhan-48

24. BRRI Dhan-85

Figure 8: Grain characteristics of the genotypes used in the experiment (Cont.)

25.Purple 2

26.BRRI dhan- 28

27. BRRI Dhan-72

28. BRRI Dhan-29

29. Kali boro 1

30. Baran boro

31. Bawoi boro

32. Kaikka boro

33. Tepi boro-2

34. Jamir boro

35. Jagli boro-1

36. Sada boro

Figure 8: Grain characteristics of the genotypes used in the experiment

The following error bar charts show the analysis of mean performances of various yield contributing and nutritional characteristics of the data of various parameters:

Plant height

For plant height, a broad range of variances were noted. 114.43±2.74 cm was the mean value, while the range of plant height was 162.38 cm to 81.91 cm. Nara bet (162.38 cm) has the tallest genotype. As opposed to this, BRRI Dhan-28 (81.91 cm) had the shortest genotype. The height of the plants varied from 93.40 cm to 107.90 cm, according to Ashrafuzzaman *et al*. (2009). The plant height range across 41 rice genotypes was similarly recorded by Tonlong *et al*. (2018), with a minimum value of 134.1 cm and a high of 191 cm length. Taller rice plants compete with weeds better than shorter plants, and grain yield increases quadratically with increasing plant height (Fageria, Castro and Baligar, 2004) up to a point. Taller plants have a higher chance of lodging (Kato *et al*., 2019), and smaller plants have been linked to a little increase in grain output (Evans *et al*., 1984).

Productive tillers per plant

One significant factor that contributes to output is the number of productive tillers per plant. The mean value of 28.85±1.11 represented the range of productive tillers per plant, which was on average 44.22 to 16. Tepi boro-2 (44.22) produced the most productive tillers per plant per plant. In Noroi-4 aus (16), the least number of productive tillers per plant was observed.

Unproductive tillers per plant

The quantity of unproductive tillers on each plant is a significant factor that affects productivity. With a mean value of 2.82±0.25, the average range of the number of unproductive tillers per plant was 6.78 to 1.56. Orabet aus produced the greatest number of unproductive tillers per plant (6.78). Begun Bichi aus has the fewest effective tillers per plant on record.

Total tillers per plant

There was a considerable difference in the total number of tillers per plant, ranging from 16.56 to 48.44, with a mean value of 31.73-0.97. The Noroi-4 aus displayed the lowest number of total tillers per plant (16.56), while Tepi Boro-2 had the largest number of total tillers 48.44.

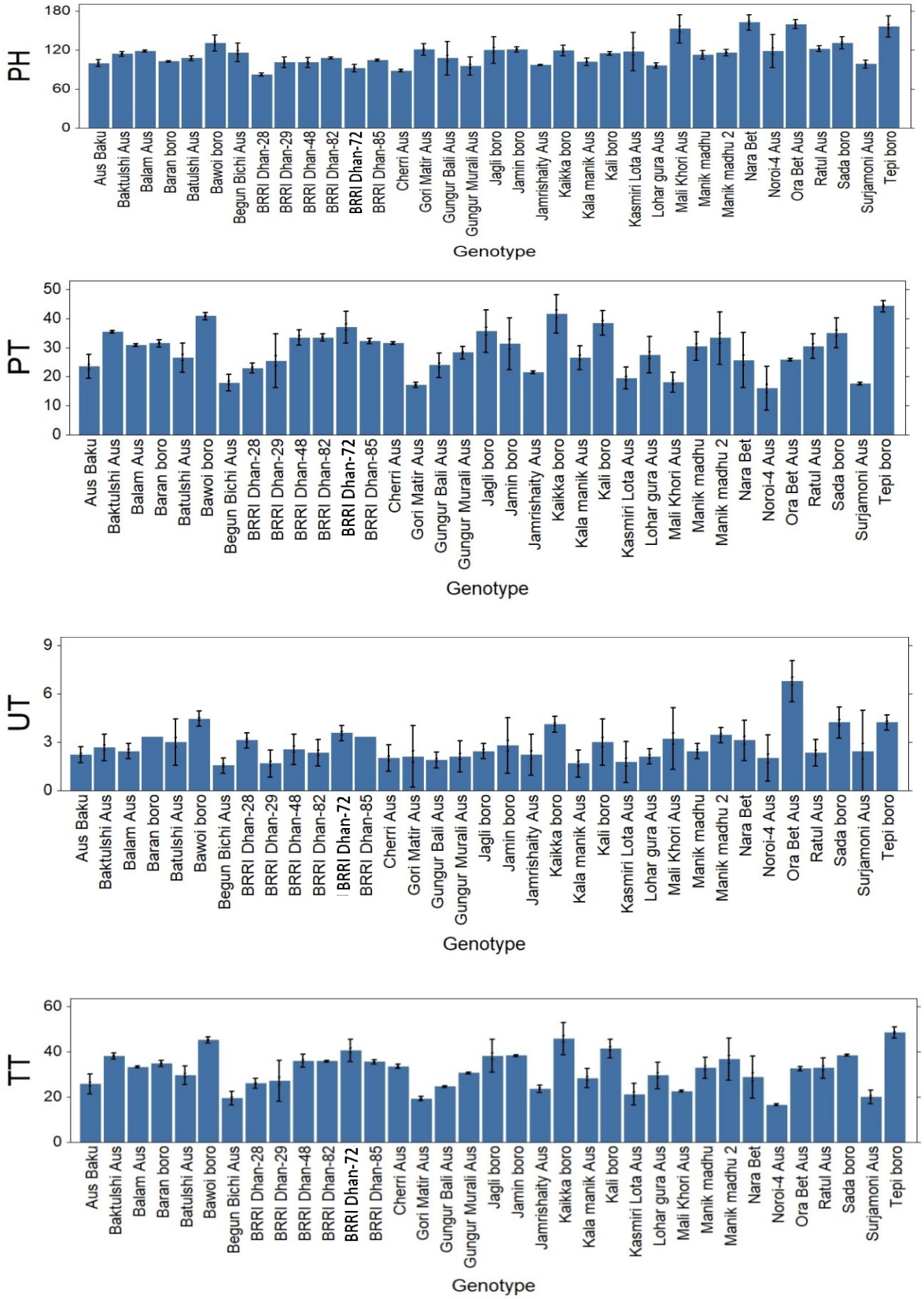


Figure 9: The mean performance of rice genotypes in terms of Plant height, Productive tiller per plant, Unproductive tiller per plant and Total tillers per plant.

Panicle length

When the plant reaches maturity, the length of the panicles is estimated. With a mean value of 21.13±0.68, the average range of panicle length per stem was measured, ranging from 11.52 cm to 32.30 cm. The largest panicle length was observed in Tepiboro-2 (32.30 cm), which was followed by Kaikka boro (30.44 cm), Bawoiboro (30.15 cm), Kaliboro (27.63 cm), and BRRI dhan-72 (27.04 cm). Noroi-4 aus had the shortest panicle length, measuring 11.52 cm. Panicules that were moderately to highly exerted were seen in all genotypes. When Kanushree *et al*. (2020) assessed the diversity of agromorphological and qualitative characterisation of 48 rice germplasm accessions, they found that the length of the panicles ranged from 21.5 cm to 29.95 cm, with a mean value of 25.16 cm.

Spike per panicle

The number of spikes per panicle showed significant variation as well, ranging from 15.33 to 5.89 with a mean value of 8.67±0.31. Mali khori aus (15.33) had the most spikes per panicle, followed by BRRI dhan-29 (11.44), Begun bichi aus (10.44), Orabet aus (10.22), Tepi boro-2 (10.11), and Jamrishaity aus (5.89) had the fewest spikes per panicle.

Straw weight

Straw weight ranged from 69.84 (g) to 565.47 (g) on average, with a mean value of 189.17±7.59. Ora bet aus had the highest straw weight (565.47g), followed by Mali khori aus (544.95g), Narabet aus (536.25g), and Bawoi boro (227.90g). Kashmiri lota aus had the lowest weight (69.84g).

Thousand seed weight

The thousand seed weight (g) ranged from 16.72 (g) to 35.75 (g) on average, with a mean value of 24.13±0.40. Kashmiri lota had the highest thousand seed weight (35.75g), followed by Noroi-4 aus (32.31g), Jamrishaity aus (31.57g), and Manikmadhu aus (31.44g). Kaikka boro had the lowest value (16.72g).

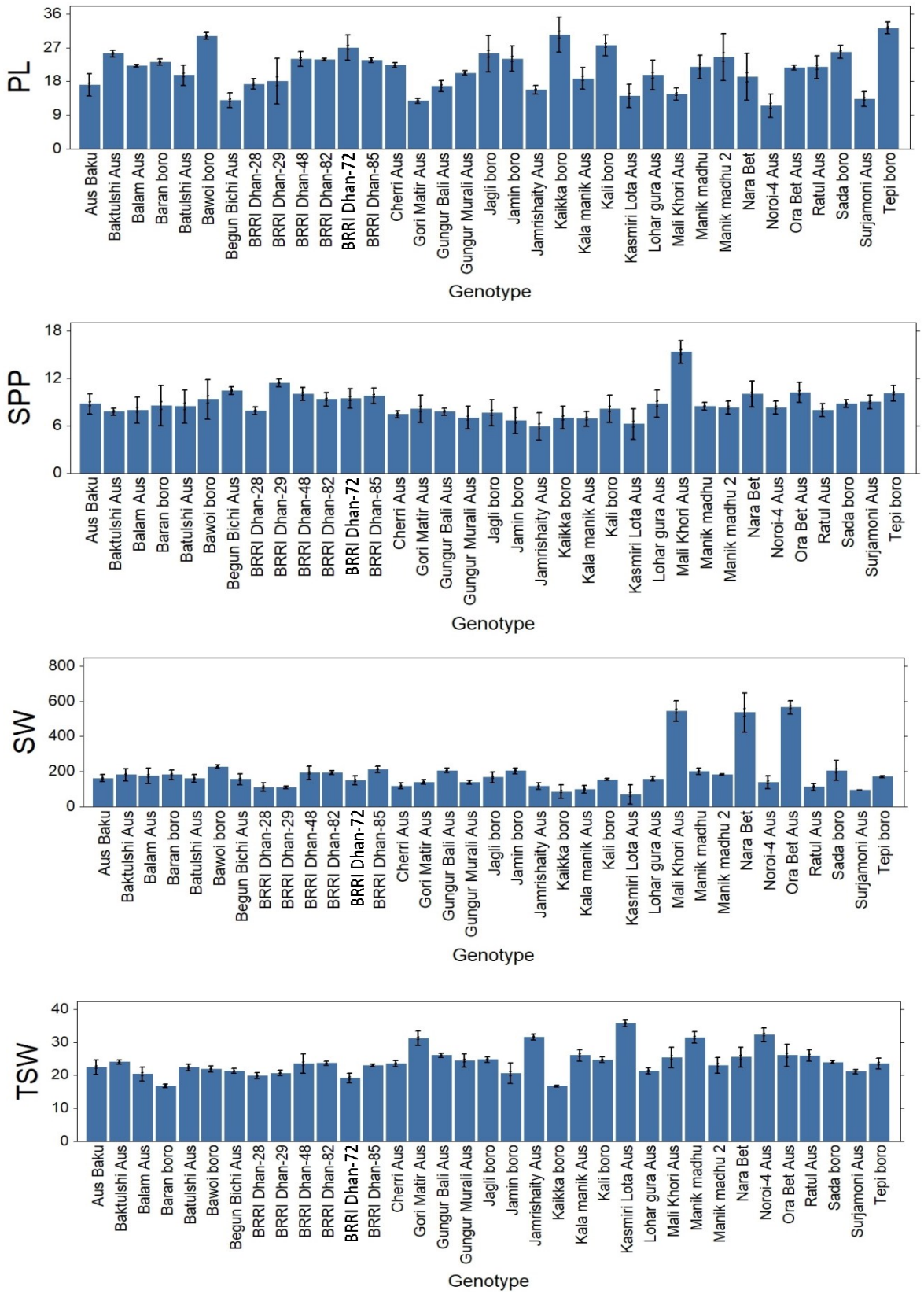


Figure 10: The mean performance of rice genotypes in terms of total flavonoid content, total phenolic content, and DPPH content are shown.

Days to 50% flowering

Days to 50% blooming ranged from 63.33 to 198, with Baran boro and Ratul aus genotype having the earliest flowering time of 63.33 days. Days to 50% flowering was 78.71±0.39 days on average. On the other hand, Orabet aus genotype required 198 days for late flowering. According to Tiruneh *et al*.'s (2021) study on the performance evaluation of upland areas in southwest Ethiopia, there is a variation of 72 to 117 days to 50% heading among 13 improved rice varieties.

Yield per plant

Regarding the yield per plant, the average range (in grams) was found to be 3.75–69.02, with a mean of 18.82±1.10. Malikhori aus had the greatest yield per plant (g) at 69.02g, while Surjamoni aus had the lowest at 3.75g. According to WAKARIA *et al*. (2021), there was a significant difference ($P \leq 0.05$) in grain weight, with values ranging from 0 to 4.93 t per ha. Lipi *et al*. (2020) reported a substantial difference and a mean of 5.5 t per ha of grain yield across eleven rice hybrids, which is in line with the findings.

Zinc content

The zinc concentration ranged from 21.20 mg/kg to 78.04 mg/kg on average, with a mean value of 40.28±0.21. Orabet aus has the greatest zinc concentration (78.04 mg/kg), followed by Manik madhu-2 (53.36 mg/kg), Jamrishaity (53.04 mg/kg), and Aus baku (52.2 mg/kg). BRRI Dhan-29 has the lowest zinc level, measuring 21.20 mg/kg. Velprabakaran *et al*. (2021) observed comparable outcomes in rice.

Iron content

With a mean value of 51.78±0.16, the typical range of iron concentration was 6.42 mg per kg to 150.46 mg per kg. At 150.46 mg/kg, Orabet aus had the highest iron concentration, followed by Lohar gura aus (115.9 mg/kg), Manik madhu-2 aus (107.22 mg/kg), Baktulshi aus (106.54 mg/kg), and Kaikka boro (76.52 mg/kg). Jagli boro had the lowest iron level (6.42 mg per kilogram). Yodmanee *et al*. (2011) reported similar outcomes in rice.

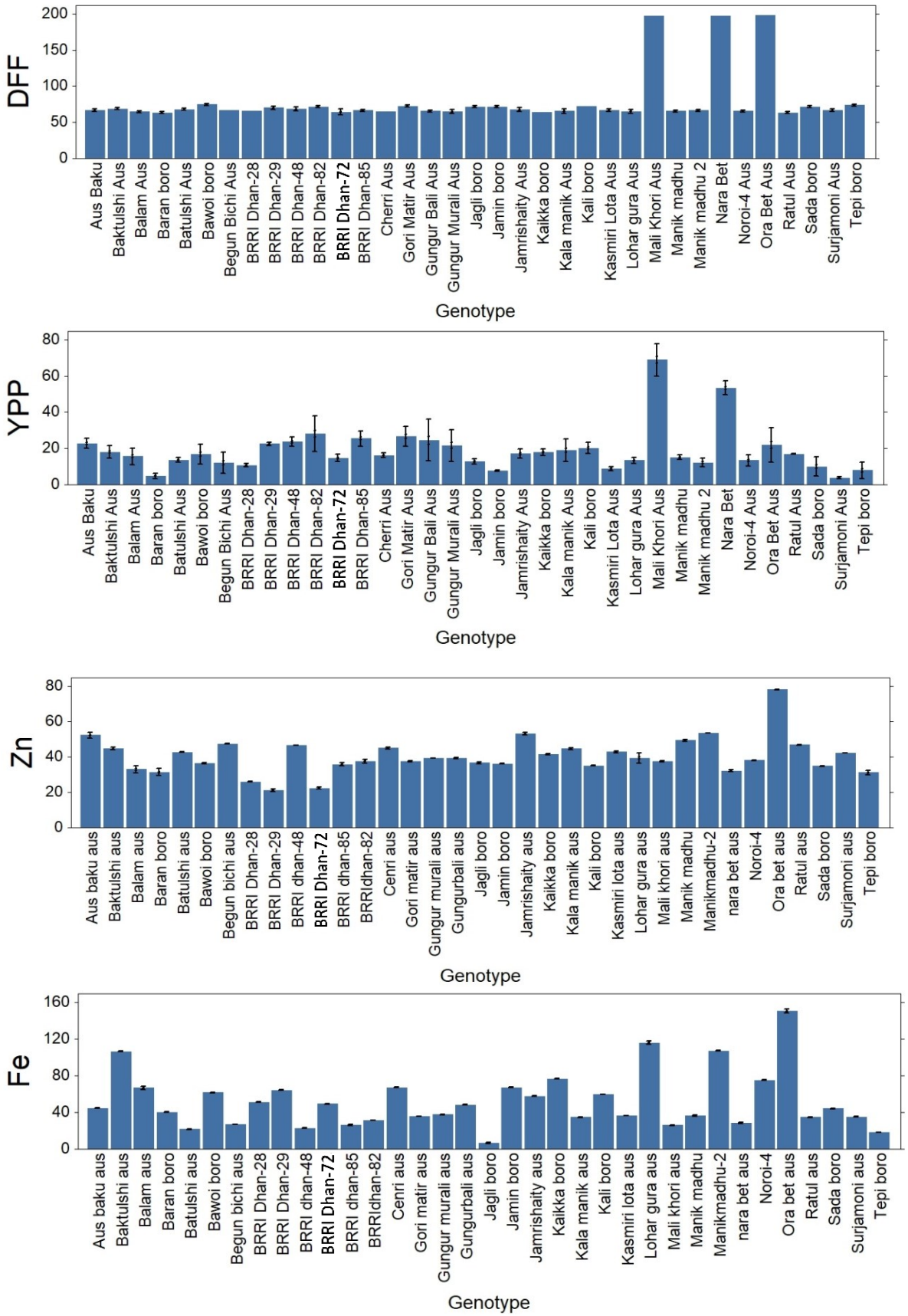


Figure 11: The mean performance of rice genotypes in terms of total flavonoid content, total phenolic content, and DPPH content are shown.

Total flavonoid content

The total flavonoid content had a mean value of 29.19±2.69. The average range was 11.57 mg per g to 56.90 mg per g. Balam aus (40.90 mg per g), Begun bichi aus (41.08 mg per g), Narabet aus (56.90 mg per g), and Orabet aus (40.07 mg per g) were the next highest total flavonoid content. With 11.57 mg/g, BRRI dhan-85 had the lowest total flavonoid content found in any study. The findings in pigmented rice by Islam *et al*. (2022) were found to be comparable to our results.

Total phenolic content

The total phenolic content was found to vary greatly as well, ranging from 1.12 mg per g to 28.19 mg per g, with a mean value of 9.28±0.53. Mali khori aus had the highest total phenolic content (28.19 mg/g), followed by Narabet aus (25.39 mg/g), Orabet aus (18.83 mg/g), and Baran boro (17.78 mg/g). BRRI dhan-85 had the lowest overall phenolic content (1.12 mg/g). The current result in rice is consistent with the findings of Sanghamitra *et al*. (2022).

DPPH content

The mean value of DPPH radical scavenging activity was 108.64±9.19, with a substantial range of 14.63mmol/100g to 322.57mmol/100g. Mali Khori Aus (322.57 mmol/100 g) had the highest DPPH radical scavenging activity, followed by Orabet Aus (244.75 mmol/100 g), Narabet Aus (236.90 mmol/100 g), and Noroi-4 aus (189.31 mmol/100 g). BRRI dhan-28 has the lowest DPPH radical scavenging activity (14.63 mmol per 100 g). Islam *et al*. (2022) similarly showed similar outcomes with colored rice.

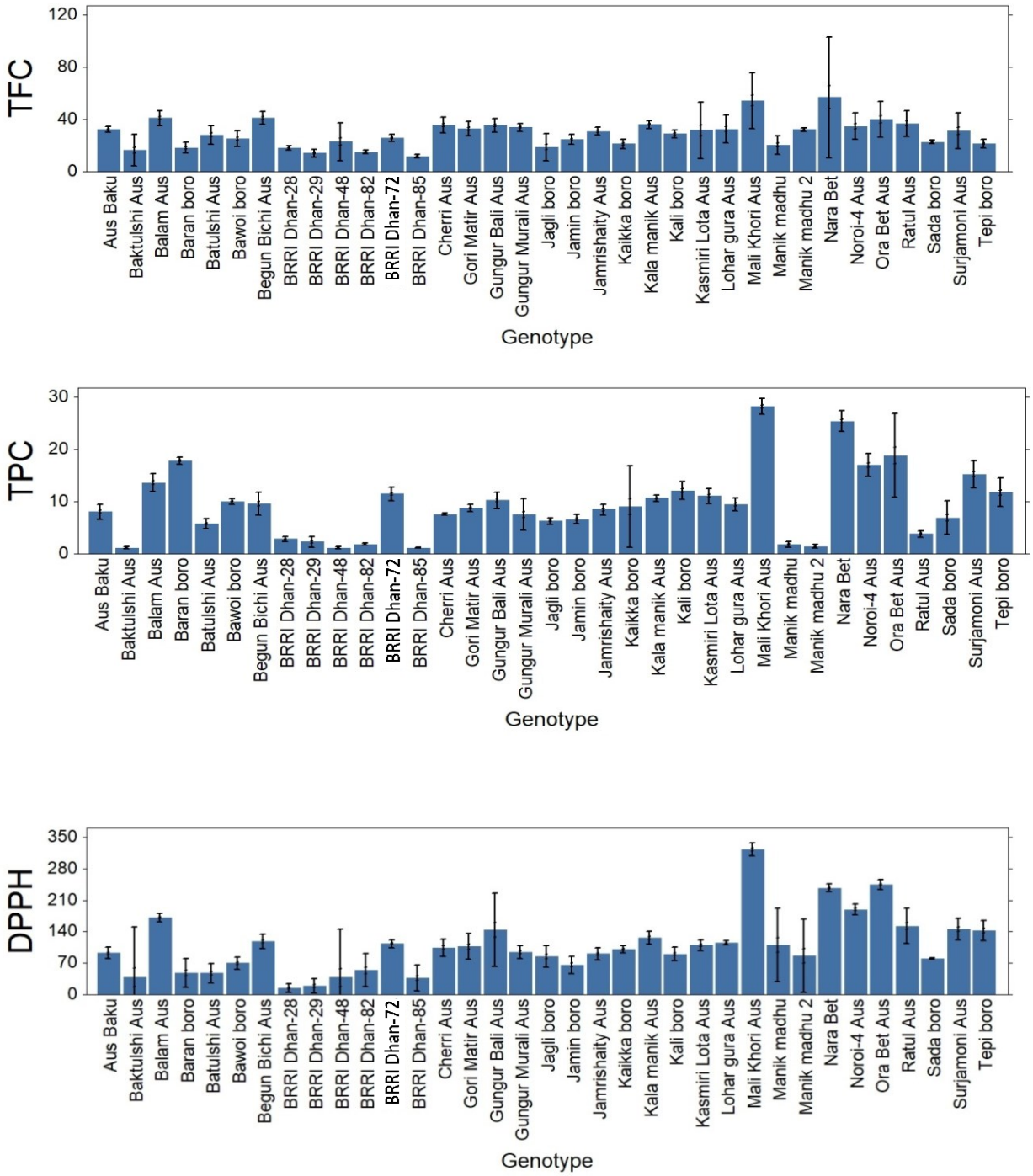


Figure 12: The mean performance of rice genotypes in terms of total flavonoid content, total phenolic content, and DPPH content are shown.

4.1.3 Characteristics chosen for pigmented rice based on genetic parameter

A variety of parameters were estimated to observe the variability among the 15 characters, including genotypic variance (σ^2g), phenotypic variance (σ^2p), heritability (h2b), genotypic coefficient of variation (GCV %), phenotypic coefficient of variation (PCV %), genetic advance (GA), and genetic advance as a percent of mean (GAM). The results presented in (Table 6) revealed that the phenotypic variances for all the characters were higher than the genotypic variances for all the characters. The most significant differences in iron content (938.47 and 947.76), days to 50% blooming (1367.09 and 1367.56), plant height (373.12 and 395.66), and straw weight (13985.59 and 14158.47) were found to have the largest genotypic and phenotypic variations. The traits of Unproductive tiller per plant (1.20 and 1.02), Spike per panicle (3.11 and 2.82), and Thousand seed weight (18.21 and 17.73) were associated with the lowest values of phenotypic and genotypic variations.

For every characteristic, PCV was greater than the matching GCV, suggesting that the environment had an impact. Saha *et al*. (2019) previously reported data that were similar. The plant height had a phenotypic coefficient of variation (PCV) of 17.38%, while the total phenolic content had a PCV of 70.99%. The plant height had a genotypic coefficient of variance (GCV) of 16.88%, while the total phenolic content had a GCV of 70.31%. The total phenolic content (70.99 and 70.31) showed the highest PCV and GCV. The high estimates of PCV and GCV for these traits suggested the possibility of yield improvement through the selection of these traits (Saha *et al.,* 2019). According to Subramanian and Menon (1973), PCV and GCV values more than 20% are regarded as high, whereas values of less than 10% are considered to be low and values between 10 and 20% to be moderate. Based on this delineation, high PCV and GCV values were noted for the following: days to 50% flowering, total flavonoid content, productive tiller per plant, total tillers per plant, panicle length, zinc content, spike per panicle, total phenolic content, yield per plant (g), straw weight, DPPH scavenging capacity, iron content, and so on. For plant height and thousand seed weight, PCV and GCV values were modest. Less than 10% of the GCV values were recorded, and there is no PCV.

Table 5. Genetic parameters on the 15 traits of pigmented aus and boro rice genotypes

Characters	Genotype Variance (σ^2_g)	Phenotype Variance (σ^2_p)	GCV (%)	PCV (%)	Heritability (%)	GA	GAM (%)
PH	373.12	395.66	16.88	17.38	94.30	38.64	33.77
PT	53.76	57.44	25.42	26.27	93.58	14.61	50.65
UT	1.02	1.20	35.76	38.90	84.52	1.91	67.73
TT	63.68	66.49	25.15	25.69	95.78	16.09	50.70
PL	27.79	29.18	24.94	25.56	95.22	10.59	50.14
SPP	2.82	3.11	19.38	20.34	90.78	3.29	38.04
SW	13985.59	14158.47	62.52	62.90	98.78	242.13	127.99
TSW	17.73	18.21	17.45	17.68	97.36	8.56	35.46
YPP	151.63	155.27	65.41	66.19	97.65	25.07	133.16
DFF	1367.09	1367.56	46.97	46.98	99.97	76.15	96.75
Zn	103.67	103.80	25.27	25.29	99.88	20.96	52.03
Fe	941.53	941.61	59.25	59.26	99.99	63.21	122.06
TFC	101.17	122.96	34.46	37.99	82.28	18.79	64.39
TPC	42.54	43.37	70.31	70.99	98.08	13.31	143.45
DPPH	4216.64	4470.27	59.77	61.54	94.33	129.58	119.58

Here, GCV (%) = Genotypic coefficient of variation, PCV (%) = Phenotypic coefficient of variation, GA = Genetic advance, GAM= genetic advance as percent of mean, PH= Plant height , PT=Productive tiller per plant, UT=Unproductive tiller per plant, TT=Total tillers per plant, PL=Panicle length, SPP=Spike per panicle, SW=Straw weight, TSW=Thousand seed weight, YPP= Yield per plant, DFF= Days to 50% flowering, Zn= Zinc content,Fe= Iron content, TFC= total flavonoid content, TPC= total phenolic content, DPPH= 2,2-Diphenyl-1-Picrylhydrazyl scavenging capacity.

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

The relative contributions of variations in genetic and non-genetic factors to the total phenotypic variances in the population are estimated via heritability. In quantitative genetics, especially in selective breeding, it is a crucial idea. For days to 50% blooming and total flavonoid content (TFC), the heritability estimation ranged from 82.28% to 99.99%, respectively. Among fifteen characters, thirteen characters showed higher heritability (>90%). This means those characters could be easily improved by selection. The most important function of the heritability in the genetic study of quantitative characters is its predictive role to indicate the reliability of the phenotypic value as a guide to breeding value. High heritability estimates for those traits indicated a high response to selection in these traits. The high heritability values of the traits under consideration in this study suggested that there was room for genetic improvement and that the traits were less affected by the environment. As a result, the traits could be effectively selected using a straightforward selection process based on phenotypic expression (Saha *et al*., 2019).

According to Wolie *et al*. (2013), genetic advance (GA) under selection is the improvement of features in genotypic value for the new population relative to the base population during one cycle of selection at a specific selection intensity. The difference between the original population's mean genotypic values and the population from which the selected population was drawn is known as genetic progress (Pandey *et al*., 2010). The high value of GA was revealed in the straw weight (242.13) and the low in unproductive tiller per plant (1.91). The range of the genetic advance as a percent mean anticipated GAM was 33.77% for plant height and 143.45% for total phenolic content. Plant height, productive and unproductive tillers per plant, total tillers per plant, panicle length, spike per panicle, straw weight, thousand seed weight, yield per plant (g), days to 50% flowering, zinc content, iron content, total flavonoid content, total phenolic content, and DPPH scavenging capacity were among the characters with high values of GAM (>20%).

Important selection characteristics include also heritability and genetic advancement. When taken into account in conjunction with heredity, it becomes a more effective selection technique. Vanlalrinngama *et al*. (2023) proposed that high heritability estimates

in conjunction with a high genetic advance are more useful in predicting gain under selection than heritability estimates alone. The estimates of genetic advance can aid in understanding the type of gene action of different polygenic characters. Therefore, if there are significant genetic advancements, the heritability estimations will be accurate.

The results of this study demonstrated a high degree of genetic advance along with high heritability as a percentage of the mean for plant height, panicle length, spike per panicle, yield per plant (g), days to 50% flowering, zinc content, iron content, total flavonoid content, total phenolic content, and DPPH scavenging capacity. Additive gene action and early generational selection may account for these outcomes (Panse and Sukhatme, 1957). Prior research (Saha *et al*., 2019, Edukondalu *et al*., 2017) supported similar findings for the number of filled grains per panicle and the number of total grains per panicle. So direct selection of these characters based on phenotypic expression by simple selection method would be effective due to accumulation of more additive genes leading to further improvement.

Johnson *et al*. (1955) have demonstrated that high heritability should be accompanied with high genetic advance to arrive at a more solid result. A character exhibiting high heritability may not necessarily give significant genetic advance. As such, it ought to be paired with knowledge about genetic advancements. A character with both strong genetic advancement and high heritability will therefore be valued in the selection process. For the number of spikes per panicle, substantial genetic advancement and great heritability were observed. Selection may therefore be successful in dividing generations using these characteristics (Paramasivam *et al*., 1996). In the selection program, a character with strong genetic advancement and high heritability will be valued.

4.1.4 Hierarchical cluster analysis based on morphological traits

A popular method for analyzing genetic diversity is the hierarchical clustering technique. The people that are closest to one another are grouped together first by this procedure, and these initial groups are then further blended based on their shared characteristics. This method is commonly referred to as the "agglomerative method". A dendrogram, a two-dimensional diagram that shows the hierarchical structure of clusters, is a visual representation of the results of a cluster analysis. The hierarchical agglomeration approaches used in clustering algorithms are diverse. In this case, a hierarchical agglomerative technique called Ward's method employed Euclidean distance. Since the goal of this hierarchical grouping is to decrease within-group variance, the technique is also known as Ward's minimum variance method (Ward 1963).

Significant variations in the genotypes for every character were found by the analysis of variance, suggesting that there is diversity in the genotypes for the genotypes under study. We used the relative mean values for each trait to do a cluster analysis. Based on fifteen traits, Euclidean distance coefficients were computed for every genotype of rice. Based on multivariate analysis, the dendrogram from UPGMA clustering showed that genotypes of rice were grouped into three clusters (Fig. 17). According to Table 7, Cluster I, II, and III each had 3, 16, and 16 genotypes.

With three genotypes, the smallest number among the three clusters was found in Cluster I. In terms of DPPH content and straw weight, the cluster had the highest mean value. The TFC content, iron content, zinc content, days to 50% blooming, plant height, and yield per plant all had moderate mean values in this cluster. The majority of genotypes in this cluster performed the worst in terms of panicle length, spike per panicle, TPC content, total tillers per plant, thousand seed weight, productive tiller per plant, and unproductive tiller per plant.

The genotypes aus baku, balam, batulshi, started bichi, chenrri, gori matir, gungur bali, gungur murali, jamrishaity, kala manik, kasmiri lota, lohar gura, manik madhu, noroi-4, ratul, and surjamoni aus comprised Cluster II. In terms of DPPH content and straw weight, the cluster had the highest cluster mean. For days to 50% flowering, plant height, TFC content, iron content, zinc content, and yield per plant, the cluster's mean value was modest. The cluster had the lowest mean value for total tillers per plant, thousand seed weight, TPC content, productive tiller per plant, panicle length, spike per panicle and

unproductive tiller per plant. Similar findings were found by (Bekis *et al.,* 2021) for panicle weight per stem, panicle length per stem, filled grain number per panicle, and thousand-grain weight. Cluster II had many sub-groups.

The properties in Cluster III are baktulshi aus, baran boro, bawoi boro, brri dhan-72, jagli boro 1, jamir boro, kaikka boro, kali boro 1, sada boro, tepi boro 2, brri dhan-28, brri dhan-29, brri dhan-48, brri dhan-82, brri dhan-85, and manik madhu 2. Regarding the weight of straw and the amount of DPPH, Cluster III displayed the largest cluster means. The 50% blooming, plant height, TFC content, iron content, zinc content, and yield per plant cluster values were all reasonable. For panicle length, spike per panicle, TPC content, productive tiller per plant, total tillers per plant, thousand seed weight, and unproductive tiller per plant, the cluster had the lowest mean values. Similar results for panicle weight per stem were reported by Bekis *et al*. in 2021.

Table 6. Cluster groups and genotypes in aus and boro rice

Cluster	Size	Genotypes
I	3	Ora Bet Aus, Mali Khori Aus, Nara Bet.
II	16	Aus Baku, Balam Aus, Batulshi Aus, Begun Bichi Aus, Chenrri Aus, Gori Matir Aus, Gungur Bali Aus, Gungur Murali Aus, Jamrishaity Aus, Kala manik Aus, Kasmiri Lota Aus, Lohar gura Aus, Manik madhu, Noroi-4 Aus, Ratul Aus, Surjamoni Aus.
III	16	Baktulshi aus, Baran boro, Bawoi boro, BRRI Dhan-72, Jagli boro 1, Jamir boro, Kaikka boro, Kali boro 1, Sada boro, Tepi boro 2, BRRI Dhan-28, BRRI Dhan-29, BRRI Dhan-48, BRRI Dhan-82, BRRI Dhan-85, Manik madhu 2

Table 7. The mean values derived from 15 quantitative traits found in rice genotypes for three clusters

Characters	Cluster mean		
	I	II	III
PH	158.21 (H)	107.32 (L)	113.33 (I)
PT	23.19 (L)	24.29 (L)	34.47 (H)
UT	4.37 (H)	2.15 (L)	3.20 (I)
TT	27.96 (L)	26.28 (L)	37.88 (H)
PL	18.51 (L)	17.57 (L)	25.18 (H)
SPP	11.85 (H)	7.97 (L)	8.76 (L)
SW	548.89 (H)	140.51 (L)	170.38 (L)
TSW	25.65 (H)	26.10 (H)	21.89 (L)
YPP	48.18(H)	16.28 (L)	15.86 (L)
Zn	49.23 (H)	43.26 (I)	35.63 (L)
Fe	68.32 (H)	48.39 (L)	52.07 (I)
TFC	50.43 (H)	33.37 (I)	21.03 (L)
TPC	24.14 (H)	9.28 (L)	6.48 (L)
DPPH	268.07 (H)	119.83 (I)	67.56 (L)
DFF	197.33 (H)	66.19 (L)	69.00 (L)

Here, PH= Plant height, PT=Productive tiller per plant, UT=Unproductive tiller per plant, TT=Total tillers per plant, PL=Panicle length, SPP=Spike per panicle, SW=Straw weight, TSW=Thousand seed weight, DFF= Days to 50% flowering, Zn= Zinc content, Fe= Iron content, YPP= Yield per plant, TPC= total phenolic content, TFC= total flavonoid content, DPPH= 2,2-Diphenyl-1-Picrylhydrazyl.

Table 8. Genotypes of aus and boro rice were analyzed for intra- and inter-cluster (colored) distances.

Cluster	I	II	III
I	4.05		
II	9.24	2.29	
III	9.86	4.75	2.53

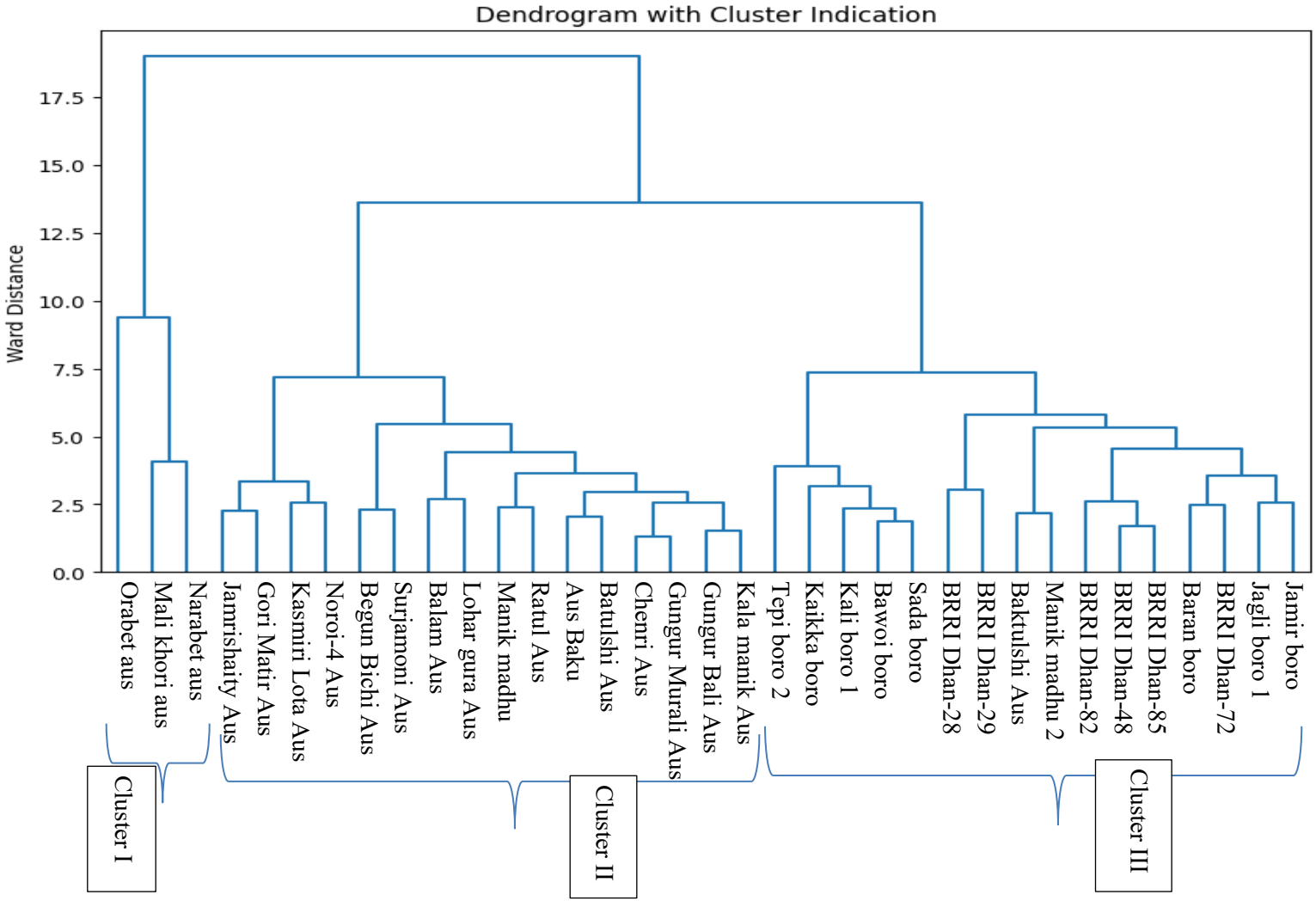


Figure 13. Using Euclidean genetic distance and twelve yield-contributing characters, a dendrogram was generated from UPGMA clustering for rice genotypes.

4.1.5 Principal component analysis for morphological traits

Since Principal Component Analysis (PCA) is a well-known multivariate statistical technique used to rank genotypes based on PC scores and determine the minimum number of components that can explain maximum variability out of the total variability (Gour *et al*., 2017), PCA is a powerful tool in modern data analysis.

PCA was used to analyze 18 rice genotype features. Of the qualities examined, only the first three principal components (PCs) had an eigenvalue of greater than 1.00 (Table 9) and a maximum cumulative variance of almost 38.5%. A scree plot that illustrates the dimensionality of the data is shown in (Figure 22). A sample line segment plot known as a scree plot shows the number of principle components that were extracted from the data as well as the cumulative proportions of the explained variance (Cattell, 1966).

Eigenvalues are variances of the principal components. The scree plot orders the eigenvalues from largest to smallest. According to (Figure 22) the horizontal axis shows the principal component number, labeled 1 to 10.

About 5.77 is the eigenvalue of Principal Component 1 (PC1), which accounts for about 38.5% of the variance. Principal component 2 (with an eigenvalue of roughly 3.81), component 3 (with an eigenvalue of roughly 1.78), component 4 (with an eigenvalue of roughly 0.99), and component 5 (with an eigenvalue of roughly 0.80) come next.

The first principal component in this study, which included the variables yield per plant (0.7337), number of productive tillers per plant (0.4665), panicle length (0.3999), spike per panicle (0.5793), and days to 50% flowering (0.9091), explained 38.5% of the total variability. Twenty-five percent of the overall variability was explained by the second main component. Plant height (0.4886), spike per panicle (0.2571), and days to 50% flowering (0.2940) were the variables that contributed most favorably.

The third component accounted 11.9% to the variance, in which the variable DPPH content (0.0785), number of productive tillers per plants per plant (0.0620), number of total tillers per plant (0.0254), days to 50% flowering (0.0022). 6.6% of the variation in the total variability was explained by the fourth principal component, which was determined by the following factors: plant height (0.3426), DPPH content (0.1688), number of productive tillers per plant (0.0620), number of total tillers per plant (0.0254), number of spikes per panicle (0.3473), number of productive tillers per plant (0.2136), number of total tillers

per plant (0.1741), and days to 50% flowering (0.0813). The main features so converge in several principal components, help to explain the variability, and have a tendency to stick together. When using these characters' inbreeding programs, this may be taken into account.

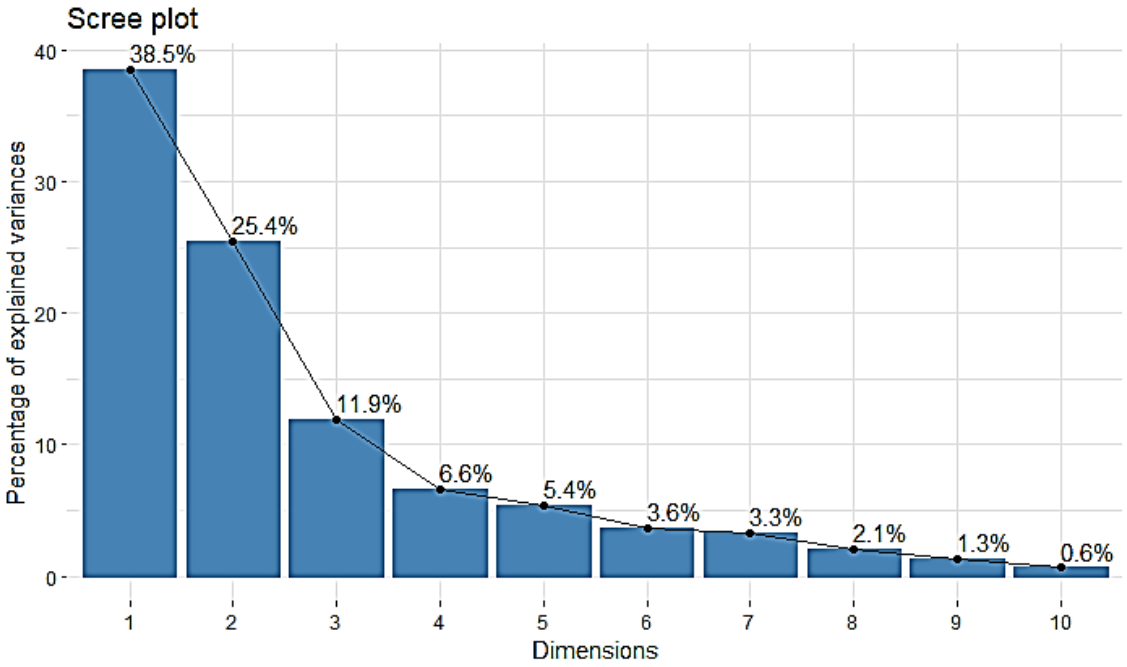


Figure 14. Scree plot of principal component analysis (PCA) for morphological features in rice genotypes.

Table 9. Evaluation of 18 phenotypic variables in pigmented rice genotypes using a rotated component matrix.

Characters	PC1	PC2	PC3	PC4	PC5
2,2-Diphenyl-1-Picrylhydrazyl	0.90	0.02	0.08	0.17	-0.25
total phenolic content	0.81	0.05	-0.10	0.05	-0.48
total flavonoid content	0.81	-0.23	0.09	0.10	-0.35
Zn content	0.29	0.00	0.82	-0.02	0.13
Iron content	0.07	0.23	0.70	-0.46	0.01
Days to 50% flowering	0.91	0.29	0.00	-0.08	0.13
Plant height (cm)	0.69	0.49	0.04	0.34	0.05
Panicle length (cm)	-0.40	0.89	-0.02	0.18	-0.04
Productive tiller per plant	-0.47	0.83	-0.06	0.21	-0.04
Unproductive tiller per plant	0.25	0.81	0.30	-0.11	0.02
Total tillers per plant	-0.40	0.89	-0.03	0.17	-0.04
Spike per panicle	0.58	0.26	-0.49	-0.35	0.25
Thousand seed weight (g)	0.29	-0.48	0.31	0.60	0.40
Straw weight (g)	0.85	0.41	-0.01	-0.11	0.19
Yield per plant (g)	0.73	0.07	-0.40	-0.01	0.30
Eigen value	5.77	3.81	1.78	0.99	0.80
Percentage of variance (%)	38.49	25.41	11.87	6.63	5.36
Cumulative percentage (%)	38.49	63.90	75.77	82.40	87.76

4.1.6 Biplot analysis

Using the relative value of each trait, a biplot representing the genotype × traits was produced from a two-way matrix containing ten morpho-physiological traits and five nutritional traits from rice genotypes (Figure 23). The cosine of the angle between vectors connecting traits to the origin is proportional to the correlation coefficient between those traits, and this graphic represents the information from this matrix into main components. Once more, qualities close to one another have a positive correlation whereas traits on opposing sides of the origin have a negative correlation. Furthermore, there is no correlation between features that are 90 degrees apart in terms of origin. When it came to distinguishing between genotypes, the narrow angles formed by two neighboring vectors pointing in the same direction demonstrated a high association.

Again, loading plots show how the traits correlate with one another; in the plot, a minimum angle indicates positive correlation, a large one implies negative correlation, and a 90° angle indicates no correlation between two characteristics (Masuda *et al*., 2021). A principal component (PC) biplot displays both PC scores of genotypes and loadings of traits. Here, the traits that are further away from PC origin indicate more influence they have on that PC.

Figure 23 revealed that all axes showed a scattering of genotypes. Positive loadings were seen in PC1 and PC2 for the following traits: days to 50% blooming, plant height, weight of straw, yield per plant, unproductive tiller per plant, total phenolic content, iron content, zinc content, and spike per panicle. The genotypes G3 (Lohar gura aus) and G29 (Kali boro 1) stayed close to these characteristics and were linked to these characteristic behaviors. Positive PC1 and negative PC2 scores are indicated by the traits productive tiller per plant, total tillers per plant, and panicle length, which have a strong correlation with one another. In this case, these features were preferred by the genotypes G21 (BRRI dhan 82), G23 (BRRI dhan 48), G33 (Tepiboro 2), G12 (Nara Bet), and G28 (BRRI dhan 29). Once more, PC2 displayed positive loading for DPPH content, total flavonoid content, and thousand seed weight, but PC1 displayed negative scoring.

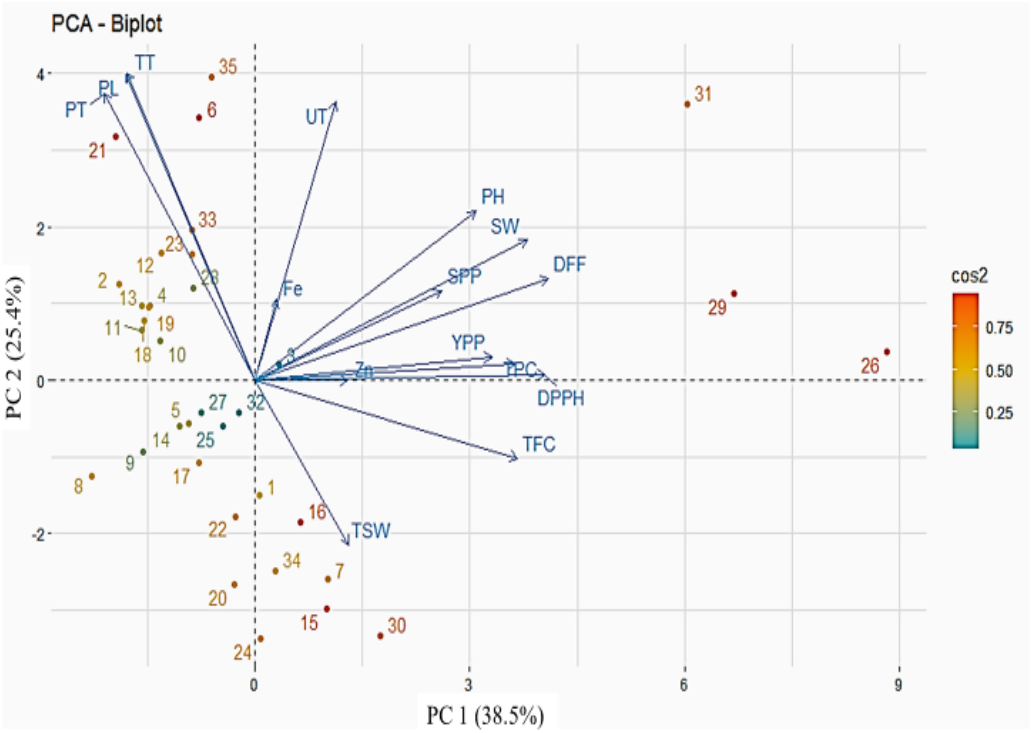


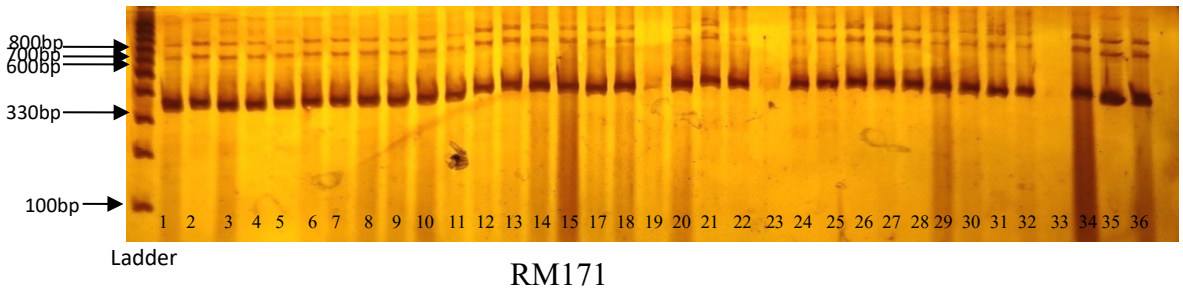
Figure 15: The PCA-biplot shows the relationship between aus and boro rice genotypes with morpho-physiological and nutritional traits.

Here, PH= Plant height, PT=Productive tiller per plant, UT=Unproductive tiller per plant, TT=Total tillers per plant, PL=Panicle length, SPP=Spike per panicle, SW=Straw weight, TSW=Thousand seed weight, DFF= Days to 50% flowering, Zn= Zinc content, Fe= Iron content, YPP= Yield per plant, TPC= total phenolic content, TFC= total flavonoid content, DPPH= 2,2-Diphenyl-1-Picrylhydrazyl

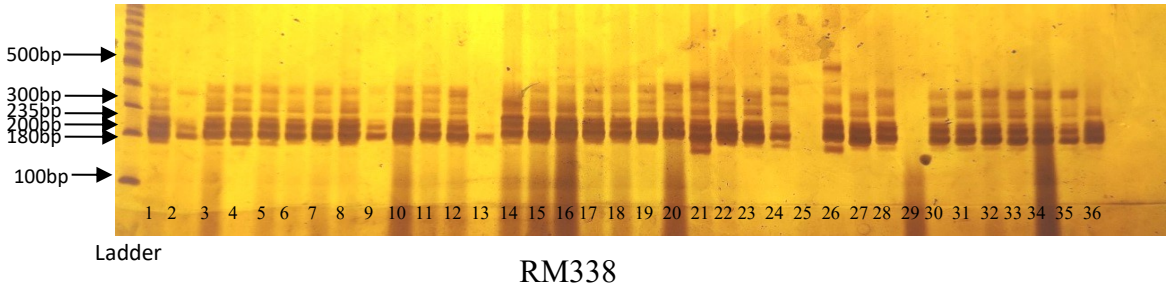
4.2 Molecular characterization of pigmented rice germplasms

4.2.1 Analysis of DNA fingerprinting using SSR markers.

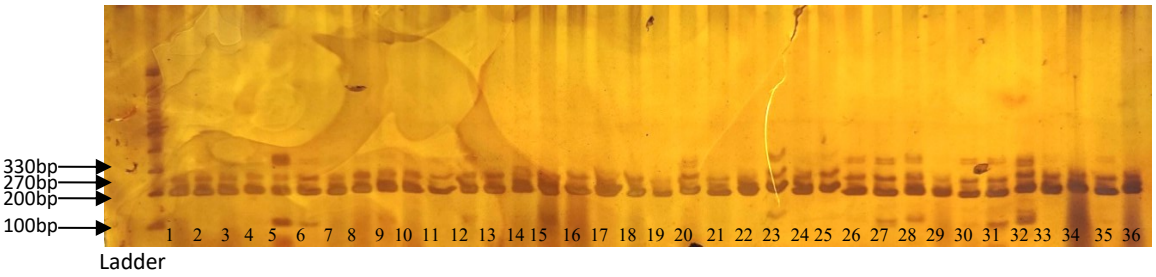
DNA was first isolated from 2 cm young leaves of 3-week-old seedlings of pigmented rice genotypes. The DNA was extracted with a modified CTAB technique. Prior to PCR amplification, DNA samples were quantified using a Thermo Scientific NanoDrop^TM1000 Spectrophotometer to ensure their quality. The DNA contents of pigmented rice genotypes varied from 275 to 2150 ng per µl. After polymerase chain reaction (PCR) and polyacrylamide gel electrophoresis (PAGE) examination, all 14 SSRs/microsatellite markers (RM1, RM27, RM452, RM338, RM249, RM585, RM162, RM234, RM107, RM316, RM171, RM19, RM20, and RM481) displayed polymorphic bands. Here, the distance between the DNA bands of SSRs and microsatellite markers was measured using 100 bp DNA ladders. Figure 22 displays gel images of PCR generated fragments utilizing those SSR markers.



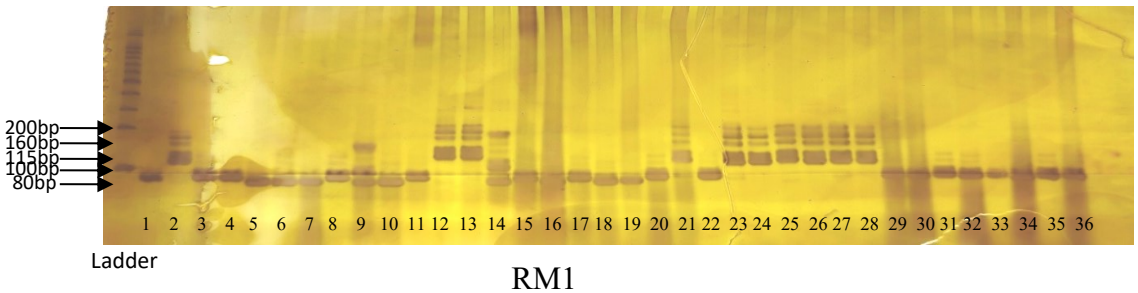
RM171



RM338

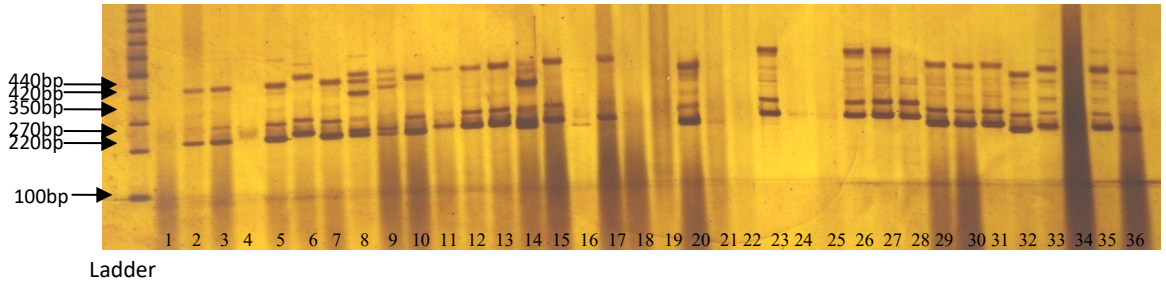


RM107

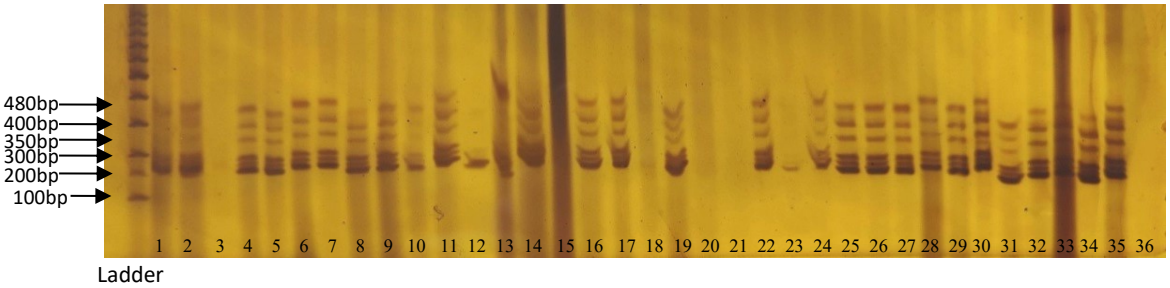


RM1

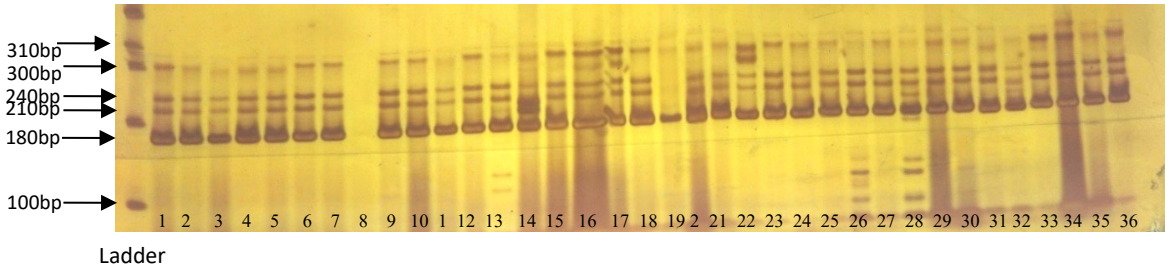
Figure 16: Gel image of microsatellite markers derived from polyacrylamide gel electrophoresis using 100 bp DNA ladder (Continued).



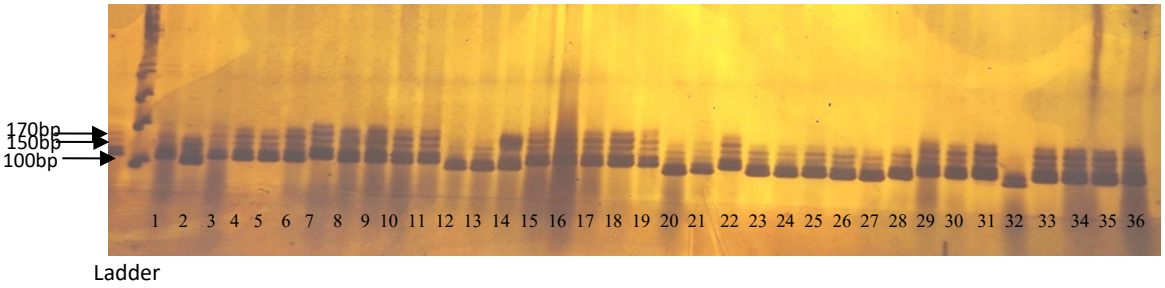
RM19



RM234



RM316



RM249

Figure 16: Gel image of microsatellite markers derived from polyacrylamide gel electrophoresis using 100 bp DNA ladder (Continued).

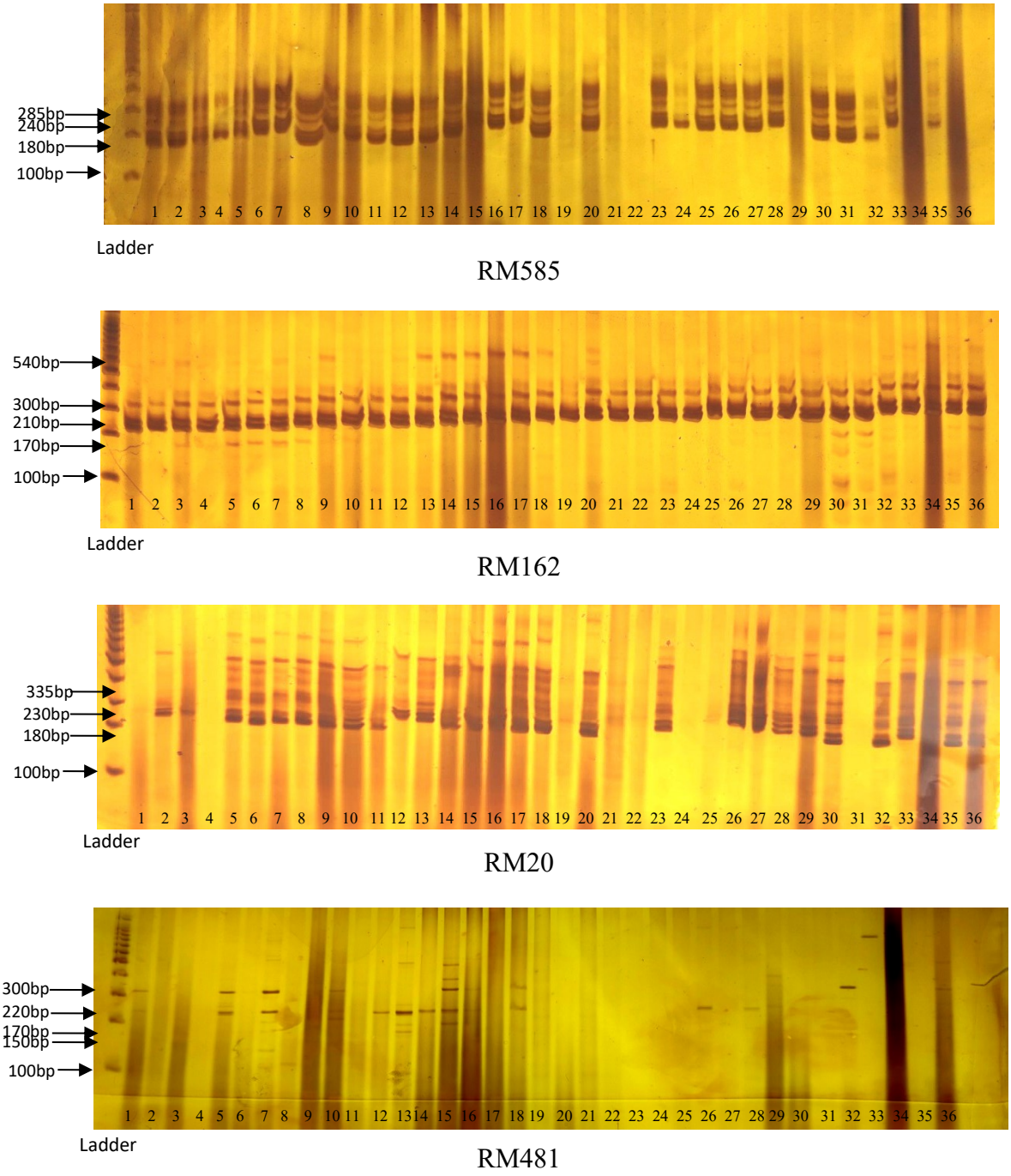


Figure 16: Gel image of microsatellite markers derived from polyacrylamide gel electrophoresis using 100 bp DNA ladder (Continued).

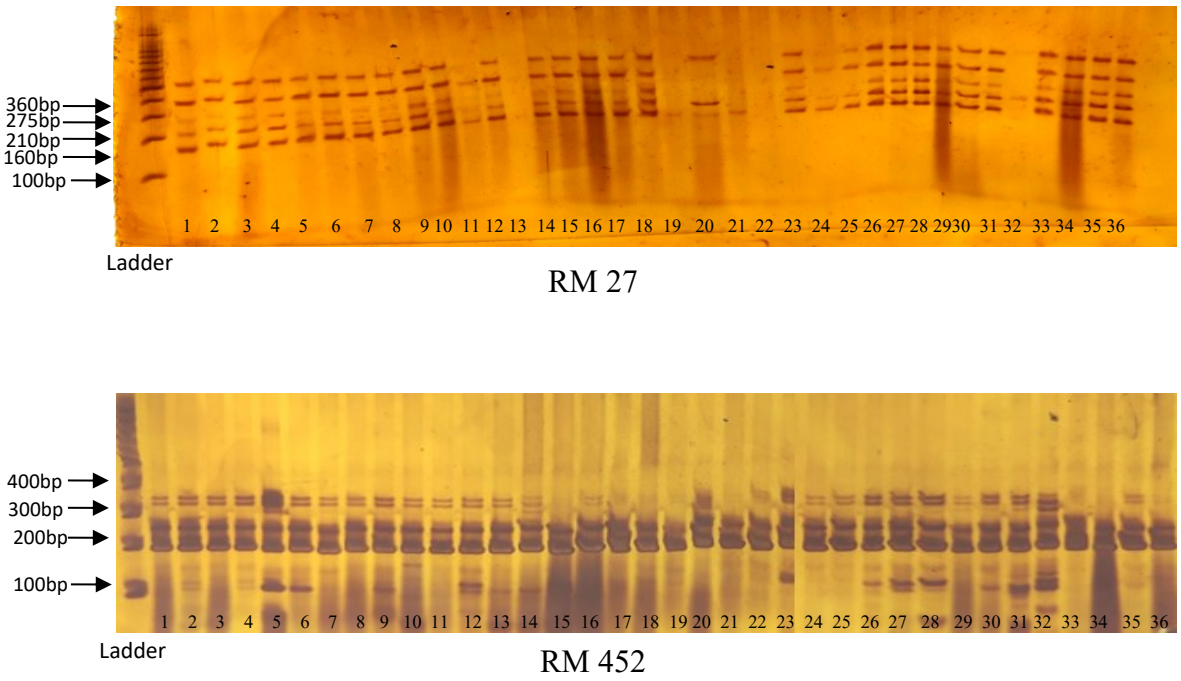


Figure 16: Gel image of microsatellite markers derived from polyacrylamide gel electrophoresis using 100 bp DNA ladder.

Legends, V1= Chenri Aus, V2= Balam Aus, V3= Lohar gura Aus, V4= Gungur Murali Aus, V5= Gungur Bali Aus, V6= Begun Bichi Aus, V7= Kala manik Aus, V8= Gori Matir Aus, V9= Noroi-4 Aus, V10= Kasmiri Lota Aus, V11= Surjamoni Aus, V12= Nara Bet, V13= Mali Khori Aus, V14= Ora Bet Aus, V15= Aus Baku, V16= JamrishaityAus,V17= Batulshi Aus, V18= Baktulshi Aus, V19= Manik madhu 2, V20= Manik madhu, V21= BRRI Dhan-82, V22= Ratul Aus, V23= BRRI Dhan-48, V24= BRRI Dhan-85, V25= Purple-2, V26= BRRI Dhan-28, V27= BRRI Dhan-72, V28= BRRI Dhan-29, V29= Kali boro-1, V30= Kaikka boro, V31= Bawoi boro, V32= Baran boro, V33= Tepi boro-2, V34= Jamir boro, V35= Jagli boro-1, V36= Sada boro.

4.2.2 Evaluation of polymorphism based on SSR profiles

Table 10 shows that 57 alleles of pigmented rice were found at 14 SSR markers. The number of alleles per locus varied from 3 to 6, with an average of 4.1 alleles per locus, out of the 57 total alleles that were found (Table 15). The greatest number of polymorphic alleles (6) was produced by RM316 out of the 14 markers. Replication numbers for RM452, RM249, RM585, and RM20 yielded the fewest polymorphic alleles (3). With an average of 0.70, the PIC (Polymorphism Information Content) values of SSRs varied from 0.59 to 0.80. With RM234 having the highest PIC value (0.80), RM1 (0.79), RM316 (0.78), RM19 (0.77), RM27 and RM338 (0.75), RM171 (0.68), RM452 (0.67), RM249, RM162 and RM107 (0.66), RM585 (0.65), RM481 (0.64), and RM20 (0.59) were following that in order. (Table 10)

Table 10. Number of alleles, allele range, and PIC values for 14 polymorphic markers

SSR loci	Number of alleles	Range of allele size (bp)	PIC
RM1	5	80-200	0.79
RM27	4	160-360	0.75
RM452	3	200-400	0.67
RM338	4	180-500	0.75
RM249	3	100-170	0.66
RM585	3	180-285	0.65
RM162	4	170-540	0.66
RM234	5	200-480	0.80
RM107	4	100-330	0.66
RM316	6	100-310	0.78
RM171	4	330-800	0.68
RM19	5	220-440	0.77
RM20	3	180-335	0.59
RM481	4	150-300	0.64
Average	4.1		0.70

Here, PIC= Polymorphism Information Content.

4.2.3 Model-based population structure

Using Structure software, all genotypes of pigmented rice were evaluated for the purpose of estimating population structure based on 14 markers. The estimated membership fractions of the rice genotypes for various k values ranged from 2 to 9 (Figure 17). The analysis of population structure revealed that the log-likelihood value (ΔK) maximized to the highest value at K= 4 (Figure 18), showing a sharp peak expressing the classification of all genotypes into four distinct sub-populations, referred to as Population I, Population II, Population III, and Population IV, respectively. Population I consisted of 19.4% of genotypes, Population II consisted of 16.7% of genotypes, Population III consisted of 13.9% of genotypes, and Population IV consisted of 50% of genotypes.

Genes were further classified as pure or admixture in the structure analysis; accessions scoring more than 0.80 were deemed pure, and those scoring less than 0.80 were deemed admixture. Five of the seven genotypes in population I were pure, whereas the other two were admixed. Six genotypes in total made up Population II; two were determined to be mixed in and four to be pure. Five genotypes in all made up Population III; two genotypes were discovered to be mixed in and three genotypes were found to be pure. Eight of the eight pure genotypes and ten admixed genotypes were found in population IV.

Table 11. The grouping of rice genotypes into four distinct sub-populations.

Sub-population	Size	Genotypes
I	7	Baktulshi aus, Lohar gura Aus, Manik madhu, Aus Baku, Ora Bet Aus, Nara Bet, Chenri Aus.
II	6	Ratul Aus, BRRI Dhan-85, Manik madhu 2, Purple-2, BRRI Dhan-82, Balam Aus.
III	5	BRRI Dhan-28, BRRI Dhan-72, BRRI Dhan-29, BRRI Dhan-48, Mali Khori Aus.
IV	18	Gungur Murali Aus, Gungur Bali Aus, Begun Bichi Aus, Kala manik Aus, Gori Matir Aus, Noroi-4 Aus, Kasmiri Lota Aus, Surjamoni Aus,Jamrishaity Aus, Batulshi Aus, Kali boro-1, Kaikka boro, Bawoi boro, Baran boro, Tepi boro-2, Jamir boro, Jagli boro-1, Sada boro.

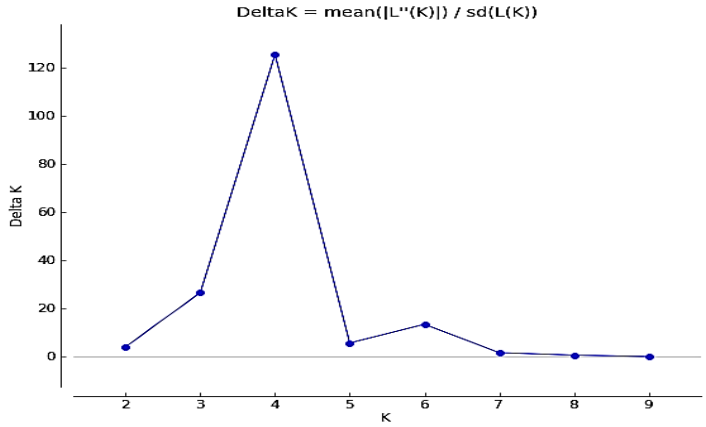


Figure 17. The optimal number of groups among sites as determined by the Evanno test methods.

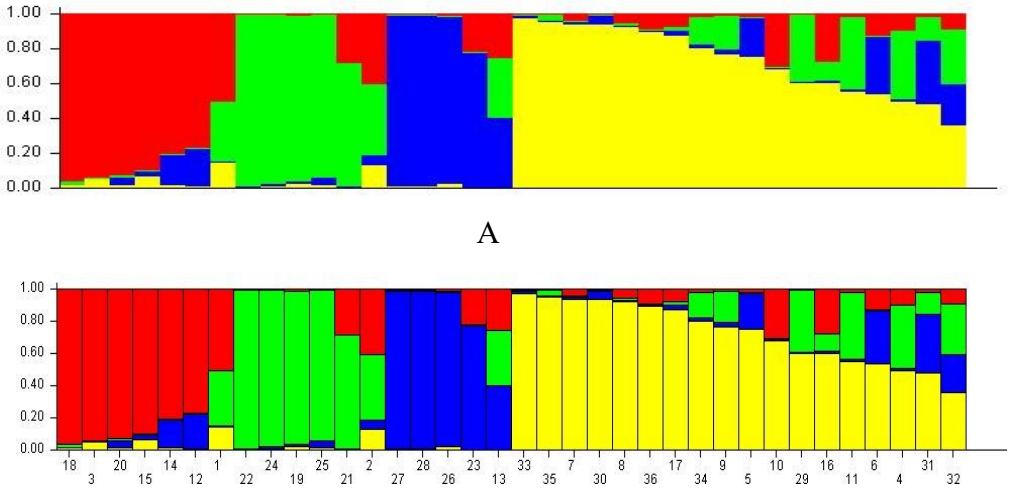


Figure 18. Model based population structure plot for each isolate with K=4, using Structure with SSR markers data.

Color codes are as follows: Population I red, Population II green, Population III blue and Population IV yellow Code of each genotype corresponds to description in Table 1.

Legend, 1= Chenri Aus, 2= Balam Aus, 3= Lohar gura Aus, 4= Gungur Murali Aus, 5= Gungur Bali Aus, 6= Begun Bichi Aus, 7= Kala manik Aus, 8= Gori Matir Aus, 9= Noroi-4 Aus, 10= Kasmiri Lota Aus, 11= Surjamoni Aus, 12= Nara Bet, 13= Mali Khori Aus, 14= Ora Bet Aus, 15= Aus Baku, 16=Jamrishaity Aus, 17= Batulshi Aus, 18= Baktulshi Aus, 19= Manik madhu 2, 20= Manik madhu, 21= BRRI Dhan-82, 22= Ratul Aus, 23= BRRI Dhan-48, 24= BRRI Dhan-85, 25= Purple-2, 26= BRRI Dhan-28, 27= BRRI Dhan-72, 28= BRRI Dhan-29, 29= Kali boro-1, 30= Kaikka boro, 31= Bawoi boro, 32= Baran boro, 33= Tepi boro-2, 34= Jamir boro, 35= Jagli boro-1, 36= Sada boro

Table 12. Mean FST values and average distances between individuals in clusters.

	Mean values of FST	Average distances between clusters
Population 1	0.6076	0.1375
Population 2	0.4622	0.2005
Population 3	0.7395	0.0917
Population 4	0.5471	0.1331

The FST population values were 0.6076 for Population I, 0.4622 for Population II, 0.7395 for Population III, and 0.5471 for Population IV, with an average alpha of 0.1233 indicating significant differences in the population structure. We estimated the average distance (expected heterozygosity) between individuals in the same populations. The average intra-population distances within the same cluster were 0.1375 for Population I, 0.2005 for Population II, 0.0917 for Population III, and 0.1331 for Population IV.

4.2.4 Molecular genetic diversity among the genotypes

Out of the total genotypes of pigmented rice with a similarity coefficient, the genetic distance-based results in the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) cluster analysis showed four significant clusters (population I, population II, population III, and population IV). Orabetaus, Narabetaus, BaktulshiAus, AusBaku, Manikmadhu, LoharguraAus, and ChenriAus were the seven genotypes of pigmented rice that made up population I. Six genotypes of pigmented rice were included in population II, which included Ratul Aus, BRRI Dhan-85, Manik Madhu 2, Purple-2, BRRI Dhan-82, and Balam Aus. 18 genotypes, including Gungur Murali Aus, Gungur Bali Aus, Begun Bichi Aus, Kala manik Aus, Gori Matir Aus, Noroi-4 Aus, Kasmiri Lota Aus, Surjamoni Aus, Jamrishaity Aus, Batulshi Aus, and 5 genotypes, namely BRRI Dhan-28, BRRI Dhan-72, BRRI Dhan-29, BRRI Dhan-48, and Mali Khori Aus, are in population III. population IV includes the following boros: Kali boro-1, Kaikka boro, Bawoi boro, Baran boro, Tepi boro-2, Jamir boro, Jagli boro-1, and Sada boro. Based on the level of variety, the UPGMA analysis can result in the formation of eight clusters (Shivani *et al*., 2021). Moreover, research on genetic diversity have revealed as many as 8 (Rachana *et al*., 2019) and 3 clusters (Vabna *et al*., 2021).

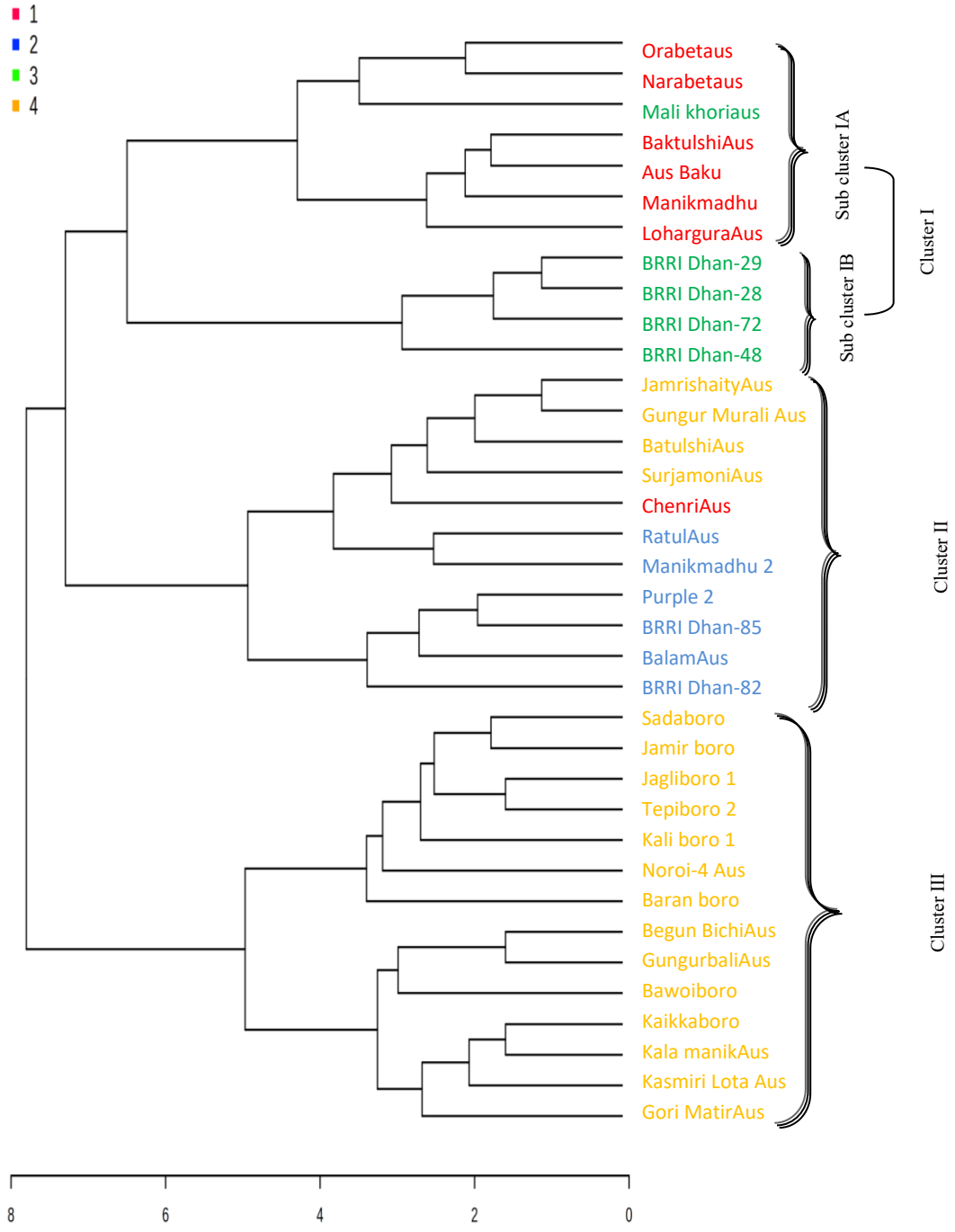


Figure 19. Based on the alleles found by 14 microsatellite markers, a UPGMA cluster dendrogram illustrates the genetic links of pigmented rice landraces in Bangladesh.

4.2.5 Analysis of molecular variance (AMOVA)

Analysis of molecular variance (AMOVA) is a suitable criterion to assess overall distribution of diversity within and among populations (Fig.20). AMOVA results showed a higher level of genetic variation within populations than among them. Similar results were reported by Singode and Prasanna (2010) and Da Silva *et al*. (2015). The largest genetic variability 90% was attributed to variation within population, while 10% of the total variation was explained by variation among population (Table-13). This result is consistent with previous studies carried out on this species (Hoxha *et al*., 2004) and more generally on allogamous species (Wanjala *et al*., 2013).

Table 13. Analysis of molecular variance (AMOVA) for pigmented rice genotypes with 4 populations

Source	df	SS	MS	Est.Var.	%
Among Pops	3	39.337	13.112	0.779	10%
Within Pops	32	220.746	6.898	6.898	90%
Total	35	260.083		7.677	100%

Df= degrees of freedom, SS= sum of square, MS= mean sum of squre, Est.Var.= estimated variations

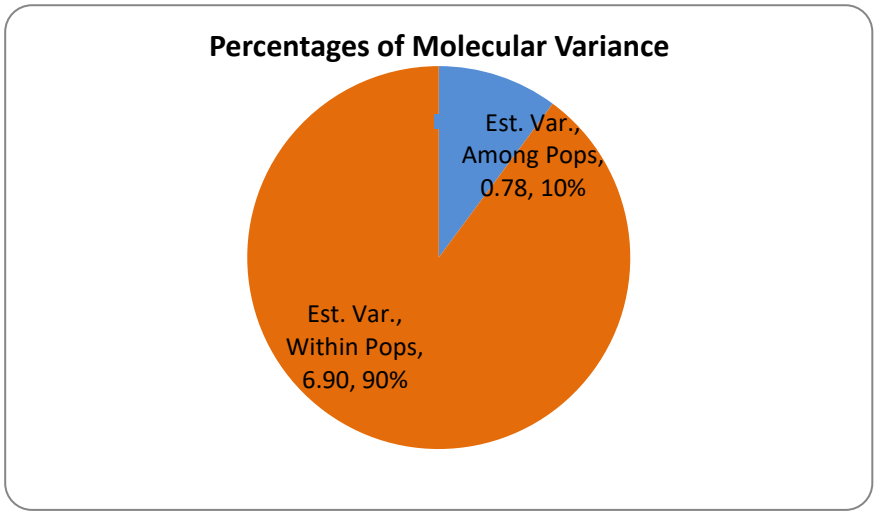


Figure 20. Analysis of molecular variances

4.2.6 Principal component analysis

Analysis of Principal Components Using PCA, associations between rice genotypes were calculated. Here, the genetic distances between the rice genotypes are shown by the localization of genotypes in a 2D PCA plot, where the first principal component (PC1) and the second principal component (PC2), respectively, explained 15.1% and 13.3% of the overall variation (Figure 21). Here, genotypes G1, G2, G15, and G18 were combined with Population I and Population II. In Population I and Population III, the genotypes G12, G14, G23, G26, G27, and G28 were combined.

The blue line beneath the green line in the PCA scree plot (Figure 22) represented the variation explained by each individual PC, while the green line on top represented the total variance explained. The first three eigenvalues in the PCA scree plot, which were subsequently shown to show the genotype diversity, explained 40.8% of the cumulative variation (Figure 22). The eigenvalues of the first three principal components were 15.1%, 13.3%, and 12.5%, respectively. The first three principal coordinate components showed an overall maximum cumulative variance of 40.8% (Figure 22).

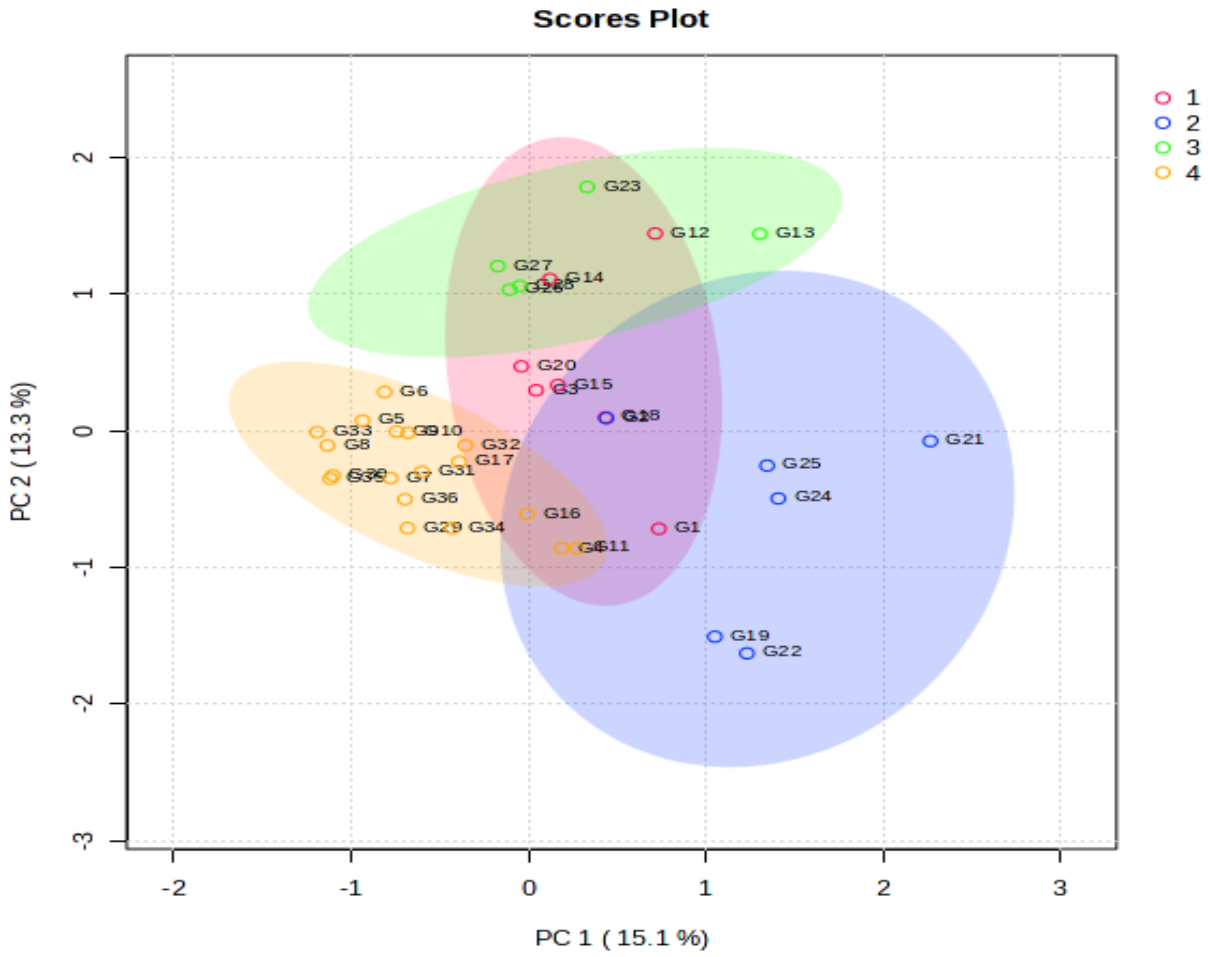


Figure 21: Two-dimensional principal component analysis (PCA) using SSR polymorphisms in rice genotypes

Legends, V1= Chenri Aus, V2= Balam Aus, V3= Lohar gura Aus, V4= Gungur Murali Aus, V5= Gungur Bali Aus, V6= Begun Bichi Aus, V7= Kala manik Aus, V8= Gori Matir Aus, V9= Noroi-4 Aus, V10= Kasmiri Lota Aus, V11= Surjamoni Aus, V12= Nara Bet, V13= Mali Khori Aus, V14= Ora Bet Aus, V15= Aus Baku, V16=JamrishaityAus,V17= Batulshi Aus, V18= Baktulshi Aus, V19= Manik madhu 2, V20= Manik madhu, V21= BRRI Dhan-82, V22= Ratul Aus, V23= BRRI Dhan-48, V24= BRRI Dhan-85, V25= Purple-2, V26= BRRI Dhan-28, V27= BRRI Dhan-72, V28= BRRI Dhan-29, V29= Kali boro-1, V30= Kaikka boro, V31= Bawoi boro, V32= Baran boro, V33= Tepi boro-2, V34= Jamir boro, V35= Jagli boro-1, V36= Sada boro.

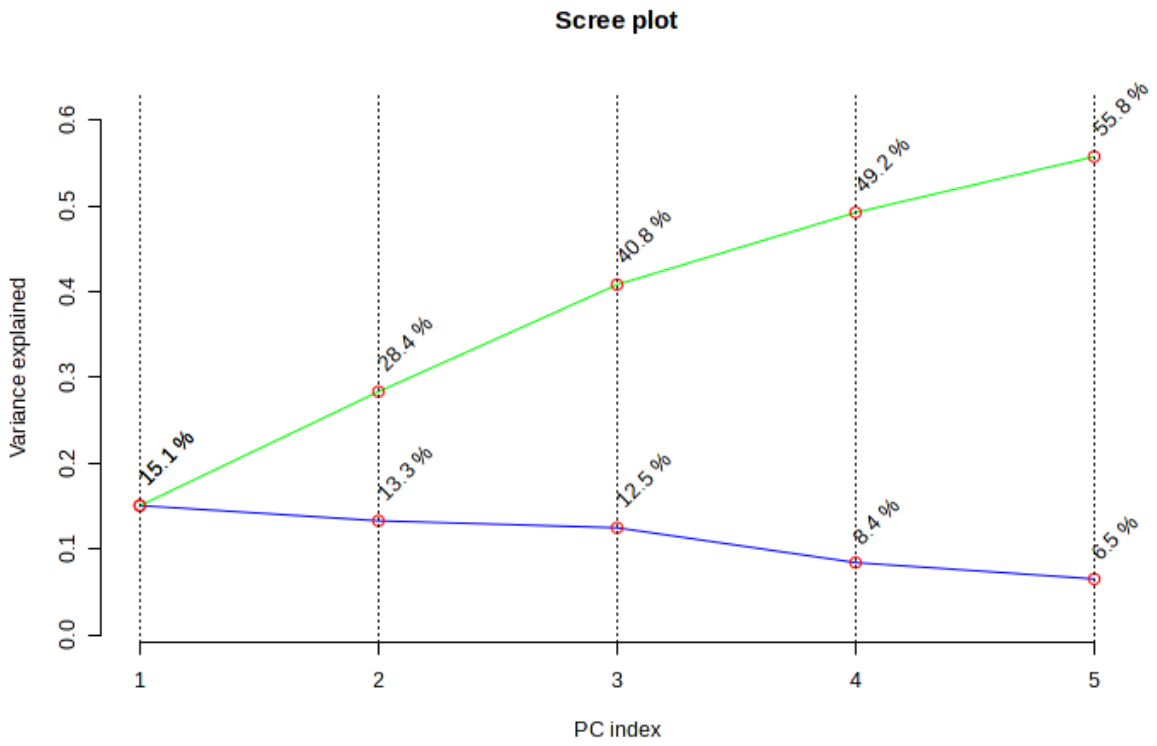


Figure 22: The scree plot shows Principal coordinate analysis of top 5 PCs for pigmented rice genotypes using SSR marker data.

4.3 Interrelationship of morphological and molecular outcome

The rice genotypes were divided into three distinct clusters according to morphological traits by the dendrogram produced from the ward's grouping in experiment 1. In the second experiment, the molecular profiles of rice genotypes were sorted into three distinct groups using the dendrogram. The variation in the number of clusters observed here is accompanied by similarities in the genotypic composition of the clusters at the molecular and morphological levels.

The study's integration of genetic and morphological data showed that although the number of clusters varied, the clusters' genotypes were the same on both the molecular and morphological levels. Orabet aus, Narabet aus, and Malikhori aus were prevalent genotypes in molecular Sub cluster IA, which shared genotypic similarities with Morphological Cluster I (Table 14). Seven genotypes—Balam Aus, Batulshi Aus, Chenrri Aus, Gungur Murali Aus, Jamrishaity Aus, Ratul Aus, and Surjamoni Aus—that were all present in molecular Cluster II made up Morphological Cluster II. Morphological Cluster III shared genes with molecular Cluster III, which included common genotypes in Baran boro, Bawoi boro, Jagli boro 1, Jamir boro, Kaikka boro, Kali boro 1, Sada boro, and Tepi boro 2. Molecular Sub-cluster IB (BRRI Dhan-28, BRRI Dhan-29, BRRI Dhan-48, BRRI Dhan-72) and Morphological Cluster III shared genotypic similarities.

Table 14. Comparative analysis of molecular clustering and morphological clustering

Genotype	Morphological	Molecular
Orabet aus, Narabet aus, Malikhori aus	Cluster I	Sub cluster IA
Balam Aus, Batulshi Aus, Chenrri Aus, Gungur Murali Aus, Jamrishaity Aus, Ratul Aus and Surjamoni Aus.	Cluster II	Cluster II
Baran boro, Bawoi boro, Jagli boro 1, Jamir boro, Kaikka boro, Kali boro 1, Sada boro, Tepi boro 2	Cluster III	Cluster III
BRRI Dhan-28, BRRI Dhan-29, BRRI Dhan-48, BRRI Dhan-72	Cluster III	Sub cluster IB

CHAPTER V

SUMMARY AND CONCLUSION

The results from the Mean performance of different yield and yield contributing characters indicated that there were significant variations among the pigmented rice genotypes for all the traits studied. The analysis of variance revealed that the differences among the genotypes were highly significant for all the characters, which included days to 50% flowering, plant height, productive tiller per plant, unproductive tiller per plant, total tillers per plant, panicle length, spike per panicle, straw weight, thousand seed weight, and yield per plant. This indicates that there is a considerable amount of genetic variability present among the genotypes, which is essential for the selection of better parental types to improve grain yield through breeding programs. The study also found that the mean squares against three replications were non-significant for all the characters except Total Phenol Content (TPC), suggesting that the variations observed were primarily due to genetic differences rather than experimental error. The coefficient of variation (CV%) was used to measure the dispersion of the variable, providing additional insights into the extent of variability within the population of rice genotypes studied.

Traits such as plant height (114.43±2.74 cm), productive tiller per plant (28.85±1.11), unproductive tiller per plant (2.82±0.25), and yield per plant (18.82±1.10 gm) exhibited substantial variances in the mean performance of the various yield and yield contributing characters. Estimates were made for genetic factors such heritability, genetic advance, phenotypic variation, genotypic variance, and genetic advance as a percentage of the mean. The largest genotypic and phenotypic variations were found with Straw weight (13985.59 and 14158.47), DPPH (4216.64 and 4470.27), Days to 50% blooming (1367.09 and 1367.56), Iron content (938.47 and 947.76), Plant height (373.12 and 395.66), respectively. The traits Unproductive tiller per plant (1.20 and 1.02), Spike per panicle (3.11 and 2.82), and Thousand seed weight (18.21 and 17.73) were associated with the lowest values of phenotypic and genotypic variations. The plant height had a phenotypic coefficient of variation (PCV) of 17.38%, while the total phenolic content had a PCV of 70.99%. The plant height had a genotypic coefficient of variance (GCV) of 16.88%, while the total phenolic content had a GCV of 70.31%. The total phenolic content (70.99 and 70.31) showed the highest PCV and GCV. The total flavonoid content (TFC) and days to

50% flowering showed the highest heritability estimates, ranging from 82.28% to 99.99%. The results also showed high heritability estimates for a number of characteristics.

The genetic parameters estimated for the selected characters in pigmented aus and boro rice included genotypic variance (σ^2g), phenotypic variance (σ^2p), genotypic coefficient of variation (GCV %), phenotypic coefficient of variation (PCV %), heritability (h2b), genetic advance (GA), and genetic advance as a percent of the mean (GAM). These parameters were calculated for 15 characters to observe the variability among the traits. The results revealed that the phenotypic variances for all the characters were higher than the genotypic variances. The highest genotypic and phenotypic variances were recorded for straw weight, DPPH, days to 50% flowering, iron content, and plant height, while lower values were observed for unproductive tiller per plant, spike per panicle, and thousand seed weight. These genetic parameters provide valuable insights into the variability and potential for genetic improvement among the pigmented rice genotypes.

The Hierarchical cluster analysis based on morphological traits revealed the grouping of genotypes of rice into three clusters. The analysis was performed using the relative mean values of each trait, and Euclidean distance coefficients were calculated for all rice genotypes based on fifteen traits. The resulting dendrogram from the UPGMA clustering indicated the formation of three clusters, denoted as Cluster I, Cluster II, and Cluster III. Cluster I comprised 3 genotypes, Cluster II comprised 16 genotypes, and Cluster III comprised 16 genotypes. The analysis demonstrated significant differences among the genotypes for all the characters, indicating the existence of variability among the genotypes studied. This clustering provides valuable insights into the genetic diversity and structure of the pigmented rice genotypes based on their morphological traits.

A total of 57 alleles were identified at 14 SSR markers over pigmented rice genotypes. The number of alleles per locus ranged from 3 to 6, with an average of 4.1 alleles per locus. Among the 14 markers, RM316 produced the highest number of polymorphic alleles (6), while the lowest number of polymorphic alleles (3) were produced in RM452, RM249, RM585, and RM20. The PIC (Polymorphism Information Content) values of SSRs ranged from 0.59 to 0.80, with an average of 0.70. The highest PIC value (0.80) was recorded for RM234, followed by RM1 (0.79), RM316 (0.78), RM19 (0.77), RM27 and RM338 (0.75), RM171 (0.68), RM452 (0.67), RM249, RM162, and RM107 (0.66), RM585 (0.65), RM481 (0.64), and RM20 (0.59) respectively.

The model-based population structure analysis based on 14 markers using Structure software revealed that the pigmented rice genotypes were classified into four specific sub-populations denoted as Population I, Population II, Population III, and Population IV. Population I consisted of 19.4% of genotypes, Population II consisted of 16.7% of genotypes, Population III consisted of 13.9% of genotypes, and Population IV consisted of 50% of genotypes. Additionally, the genotypes were further categorized as pure or admixture, with the FST population values indicating significant differences among the population structure. The analysis also identified the number of pure and admixed genotypes within each population, providing valuable insights into the genetic diversity and structure of the pigmented rice genotypes.

The Analysis of Molecular Variance (AMOVA) results indicated a higher level of genetic variation within populations than among them. Specifically, the largest genetic variability, accounting for 90% of the total variation, was attributed to variation within the population, while 10% of the total variation was explained by variation among populations. This outcome is consistent with previous studies conducted on similar species and allogamous species. The AMOVA analysis provides valuable insights into the distribution of genetic diversity within and among populations, offering crucial information for understanding the genetic structure and variability of the pigmented rice genotypes.

REFERENCES

Aalim, H. and Luo, Z., 2021. Insight into rice (*Oryza sativa L.*) cooking: Phenolic composition, inhibition of α-amylase and α-glucosidase, and starch physicochemical and functional properties. *Food Bioscience*, *40*, p.100917.

Aesomnuk, W., Ruengphayak, S., Ruanjaichon, V., Sreewongchai, T., Malumpong, C., Vanavichit, A., Toojinda, T., Wanchana, S. and Arikit, S., 2021. Estimation of the genetic diversity and population structure of Thailand's rice landraces using SNP markers. *Agronomy*, *11*(5), p.995.

Acharjee, S., Chakraborty, N.R. and Das, S.P., 2021. Screening of rice landraces for potential drought tolerance through comparative studies of genetic variability and principal component analysis. *Electronic Journal of Plant Breeding*, *12*(4), pp.1091-1101.

Affre, L., Dutoit, T., Jäger, M. and Garraud, L., 2003. Ecology of reproduction and dispersal, and genetic structure in *Messicole* species: management proposals in the Luberon Regional Nature Park. *The Proceedings of the BRG*, *4*, pp.405-428.

Ahmed, M.S., Khaleda, A., Khalequzzaman, M., Rashid, E.S.M.H. and Bashar, M.K., 2010. Diversity analysis in Boro rice (*Oryza sativa L.*) accessions. *Bangladesh Journal of Agricultural Research*, *35*(1), pp.29-36.

Akshay, M., Chandra, B.S., Devi, K.R. and Hari, Y., 2022. Genetic variability studies for yield and its attributes, quality and nutritional traits in rice (*Oryza sativa L.*). *The Pharma Innovation Journal*, *11*(5), pp.167-172.

Akter, R., Sugino, H., Akhter, N., Brown, C.L., Thilsted, S.H. and Yagi, N., 2021. Micronutrient adequacy in the diet of reproductive-aged adolescent girls and adult women in rural Bangladesh. *Nutrients*, *13*(2), p.337.

Al-Shammari, A.M.A., Hamdi, G.J., Al-Mahdawi, M.A.S. and Mohammed, N.K., 2021. Genetic diversity analysis and DNA fingerprinting of tomato breeding lines using SSR markers.

Andarini, Y.N., Suwarno, W.B., Aswidinnoor, H. and Kurniawan, H., 2022, January. Genetic relationship of pigmented rice (*Oryza sativa L.*) collected from Eastern Indonesia based on morpho-agronomical traits and SSR markers. In AIP Conference Proceedings (Vol. 2462, No. 1, p. 020023). AIP Publishing LLC.

Asante, M.D., Adjah, K.L. and Annan-Afful, E., 2019. Assessment of genetic diversity for grain yield and yield component traits in some genotypes of rice (*Oryza sativa L.*). *Journal of Crop Science and Biotechnology*, *22*(2), pp.123-130.

Ashrafuzzaman, M., Islam, M.R., Ismail, M.R., Shahidullah, S.M. and Hanafi, M.M., 2009. Evaluation of six aromatic rice varieties for yield and yield contributing characters. *Int. J. Agric. Biol*, *11*(5), pp.616-620.

Basavaraj, K. and Gireesh, C., 2023. Variability Parameters, Correlation Studies and Path Analysis of Yield and Yield-Related Traits in Rice (*Oryza sativa L.*): A Comprehensive Review. *International Journal of Environment and Climate Change*, *13*(11), pp.2015-2022.

Bassolino, L., Petroni, K., Polito, A., Marinelli, A., Azzini, E., Ferrari, M., Ficco, D.B., Mazzucotelli, E., Tondelli, A., Fricano, A. and Paris, R., 2022. Does plant breeding for antioxidant-rich foods have an impact on human health? *Antioxidants*, *11*(4), p.794.

Behera, P.K., Kumar, V., Sharma, S.S., Lenka, S.K. and Panda, D., 2023. Genotypic diversity and abiotic stress response profiling of short-grain aromatic landraces of rice (*Oryza sativa L.* Indica). *Current Plant Biology*, *33*, p.100269.

Behera, P.P., Singh, S.K., Sivasankarreddy, K., Majhi, P.K., Reddy, B.J. and Singh, D.K., 2022. Yield attributing traits of high zinc rice (*Oryza sativa L.*) genotypes with special reference to principal component analysis. *Environment Conservation Journal*, *23*(3), pp.458-470.

Bekis, D., Mohammed, H. and Belay, B., 2021. Genetic divergence and cluster analysis for yield and yield contributing traits in lowland rice (*Oryza sativa L.*) genotypes at Fogera, Northwestern Ethiopia. *International Journal of Advanced Research in Biological Sciences*, *8*(5), pp.1-11.

Beser, N. and Mutafcilar, Z.C., 2020. Identification of SSR markers for differentiating rice (*Oryza sativa L.*) varieties marketed in Turkey. *Journal of Agricultural Sciences*, *26*(3), pp.357-362.

Bhandari, H.R., Bhanu, A.N., Srivastava, K., Singh, M.N. and Shreya, H.A., 2017. Assessment of genetic diversity in crop plants-an overview. *Adv. Plants Agric. Res*, *7*(3), pp.279-286.

Bhati, P.K., Singh, S.K., Singh, R., Dhurai, S.Y., Sharma, A. and Kumar, V., 2015. Correlation and path analysis for yield and quality characters in rice (*Oryza sativa L.*).

Bhat, F.M., Sommano, S.R., Riar, C.S., Seesuriyachan, P., Chaiyaso, T. and Prom-u-Thai, C., 2020. Status of bioactive compounds from bran of pigmented traditional rice varieties and their scope in production of medicinal food with nutraceutical importance. *Agronomy*, *10*(11), p.1817.

Birtucan, D.T., 2021. *Genetic variability, heritability and association of morphological, yield related and quality traits in upland rice (Oryza sativa L.) genotypes at pawe, northwestern Ethiopia* (Doctoral dissertation, Bahir Dar University).

Bitew, J.M., Mekbib, F. and Assefa, A., 2016. Genetic variability among yield and yield related traits in selected upland rice (*Oryza sativa L.* and Oryza glaberrima Steud) genotypes in Northwestern Ethiopia. *world scientific news*, *47*(2), pp.62-74.

Bitew, J.M., 2016. Estimation of genetic parameters, heritability and genetic advance for yield related traits in upland rice (*Oryza sativa L.* and Oryza glaberrima Steud) genotypes in northwestern Ethiopia. *World Scientific News*, *47*(2), pp.340-350.

Blessy, V., Murugan, E., Suresh, R., Gnanamalar, R.P., Kumar, S.V. and Kanchana, S., 2022. Parent progeny regression analysis for yield and yield contributing traits in F3 and F4 generations in rice (*Oryza sativa L.*). *International Journal of Bio-resource and Stress Management*, *13*(10), pp.1021-1028.

Cattell, R.B., 1966. The scree test for the number of factors. *Multivariate behavioral research*, *1*(2), pp.245-276.

CECAP, P., IIRR. 2000.". *Highland Rice Production in the Philippine Cordillera*.

Choi, J.Y., Platts, A.E., Fuller, D.Q., Hsing, Y.I., Wing, R.A. and Purugganan, M.D., 2017. The rice paradox: multiple origins but single domestication in Asian rice. *Molecular biology and evolution*, *34*(4), pp.969-979.

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

Civáň, P., Ali, S., Batista-Navarro, R., Drosou, K., Ihejieto, C., Chakraborty, D., Ray, A., Gladieux, P. and Brown, T.A., 2019. Origin of the aromatic group of cultivated rice (*Oryza sativa L.*) traced to the Indian subcontinent. *Genome biology and evolution*, *11*(3), pp.832-843.

Da Silva, T.A., Cantagalli, L.B., Saavedra, J., Lopes, A.D., Mangolin, C.A., da Silva, M.D.F.P. and Scapim, C.A., 2015. Population structure and genetic diversity of Brazilian popcorn germplasm inferred by microsatellite markers. *Electronic Journal of Biotechnology*, *18*(3), pp.181-187

da Silva, L.R., de Carvalho, C.W.P., Velasco, J.I. and Fakhouri, F.M., 2020. Extraction and characterization of starches from pigmented rice. *International journal of biological macromolecules*, *156*, pp.485-493.

Da Silva, T.A., Cantagalli, L.B., Saavedra, J., Lopes, A.D., Mangolin, C.A., da Silva, M.D.F.P. and Scapim, C.A., 2015. Population structure and genetic diversity of Brazilian popcorn germplasm inferred by microsatellite markers. *Electronic Journal of Biotechnology*, *18*(3), pp.181-187.

Devi, K.R., Chandra, B.S., Lingaiah, N., Hari, Y. and Venkanna, V., 2017. Analysis of variability, correlation and path coefficient studies for yield and quality traits in rice (*Oryza sativa L.*). *Agricultural Science Digest-A Research Journal*, *37*(1), pp.1-9.

De Mendiburu, F. and Simon, R., 2015. *Agricolae-Ten years of an open source statistical tool for experiments in breeding, agriculture and biology* (No. e1748). PeerJ PrePrints.

Dhakal, A., Pokhrel, A., Sharma, S. and Poudel, A., 2020. Multivariate analysis of phenotypic diversity of rice (*Oryza sativa L.*) landraces from Lamjung and Tanahun Districts, Nepal. *International journal of agronomy*, *2020*, pp.1-8.

Dutta, P., Dutta, P.N. and Borua, P.K., 2013. Morphological traits as selection indices in rice: A statistical view. *Universal Journal of Agricultural Research*, *1*(3), pp.85-96.

Dwiningsih, Y. and Alkahtani, J., 2022. Phenotypic Variations, Environmental Effects and Genetic Basis Analysis of Grain Elemental Concentrations in Rice (*Oryza sativa L.*) for Improving Human Nutrition.

Embate, M.V.G., Calayugan, M.I.C., Gentallan, R.P., Sta Cruz, P.C., Hernandez, J.E. and Borromeo, T.H., 2021. Genetic diversity of selected pigmented traditional rice (*Oryza sativa L.*) varieties from Mindanao, Philippines using agromorphological traits and simple sequence repeats markers. *Journal of Crop Science and Biotechnology*, *24*, pp.259-277.

Earl, D.A. and VonHoldt, B.M., 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation genetics resources*, *4*, pp.359-361.

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

Fageria, N.K., Castro, E.M. and Baligar, V.C., 2004. Response of upland rice genotypes to soil acidity. *The red soils of China: Their nature, management and utilization*, pp.219-237.

Farvid, M.S., Cho, E., Eliassen, A.H., Chen, W.Y. and Willett, W.C., 2016. Lifetime grain consumption and breast cancer risk. *Breast cancer research and treatment*, *159*, pp.335-345.

Faiz, A., Hanafi, M.M., Hakim, M.A., Rafii, M.Y. and Akmar Abdullah, S.N., 2015. Micronutrients, Antioxidant Activity, and Tocochromanol Contents of Selected Pigmented Upland Rice Genotypes. *International Journal of Agriculture & Biology*, *17*(4).

Feng, T., Jia, Q., Meng, X., Chen, X., Wang, F., Chai, W. and Liang, Z., 2020. Evaluation of genetic diversity and construction of DNA fingerprinting in Polygonatum Mill. based on EST-SSR and SRAP molecular markers. *3 Biotech*, *10*, pp.1-13.

Fu, X., Yu, X., Ye, Z. and Cui, H., 2015. Analysis of antioxidant activity of Chinese brown rice by Fourier-transformed near infrared spectroscopy and chemometrics. *Journal of Chemistry*, *2015*.

GANA, A.S., 2006. *VARIABILITY STUDIES ON THE RESPONSE OF RICE VARIETIES TO BIOTIC AND ABIOTC STRESSES* (Doctoral dissertation, University of Ilorin).

Gana, A.S., Shaba, S.Z. and Tsado, E.K., 2013. Principal component analysis of morphological traits in thirty-nine accessions of rice (*Oryza sativa L.*) grown in a rainfed lowland ecology of Nigeria. *Journal of plant breeding and crop science*, *5*(10), pp.120-126.

Garris, A.J., Tai, T.H., Coburn, J., Kresovich, S. and McCouch, S., 2005. Genetic structure and diversity in *Oryza sativa L. Genetics*, *169*(3), pp.1631-1638.

Glaszmann, J.C., 1987. Isozymes and classification of Asian rice varieties. *Theoretical and Applied genetics*, *74*, pp.21-30.

Gour, L., Maurya, S.B., Koutu, G.K., Singh, S.K., Shukla, S.S. and Mishra, D.K., 2017. Characterization of rice (*Oryza sativa L.*) genotypes using principal component analysis including scree plot & rotated component matrix. *International Journal of Chemical Studies*, *5*(4), pp.975-83.

Gupta, R., Faruquee, M. and andSarkar, M.J., 2022. Performance of high yielding boro rice varieties in Khagrachhari district of Bangladesh. *Technology*, *18*(5), pp.1961-1972.

Gutaker, R.M., Groen, S.C., Bellis, E.S., Choi, J.Y., Pires, I.S., Bocinsky, R.K., Slayton, E.R., Wilkins, O., Castillo, C.C., Negrão, S. and Oliveira, M.M., 2020. Genomic history and ecology of the geographic spread of rice. *Nature plants*, *6*(5), pp.492-502.

Ham, H., Kim, H.J., Park, H.Y., Sim, E.Y., Oh, S.K., Kim, W.H., Jeong, H.S. and Woo, K.S., 2016. Functional components and radical scavenging activity of germinated brown rice according to variety. *The Korean Journal of Food And Nutrition*, *29*(2), pp.145-152.

Hasan, N., Choudhary, S., Naaz, N., Sharma, N. and Laskar, R.A., 2021. Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. *Journal of Genetic Engineering and Biotechnology*, *19*(1), pp.1-26.

Hashim, N., Rafii, M.Y., Oladosu, Y., Ismail, M.R., Ramli, A., Arolu, F. and Chukwu, S., 2021. Integrating multivariate and univariate statistical models to investigate genotype–environment interaction of advanced fragrant rice genotypes under rainfed condition. *Sustainability*, *13*(8), p.4555.

Hossain, M., Jaim, W.M.H., Alam, M.S. and Rahman, A.N.M., 2013. Rice biodiversity in Bangladesh: adoption, diffusion and disappearance of varieties: a statistical report from farm survey in 2005.

Hoxha, S., Shariflou, MR and Sharp, P., 2004. Evaluation of genetic diversity in Albanian maize using SSR markers. *Maydica*, *49* (2), pp.97-103.

Huang, R., Jiang, L., Zheng, J., Wang, T., Wang, H., Huang, Y. and Hong, Z., 2013. Genetic bases of rice grain shape: so many genes, so little known. *Trends in plant science*, *18*(4), pp.218-226.

Huang, X., Jang, S., Kim, B., Piao, Z., Redona, E. and Koh, H.J., 2021. Evaluating genotype× environment interactions of yield traits and adaptability in rice cultivars grown under temperate, subtropical and tropical environments. *Agriculture*, *11*(6), p.558.

Hurtada, W.A., Barrion, A.S.A. and Nguyen-Orca, M.F.R., 2018. Mineral content of dehulled and well-milled pigmented and non-pigmented rice varieties in the Philippines. *International Food Research Journal*, *25*(5).

Ilieva, V., Markova Ruzdik, N., Mihajlov, L. and Ilievski, M., 2019. Assessment of agro-morphological variability in rice using multivariate analysis. *Journal of Agriculture and Plant Sciences*, *17*(1), pp.79-85.

Islam, M.Z., Shim, M.J., Jeong, S.Y. and Lee, Y.T., 2022. Effects of soaking and sprouting on bioactive compounds of black and red pigmented rice cultivars. *International Journal of Food Science & Technology*, *57*(1), pp.201-209.

Islam, S.S., Nualsri, C. and Hasan, A.K., 2021. Character association and path analysis studies in upland rice (Oryza sativa) genotypes. *Research on Crops*, *22*(2), pp.239-245.

Jaksomsak, P., Rerkasem, B. and Prom-U-Thai, C., 2021. Variation in nutritional quality of pigmented rice varieties under different water regimes. *Plant Production Science*, *24*(2), pp.244-255.

Jun, H.I., Song, G.S., Yang, E.I., Youn, Y. and Kim, Y.S., 2012. Antioxidant activities and phenolic compounds of pigmented rice bran extracts. *Journal of food science*, *77*(7), pp.C759-C764.

Kammapana, L., 2023. Physical Characteristics, Phytochemical Contents and Antioxidant Activity of Ten Organic-Pigmented Rice Varieties from Surin Province. *Trends in Sciences*, *20*(4), pp.4566-4566.

Nandedkar, K., Sarawgi, A.K., Parikh, M., Saxena, R.R. and Rawte, S., 2020. Assessment of diversity based on agro-morphological and quality characterization of germplasm accessions of rice (*Oryza sativa L.*). *International Journal of Current Microbiology and Applied Sciences*, *9*(8), pp.2397-2408.

Karimah, A.Z., Siswoyo, T.A., Kim, K.M. and Ubaidillah, M., 2021. Genetic diversity of rice germplasm (*Oryza sativa L.*) of java island, Indonesia. *Journal of Crop Science and Biotechnology*, *24*, pp.93-101.

Kato, Y., Collard, B.C., Septiningsih, E.M. and Ismail, A.M., 2019. Increasing flooding tolerance in rice: combining tolerance of submergence and of stagnant flooding. *Annals of Botany*, *124*(7), pp.1199-1209.

Khan, M.M.H., Rafii, M.Y., Ramlee, S.I., Jusoh, M. and Al Mamun, M., 2021. Genetic analysis and selection of Bambara groundnut (Vigna subterranea [L.] Verdc.) landraces for high yield revealed by qualitative and quantitative traits. *Scientific Reports*, *11*(1), p.7597.

Khanom, M.S.R., Rani, M.H., Rahman, M.H.S., Shammy, S.A., Sharma, A.C., Akram, M.W., Begum, S.N. and Islam, M.M., 2021. Evaluation of iron and zinc enriched rice (*Oryza sativa L.*) genotypes in different locations of bangladesh. *Bangladesh J. Nucl. Agric.*, *35*, pp.21-28.

Kim, D.O., Jeong, S.W. and Lee, C.Y., 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food chemistry*, *81*(3), pp.321-326.

Kimwemwe, P.K., Bukomarhe, C.B., Mamati, E.G., Githiri, S.M., Civava, R.M., Mignouna, J., Kimani, W. and Fofana, M., 2023. Population structure and genetic diversity of rice (*Oryza sativa L.*) germplasm from the Democratic Republic of Congo (DRC) using DArTseq-derived single nucleotide polymorphism (SNP). *Agronomy*, *13*(7), p.1906.

Kishore, N.S., Srinivas, T., Nagabhushanam, U., Pallavi, M. and Sameera, S.K., 2015. Genetic variability, correlation and path analysis for yield and yield components in promising rice (*Oryza sativa L.*) genotypes. *SAARC Journal of Agriculture*, *13*(1), pp.99-108.

Kumar, B.S. and DAS, J.C., 2019. Effect of irrigation and nutrient management on growth, yield and evapotranspiration of direct-seeded autumn rice (oryza sativa) under the agro-climatic conditions of assam. *The Bioscan. A quarterly journal of life sciences*, pp.239-248.

Nithya, N., Beena, R., Abida, P.S., Sreekumar, J., Stephen, R., Jayalekshmi, V.G., Manju, R.V. and Viji, M.M., 2021. Genetic diversity and population structure analysis of bold type rice collection from Southern India. *Cereal Research Communications*, *49*, pp.311-328.

Nybom, H., 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology*, *13*(5), pp.1143-1155.

Pandey, P. and Anurag, P.J., 2010. Estimation of genetic parameters in indigenous rice. *Advances in Agriculture & Botanics*, *2*(1), pp.79-84.

Parimala, K., Raju, C.S., Prasad, A.H., Kumar, S.S. and Reddy, S.N., 2020. Studies on genetic parameters, correlation and path analysis in rice (*Oryza sativa L.*). *Journal of Pharmacognosy and Phytochemistry*, *9*(1), pp.414-417.

Poonia, A. and Pandey, S., 2021. Bioactive compounds, nutritional benefits and food applications of black rice: a review. *Nutrition & Food Science*, *52*(3), pp.466-482.

Prajapati, M.K., Singh, C.M., Babu, G.S., Lavanya, G.R. and Jadhav, P., 2011. Genetic parameters for grain yield and its component characters in rice. *Electronic Journal of Plant Breeding*, *2*(2), pp.235-238.

Pratap, A., Bisen, P., Loitongbam, B. and Singh, P.K., 2018. Assessment of genetic variability for yield and yield components in rice (*Oryza sativa L.*) germplasms. *International Journal of Bio-resource and Stress Management*, *9*(Feb, 1), pp.087-092.

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

Purugganan, M.D. and Fuller, D.Q., 2009. The nature of selection during plant domestication. *Nature*, *457*(7231), pp.843-848.

Rachana, B., Eswari, K.B., Jyothi, B., Devi, L.G., Vidhya, J.L., Bhavani, L.P., Bharath, M., Rao, R.P., Kumar, A.J., Abdul, F.R. and Neeraja, C.N., 2019. Characterization of new plant type core set of rice (*Oryza sativa L.*) using QTL/gene-linked markers. *ORYZA-An International Journal on Rice*, *56*(4), pp.352-360.

Rahman, S.W., Bhagawati, S. and Gogoi, B., 2017. Assessment of nutritive and antioxidant properties of some indigenous pigmented hill rice (*Oryza sativa L.*) cultivars of Assam. *Indian Journal of Agricultural Research*, *51*(3), pp.214-220.

Rahman, M.A., Islam, M.R., Islam, A.S., Akter, N., Munna, M.A.U.R. and Rana, M.M., 2023. Genetic analyses of advanced breeding lines of rice (*Oryza sativa L.*) based on morphological traits. *Journal of Bioscience and Agriculture Research*, *30*(02), pp.2559-2569.

Rajesh, T., Paramasivam, K., Thirumeni, S., Raja Rajan, D, Kumar S.M., 2022. Molecular characterization of rice land races by SSR markers. *Journal of Plant Genetics and Breeding,* 6(1), pp.1-5.

Ranjkesh, N., Daliri, M.S., Mazloum, P., Mousavi, A. and Rameeh, V., 2021. Evaluation of physicochemical characteristics and antioxidant properties of elite rice (*Oryza sativa L.*). *Cereal Research Communications*, *49*, pp.485-491.

Rashid, M.M., Nuruzzaman, M., Hassan, L. and Begum, S.N., 2017. Genetic variability analysis for various yield attributing traits in rice genotypes. *Journal of the Bangladesh Agricultural University*, *15*(1), pp.15-19.

Ravi, M., Geethanjali, S., Sameeyafarheen, F. and Maheswaran, M., 2003. Molecular marker based genetic diversity analysis in rice (*Oryza sativa L.*) using RAPD and SSR markers. *Euphytica*, *133*, pp.243-252.

Reddy, C.K., Kimi, L. and Haripriya, S., 2016. Variety difference in molecular structure, functional properties, phytochemical content and antioxidant capacity of pigmented rice. *Journal of Food Measurement and Characterization*, *10*, pp.605-613.

Rerkasem, B., Sangruan, P. and Thebault Prom-u-thai, C., 2015. Effect of Polishing Time on Distribution of Monomeric Anthocyanin, Iron and Zinc Content in Different Grain Layers of Four Thai Purple Rice Varieties. *International Journal of Agriculture & Biology*, *17*(4).

Rocha, R.S., Nascimento, M.R., Chagas, J.T.B., de Almeida, R.N., Dos Santos, P.R., da Cruz, D.P., da Silva Costa, K.D., de Amaral Gravina, G. and Daher, R.F., 2019. Association among Agro-morphological Traits by Correlations and Path in Selection of Maize Genotypes. *Journal of Experimental Agriculture International*, *34*(2), pp.1-12.

Roy, S.C. and Sharma, B.D., 2014. Assessment of genetic diversity in rice [*Oryza sativa L.*] germplasm based on agro-morphology traits and zinc-iron content for crop improvement. *Physiology and Molecular Biology of Plants*, *20*, pp.209-224.

Sabri, R.S., Rafii, M.Y., Ismail, M.R., Yusuff, O., Chukwu, S.C. and Hasan, N.A., 2020. Assessment of agro-morphologic performance, genetic parameters and clustering

pattern of newly developed blast resistant rice lines tested in four environments. *Agronomy*, *10*(8), p.1098.

Saha, S.R., Lutful, H., Haque, M.A., Islam, M.M. and Rasel, M., 2019. Genetic variability, heritability, correlation and path analyses of yield components in traditional rice (*Oryza sativa L.*) landraces. *Journal of the Bangladesh Agricultural University*, *17*(1), pp.26-32.

Saikia, S., Dutta, H., Saikia, D. and Mahanta, C.L., 2012. Quality characterisation and estimation of phytochemicals content and antioxidant capacity of aromatic pigmented and non-pigmented rice varieties. *Food Research International*, *46*(1), pp.334-340.

Salgotra, R.K. and Chauhan, B.S., 2023. Ecophysiological responses of rice (*Oryza sativa L.*) to drought and high temperature. *Agronomy*, *13*(7), p.1877.

Samyor, D., Das, A.B. and Deka, S.C., 2017. Pigmented rice a potential source of bioactive compounds: A review. *International Journal of Food Science & Technology*, *52*(5), pp.1073-1081.

Sangeetha, J., Thangadurai, D., Fayeun, L.S., Akinwale, J.A., Habeeb, J., Maxim, S.S., Hospet, R. and Islam, S., 2020. Origin and evolution of rice as domesticated food crop. *Rice Research for Quality Improvement: Genomics and Genetic Engineering: Volume 1: Breeding Techniques and Abiotic Stress Tolerance*, pp.1-14.

Sanghamitra, P., Barik, S.R., Bastia, R., Mohanty, S.P., Pandit, E., Behera, A., Mishra, J., Kumar, G. and Pradhan, S.K., 2022. Detection of genomic regions controlling the antioxidant enzymes, phenolic content, and antioxidant activities in Rice grain through association mapping. *Plants*, *11*(11), p.1463.

Sani, N.A., Sawei, J., Ratnam, W. and Rahman, Z.A., 2018. Physical, antioxidant and antibacterial properties of rice (*Oryza sativa L.*) and glutinous rice (Oryza sativa var. glutinosa) from local cultivators and markets of Peninsular, Malaysia. *International Food Research Journal*, *25*(6).

Sarif, H.M., Rafii, M.Y., Ramli, A., Oladosu, Y., Musa, H.M., Rahim, H.A., Zuki, Z.M. and Chukwu, S.C., 2020. Genetic diversity and variability among pigmented rice

germplasm using molecular marker and morphological traits. *Biotechnology & Biotechnological Equipment*, *34*(1), pp.747-762.

Sarwar, A.K.M., Haque, M.S., Haque, M.E., Hossain, M.A., Azam, M.G., Uddin, M.N., Dessoky, E.S., Basry, M.A. and Hossain, M.A., 2022. Agro-Morphological Characterization and Genetic Dissection of Linseed (Linum usitatissimum L.) Genotypes. *Phyton (0031-9457)*, *91*(8).

Sharma, V., Saini, D.K., Kumar, A. and Kaushik, P., 2019. A Review of Important QTLs for Biofortification Traits in Rice.

Shew, A.M., Durand-Morat, A., Putman, B., Nalley, L.L. and Ghosh, A., 2019. Rice intensification in Bangladesh improves economic and environmental welfare. *Environmental Science & Policy*, *95*, pp.46-57.

Shin, W.K., Lee, H.W., Shin, A., Lee, J.K., Lee, S.A., Lee, J.E. and Kang, D., 2020. Multi-grain rice diet decreases risk of breast cancer in Korean women: results from the health examinees study. *Nutrients*, *12*(8), p.2273.

Shivani, D., Jabeen, F., Chaithanya, K., Koushik, M.B.V.N., Dileep, G.D., Koti, E.P., Supriya, K., Sundaram, R.M., Kumar, J.A. and Abdul, R., 2021. Assessment of genetic diversity of rice germplasm using microsatellite markers. *The Pharma Innovation Journal*, *10*(5), pp.1393-1397.

Shrestha, J., Subedi, S., Kushwaha, U.K.S. and Maharjan, B., 2021. Evaluation of growth and yield traits in rice genotypes using multivariate analysis. *Heliyon*, *7*(9).

Shrivastava, A., Mishra, D.K. and Koutu, G.K., 2015. Estimation of genetic parameters of variability for yield and its attributing traits in parental lines of hybrid rice. *Plant Archives*, *15*(1), pp.571-574.

Singh, S.K., Pandey, V., Mounika, K., Singh, D.K., Khaire, A.R., Habde, S. and Majhi, P.K., 2020. Study of genetic divergence in rice (*Oryza sativa L.*) genotypes with high grain zinc using Mahalanobis' D2 analysis. *Electronic Journal of Plant Breeding*, *11*(02), pp.367-372.

Singh, K.S., Suneetha, Y., Kumar, G.V., Rao, V.S., Raja, D.S. and Srinivas, T., 2020. Variability, correlation and path studies in coloured rice. *Int J Chem Stud*, *8*(4), pp.2138-2144.

Singh, N., Choudhury, D.R., Tiwari, G., Singh, A.K., Kumar, S., Srinivasan, K., Tyagi, R.K., Sharma, A.D., Singh, N.K. and Singh, R., 2016. Genetic diversity trend in Indian rice varieties: an analysis using SSR markers. *BMC genetics*, *17*, pp.1-13.

Singh, R.K. and Chaudhary, B.D., 1977. Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi, India.

Singh, S.K., Singh, P., Korada, M., Khaire, A.R., Singh, D.K., Habde, S.V., Majhi, P.K. and Naik, R., 2020. Character association and path-coefficient analysis for yield and yield-related traits in 112 genotypes of rice (*Oryza sativa L.*). *Current Journal of Applied Science and Technology*, *39*(48), pp.545-556.

Singode, A. and Prasanna, B.M., 2010. Analysis of genetic diversity in the North Eastern Himalayan maize landraces using microsatellite markers. *Journal of Plant Biochemistry and Biotechnology*, *19*, pp.33-41.

Sridevi, P., Veni, B.K., Raja, D.S. and Jyothula, D.P.B., 2021. Physico-chemical, nutritional and Anti-oxidative properties of different colored grain genotypes of rice (*Oryza sativa L.*). *Int J Chem Stud*, *9*(1), pp.1769-1776.

Sudeepthi, K. and Srinivas, T., 2022. BNVSR Ravi kumar3, Y. Suneetha4, B. Bhargavi5 and T. Venkata Ratnam6. Assessment of molecular diversity in rice using anaerobic germination linked SSR markers. *Scientist*, pp.873-885.

Sudeepthi, K., Srinivas, T.V.S.R., Kumar, B.R., Jyothula, D.P.B. and Umar, S.N., 2020. Assessment of genetic variability, character association and path analysis for yield and yield component traits in rice (*Oryza sativa L.*). *Electronic Journal of Plant Breeding*, *11*(01), pp.144-148.

Sumanth, V., Suresh, B.G., Ram, B.J. and Srujana, G., 2017. Estimation of genetic variability, heritability and genetic advance for grain yield components in rice (*Oryza sativa L.*). *Journal of Pharmacognosy and Phytochemistry*, *6*(4), pp.1437-1439.

Yun SungHa, Y.S., Kim KiRang, K.K., Nam SeokJin, N.S., Kong Gu, K.G. and Kim MiKyung, K.M., 2010. The association of carbohydrate intake, glycemic load, glycemic index, and selected rice foods with breast cancer risk: a case-control study in South Korea. *Asia Pacific journal of clinical nutrition*, *19*(3), pp.383-392.

Suvi, W.T., Shimelis, H., Laing, M., Mathew, I. and Shayanowako, A.I.T., 2020. Assessment of the genetic diversity and population structure of rice genotypes using SSR markers. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science*, *70*(1), pp.76-86.

Tarang, A., Kordrostami, M., Shahdi Kumleh, A., Hosseini Chaleshtori, M., Forghani Saravani, A., Ghanbarzadeh, M. and Sattari, M., 2020. Study of genetic diversity in rice (*Oryza sativa L.*) cultivars of Central and Western Asia using microsatellite markers tightly linked to important quality and yield related traits. *Genetic Resources and Crop Evolution*, *67*, pp.1537-1550.

Temnykh, S., Park, W.D., Ayres, N., Cartinhour, S., Hauck, N., Lipovich, L., Cho, Y.G., Ishii, T. and McCOUCH, S.R., 2000. Mapping and genome organization of microsatellite sequences in rice (*Oryza sativa L.*). *Theoretical and applied genetics*, *100*, pp.697-712.

Tiozon, R.J.N., Sartagoda, K.J.D., Fernie, A.R. and Sreenivasulu, N., 2023. The nutritional profile and human health benefit of pigmented rice and the impact of post-harvest processes and product development on the nutritional components: A review. *Critical Reviews in Food Science and Nutrition*, *63*(19), pp.3867-3894.

Tripathi, S., Singh, S.K., Srivashtav, V., Khaire, A.R., Vennela, P. and Singh, D.K., 2020. Molecular diversity analysis in rice (*Oryza sativa L.*) using SSR markers. *Electronic Journal of Plant Breeding*, *11*(03), pp.776-782.

Tyagi, A., Lim, M.J., Kim, N.H., Barathikannan, K., Vijayalakshmi, S., Elahi, F., Ham, H.J. and Oh, D.H., 2022. Quantification of Amino Acids, Phenolic Compounds Profiling from Nine Rice Varieties and Their Antioxidant Potential. *Antioxidants*, *11*(5), p.839.

Vabna, F.A., Islam, M.Z., Prince, M.F.R.K. and Hoque, M.E., 2021. Molecular diversity analysis in boro rice (*Oryza sativa L.*) landraces using SSR markers. *Asian Journal of Biology*, *12*(1), pp.36-48.

Vanlalrinngama, C., Jha, B., Singh, S.K., Tigga, A., Kumar, B., Kumari, N. and Singh, M.K., 2023. Variability and divergence studies on rice genotypes for micronutrient potential and its utility in biofortification. *Environment Conservation Journal*, *24*(1), pp.151-156.

Velprabakaran, S., Rajeswari, S., Jeyaprakash, P. and Raveendran, M., 2021. Profiling Pigmented Rice Germplasm for Iron and Zinc Concentration. *Indian Journal of Plant Genetic Resources*, *34*(01), pp.82-85.

Vichit, W.A.N.N.I.S.A. and Saewan, N.I.S.A.K.O.R.N., 2015. Antioxidant activities and cytotoxicity of Thai pigmented rice. *International Journal of Pharmacy and Pharmaceutical Sciences*, *7*(7), pp.329-334.

Wakaria, N. M., 2021. Evaluation of rice genotypes for rice blast resistance and its inheritance in mwea, kirinyaga county, Kenya (Doctoral dissertation, Kenyatta University).

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

Ward Jr, J.H., 1963. Hierarchical grouping to optimize an objective function. *Journal of the American statistical association*, *58*(301), pp.236-244.

Wanjala, B.W., Obonyo, M., Wachira, F.N., Muchugi, A., Mulaa, M., Harvey, J., Skilton, R.A., Proud, J. and Hanson, J., 2013. Genetic diversity in Napier grass (Pennisetum purpureum) cultivars: implications for breeding and conservation. *AoB Plants*, *5*, p.plt022.

Wijaya, C. and Romulo, A., 2021, October. Proximate analysis and antioxidant activity of red rice (*Oryza sativa L.*) Milk. In *Journal of Physics: Conference Series* (Vol. 2049, No. 1, p. 012012). IOP Publishing.

Wolie, A., Dessalegn, T. and Belete, K., 2013. Heritability, variance components and genetic advance of some yield and yield related traits in Ethiopian collections of finger millet (Eleusine coracana (L.) Gaertn.) genotypes. *African Journal of Biotechnology*, *12*(36).

Xiongsiyee, V., Rerkasem, B., Veeradittakit, J., Saenchai, C., Lordkaew, S. and Thebault Prom-u-thai, C., 2018. Variation in grain quality of upland rice from Luang Prabang province, Lao PDR. *Rice Science*, *25*(2), pp.94-102.

Yodmanee, S., Karrila, T.T. and Pakdeechanuan, P., 2011. Physical, chemical and antioxidant properties of pigmented rice grown in Southern Thailand. *International food research journal*, *18*(3).

Zaid, I.U., Zahra, N., Habib, M., Naeem, M.K., Asghar, U., Uzair, M., Latif, A., Rehman, A., Ali, G.M. and Khan, M.R., 2022. Estimation of genetic variances and stability components of yield-related traits of green super rice at multi-environmental conditions in Pakistan. *Agronomy*, *12*(5), p.1157.

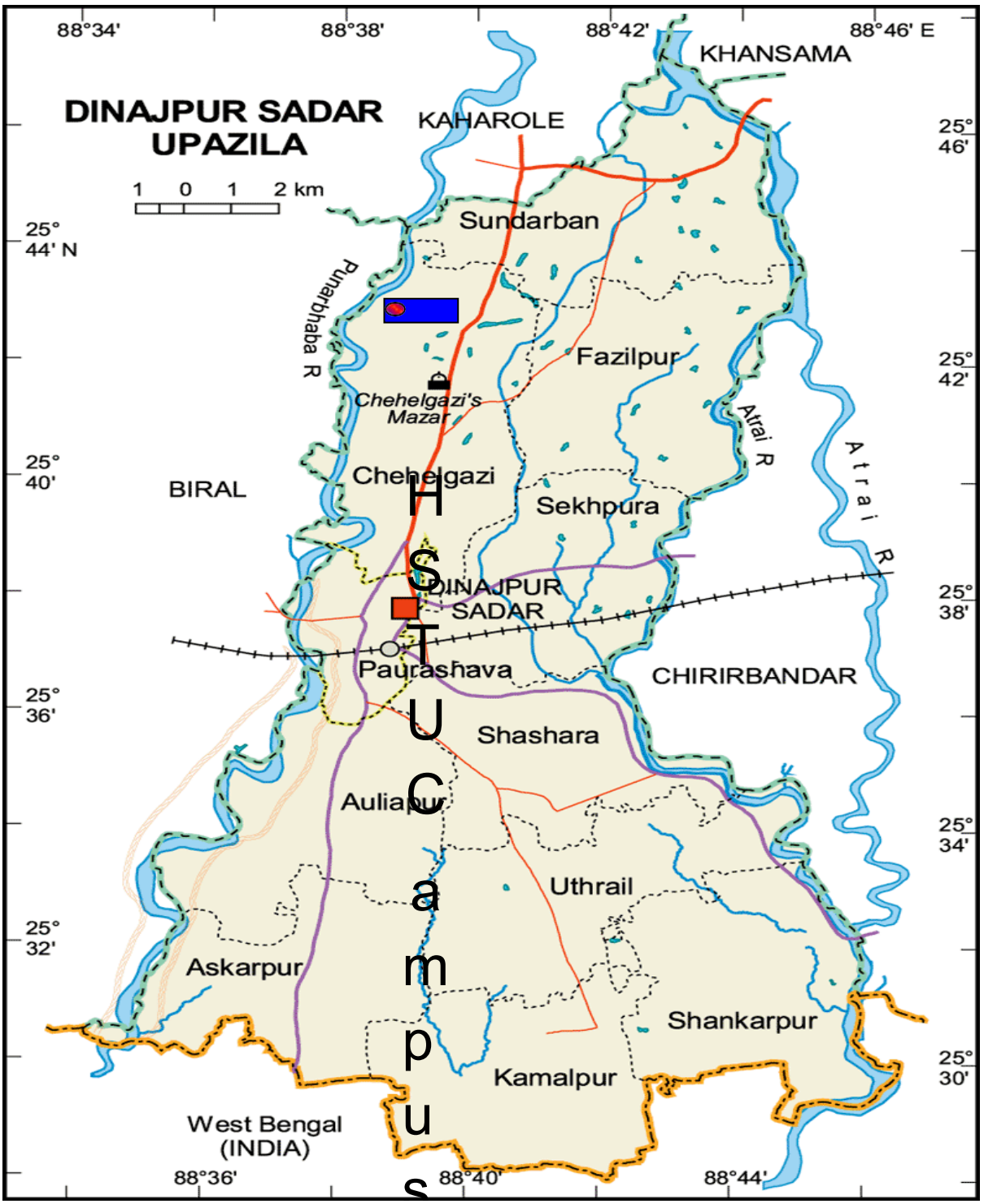
Zaupa, M., Calani, L., Del Rio, D., Brighenti, F. and Pellegrini, N., 2015. Characterization of total antioxidant capacity and (poly) phenolic compounds of differently pigmented rice varieties and their changes during domestic cooking. *Food chemistry*, *187*, pp.338-347.

Zulfiqar, A., Naseer, S., Saleem, A., Sabar, M., Ahmed, S., Sardar, R., Shahzadi, F. and Raza, Q., 2023. Genetic diversity studies for grain iron and zinc content analysis for Elite rice (*Oryza sativa L.*) genotype by using SSR markers. *Journal of Food Composition and Analysis*, *115*, p.104816.

Zulfiqar, U., Hussain, S., Maqsood, M., Ishfaq, M. and Ali, N., 2021. Zinc nutrition to enhance rice productivity, zinc use efficiency, and grain biofortification under different production systems. *Crop Science*, *61*(1), pp.739-749.

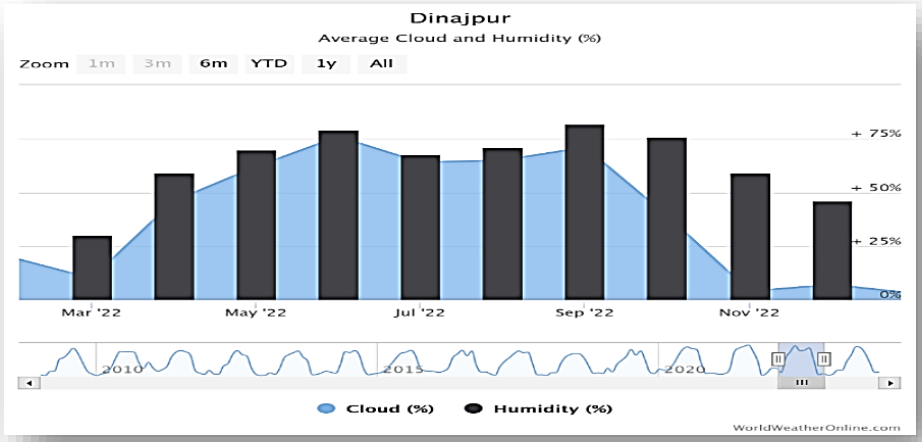
APPENDICES

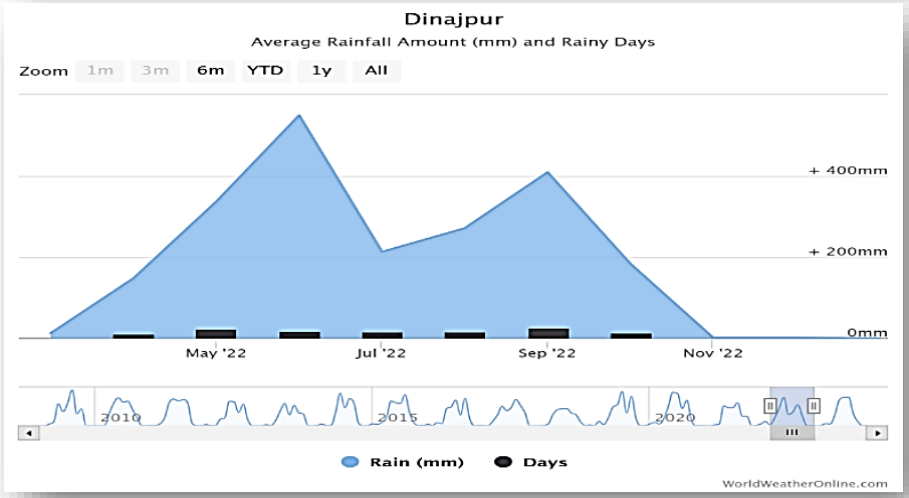
Appendix I: Map of Dinajpur Sadar Upazila showing the experimental area

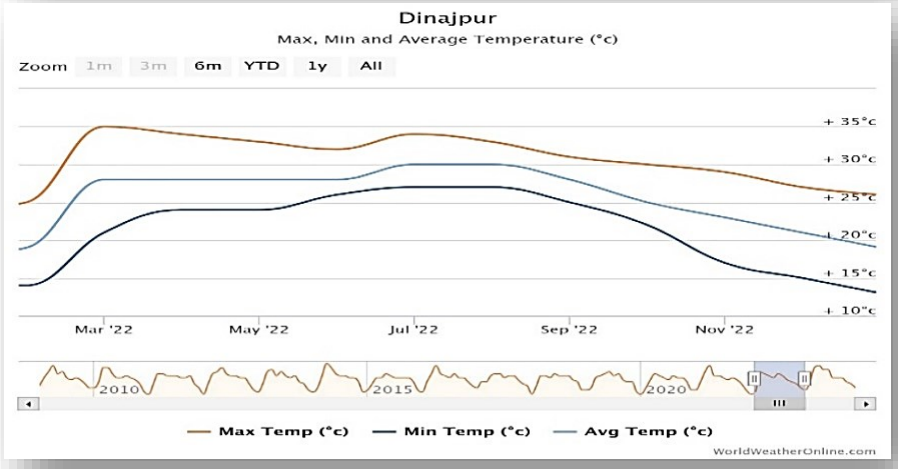


Map Courtesy: Banglapedia

Appendix II: Weather data of the experimental site during the period from, April 2022 to November, 2022







Source: www.worldweatheronline.com

Appendix III: The soil nutrient composition of Genetics and Plant Breeding research plot of HSTU, Dinajpur

Physical and chemical properties	Value
Sand (%)	65
Silt (%)	30
Clay (%)	5
Textural class	Sandy loam
pH	4.70
Organic matter (%)	0.89
Total nitrogen (%)	0.04
Magnesium (meq/ 100g)	0.66
Potassium (meq/ 100g)	0.26
Phosphorus (µg/g)	55.85
Sulphur (µg/g)	9.93
Boron (µg/g)	0.25
Zinc (µg/g)	1.43

µg: Micro gram; meq: Mili equivalent

Source: Soil Resources Development Institute (SRDI), Nashipur, Dinajpur-5200.

Appendix IV: Some photographs of research work

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