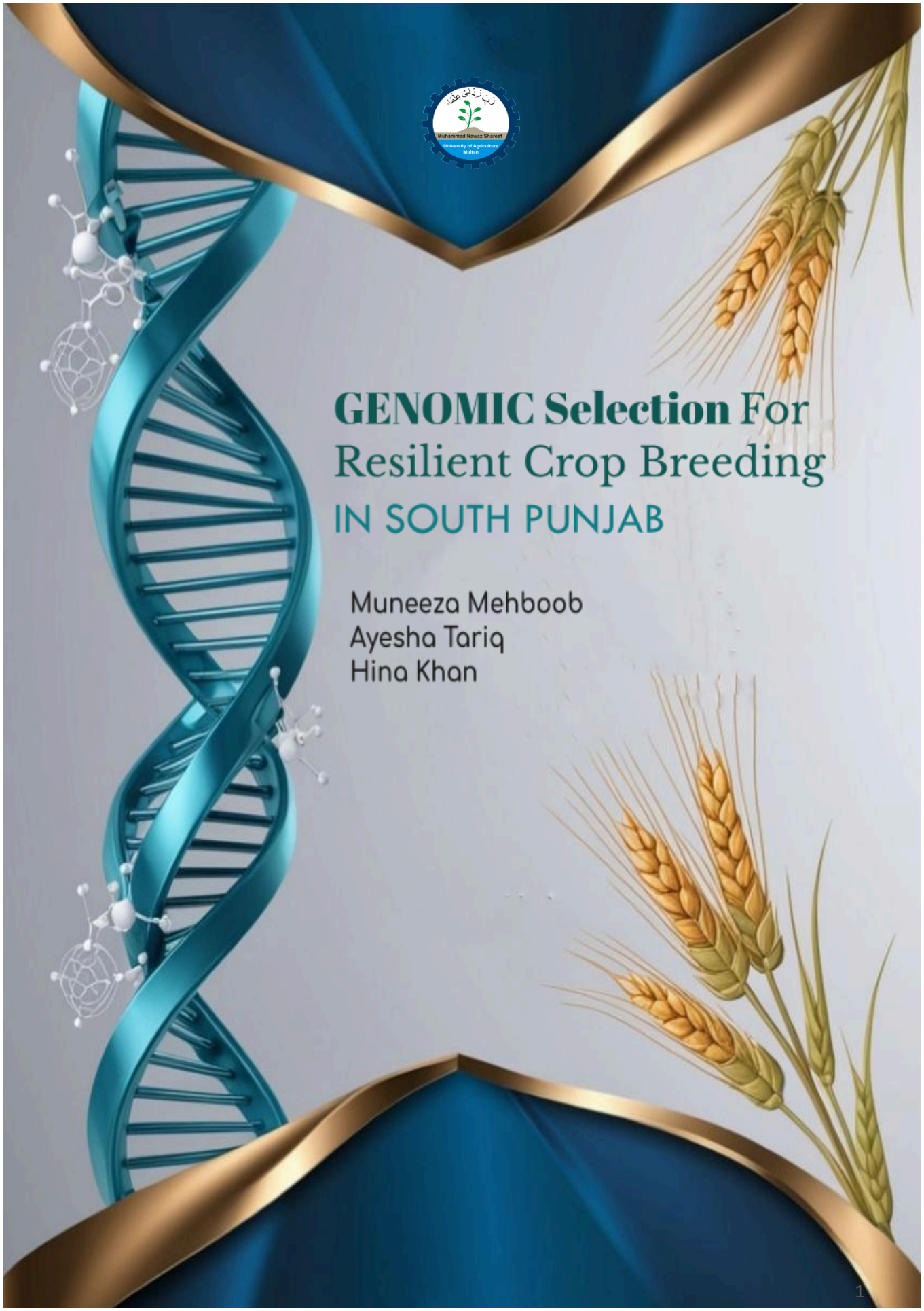
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University of Agriculture
Multan
GENOMIC Selection For
Resilient Crop Breeding
IN SOUTH PUNJAB
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Project Report

Genomic Selection For Resilient Crop Breeding In South Punjab

Submitted by

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2021-uam-4638

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Session: 2021-2025

Program: BS Data Science

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INSTITUTE OF COMPUTING

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FINAL APPROVAL

This is to certify that we have read this report submitted by *Muneeza Mehboob, Ayesha Tariq* and *Hina Khan* it is our judgment that this report is of sufficient standard to warrant its acceptance by MNS-University of Agriculture, Multan for the degree of BS Data Science.

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DEDICATION

We dedicate this project with our whole heart to our dear parents, whose unconditional support, genuine prayers, and infinite sacrifices have been the pillar of our academic life. Their love, motivation, and confidence in our abilities have been a source of inspiration, supporting us through every ordeal and triumph. This work is a small gesture of appreciation for all they have done to guide us towards reaching this milestone.

ACKNOWLEDGMENT

We would like to express our deepest gratitude to our respected supervisor **Mr. Israr Hussain** for their continuous guidance, patience, and support throughout this project. We also thank the faculty members of the Institute of Computing and our families and friends who motivated us at every step of this journey. This project would not have been possible without their encouragement and moral support.

PROJECT BRIEF

Field	Detail
Project Name	Genomic Selection For Resilient Crop Breeding In South Punjab
University Name	Muhammad Nawaz Sharif University of Agriculture, Multan
Undertaken by	Muneeza Mehbbob, Ayesha Tariq, Hina Khan
Supervised by	Mr Israr Hussain
Starting Date	January 2025
Completion Date	May 2025
Computer Used	Intel Core i5, 8GB RAM, 12 Generation
Operating System	Windows 11
Source Language(s)	Python
DBMS Used	N/A
Tools/Packages	Beagle 5.5, Plink, Pandas, Scikit-learn, XGBoost, GAPIT, Matplotlib

PLAGIARISM UNDERTAKING

We solemnly declare that the work presented in the report titled **"Genomic Selection For Resilient Crop Breeding In South Punjab**" is solely our effort with no significant contribution from any other individual. Wherever minor assistance or contribution was taken, it has been duly acknowledged, and the complete report has been written by us.

We understand the zero-tolerance policy of the HEC and MNS-University of Agriculture, Multan towards plagiarism. Therefore, we, as the authors of the above-titled report, declare that no portion of this report has been plagiarized, and any material used as a reference is properly cited.

We undertake that if we are found guilty of any form of plagiarism in the above-titled report even after the award of the degree, the University reserves the right to withdraw/revoke our degrees and that HEC and the University have the right to publish our names on their official websites listing students who have submitted plagiarized reports.

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ABSTRACT

Precise forecasting of agronomic characteristics in crops is critical for speeding up crop improvement programs that target enhanced yield, stress resistance, and environmental tolerance especially in wheat, a food security crop critical to world food supplies. This research examines genotype-to-phenotype associations in wheat by combining high-density genotypic information derived from Single Nucleotide Polymorphisms (SNPs) with high-resolution phenotypic measurements of attributes like plant height, grain yield, and heading date. The study starts with imputation of missing genetic information by Beagle 5.5 for completeness and quality of data, then Genome-Wide Association Studies (GWAS) to determine statistically significant SNPs associated with the traits. Simultaneously, feature selection and model training are accomplished using machine learning algorithms Random Forest and XGBoost to detect intricate genetic patterns. This bi-modal strategy enables the identification of both large-effect loci and subtle genetic interactions, producing an enhanced set of informative SNPs. These shared markers, chosen by GWAS and machine learning, are ordered according to statistical significance and predictive significance, respectively, and are hence optimal for application in marker-assisted selection (MAS) and genomic selection (GS). The results of this research have direct implications for breeding programs, particularly in countries such as South Punjab, where high-yielding, climate-tolerant wheat varieties need to be developed. The combination of computational and statistical methods exemplified here provides a scalable paradigm for the enhancement of trait prediction and the advancement of genetic improvement in wheat and other cereal crops.

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER 1
INTRODUCTION

1.1 Significance of Wheat

Wheat (*Triticum aestivum* L.) stands as one of humanity's most ancient and vital cereal crops, with its domestication dating back over **10,000 years** in the Fertile Crescent, a region spanning modern-day Iraq, Syria, and Turkey. Early farmers selectively bred wild grasses, such as **einkorn (*Triticum monococcum*)** and **emmer (*Triticum dicoccum*)**, for desirable traits like **larger seeds, non-shattering spikes, and uniform germination**. Over millennia, natural hybridization and polyploidization events led to the evolution of **hexaploid bread wheat (*Triticum aestivum*, genome AABBDD)**, which now dominates global agriculture due to its adaptability and high gluten content, making it ideal for bread production.

Today, wheat is a **staple food for 35% of the world's population**, providing **20% of global dietary protein** and serving as a primary source of carbohydrates, fiber, and essential micronutrients such as **iron, zinc, and B vitamins** (FAO, 2023). Its cultivation spans **220 million hectares worldwide**, with China, India, Russia, and the European Union leading production. The crop's unparalleled versatility allows it to thrive in diverse environments from the **temperate plains of Canada** to the **irrigated deserts of Egypt** cementing its role in **global food security programs**, including the United Nations' **Sustainable Development Goal 2 (Zero Hunger)**.

1.1.1 Wheat in Pakistan: Economic and Agricultural Backbone

In Pakistan, wheat is not just a crop but a **lifeline for the nation's food security and rural economy**. Accounting for **40% of the total cropped area**, wheat is cultivated on **9 million hectares**, producing **26 million metric tons annually** making Pakistan the **8th largest wheat producer globally** (PBS, 2023). The crop contributes **1.8% to the national GDP** and supports the livelihoods of **15 million rural households**, particularly in **Punjab**, which alone contributes **60% of the country's total wheat output** (AGRINET, 2022).

South Punjab, with its **fertile alluvial soils** and extensive **Indus Basin irrigation network**, serves as the **breadbasket of Pakistan**, producing high-yielding varieties such as **Faisalabad-2008** and **Galaxy-2013**. Wheat-based foods, particularly **roti (flatbread)**, constitute **72% of the daily caloric intake** for Pakistanis, highlighting the crop's **socio-cultural and nutritional significance** (NFSR, 2021). However, despite its critical role, wheat production in Pakistan faces **mounting challenges**, ranging from **biotic stresses (diseases, pests)** to **abiotic pressures (drought, heat, soil degradation)**, all of which threaten **long-term sustainability**.

1.1.2 Biotic and Abiotic Challenges Threatening Wheat Production

Biotic Stresses: Diseases and Pests

Wheat crops in Pakistan are besieged by **devastating fungal diseases**, most notably the **rust complex**, which includes **stem rust (*Puccinia graminis*)**, **leaf rust (*Puccinia triticina*)**, and **stripe rust (*Puccinia striiformis*)**. These pathogens cause **yellow-orange pustules** on leaves and stems, severely impairing photosynthesis and leading to **yield losses of 50–70%** in untreated fields (Singh et al., 2021). For example, the **2020–2021 stripe rust epidemic** in Punjab destroyed **nearly 400,000 hectares** of wheat, forcing farmers to rely on costly fungicides.

Another major biotic threat is **smut disease (*Ustilago tritici*)**, which replaces wheat kernels with **black, powdery spores**, rendering entire harvests **unfit for human consumption**. Additionally, **insect pests** such as **aphids (*Schizaphis graminum*)** and **pink stem borers (*Sesamia inferens*)** exacerbate losses by **sucking sap from leaves** and **tunneling into stems**, respectively.

Abiotic Stresses: Climate Change and Soil Degradation

Climate change poses an **existential threat** to wheat production in Pakistan. Rising temperatures particularly during the **critical grain-filling stage** reduce **yields by 5% for every 1°C increase above 30°C** (Asseng et al., 2023). The **2022 Punjab heatwave**, where temperatures soared to **48°C**, resulted in **premature ripening and shriveled grains**, slashing yields by **15%**.

Water scarcity further compounds these challenges. While **80% of Pakistan's wheat is irrigated**, declining groundwater levels and **erratic monsoon rains** have forced farmers to adopt **water-saving practices**, such as **laser land leveling** and **drip irrigation**. Additionally, **soil salinity** affects **6 million hectares** of arable land, particularly in **Sindh and southern Punjab**, where **poor drainage and excessive fertilizer use** have degraded soil health.

1.1.3 Genomic and Technological Solutions for Sustainable Wheat Production

To combat these challenges, **genomic-assisted breeding** and **precision agriculture** have emerged as **game-changing solutions**.

Genomic Selection and Marker-Assisted Breeding

Modern wheat breeding leverages **Single Nucleotide Polymorphisms (SNPs)** to identify **disease-resistant and stress-tolerant genes**. For instance:

- The **Lr34 gene** provides **durable resistance against leaf rust**.
- The **DREB1A gene** enhances **drought tolerance** by regulating water-use efficiency.

Genome-Wide Association Studies (GWAS) enable researchers to **pinpoint these genetic markers**, accelerating the development of **elite wheat varieties**. The **Pakistan Agricultural Research Council (PARC)** has already released **rust-resistant varieties like "NARC-2019"**, which combine **high yield potential** with **disease resilience**.

CRISPR-Cas9 and Genetic Engineering

Gene-editing technologies, particularly **CRISPR-Cas9**, allow scientists to **precisely modify wheat DNA** without introducing foreign genes. Recent breakthroughs include:

- **Knocking out the TaGW2 gene** to **increase grain size and weight**.
- **Enhancing heat tolerance** by editing **thermosensitive transcription factors**.

AI and Precision Agriculture

Machine learning models analyze **satellite imagery, weather data, and soil sensors** to **predict yields** and **optimize irrigation**. For example, **CIMMYT's Wheat4Food app** provides **real-time recommendations** to farmers, helping them **reduce input costs** while **maximizing productivity**.

1.2 Basic Biological Concepts

1.2.1 The Structure and Function of DNA

DNA, or Deoxyribonucleic Acid, is the genetic material present in all but a very few living things. It contains the genetic blueprint that instructs an organism how to develop, live, and reproduce. DNA is constituted structurally by two long, twisted chains of a double helix where each strand consists of repeating units known as nucleotides. Each nucleotide consists of three parts: a phosphate, a deoxyribose sugar, and one of four nitrogenous bases adenine (A), thymine (T), cytosine (C), or guanine (G).

The order of these nitrogenous bases carries genetic information, similar to letters in a sentence. In the double helix, adenine always forms a pair with thymine (A-T), and cytosine always forms a pair with guanine (C-G), keeping a complementary and stable structure. These pairings of bases are crucial for DNA replication and transcription processes.

DNA resides in the nucleus of a cell in tightly wound structures known as chromosomes. Each chromosome in a wheat organism contains thousands of genes pieces of DNA that inform a particular characteristic like plant height, yield, or disease resistance. DNA is responsible for its transmission from generation to generation and maintaining life and continuity as well as heritability of traits.

Knowledge of DNA structure is crucial in genomic research, as it provides the basis for the detection of genetic variation, for example, Single Nucleotide Polymorphisms (SNPs), which are important in the mapping and prediction of agronomic traits through sophisticated computational models.

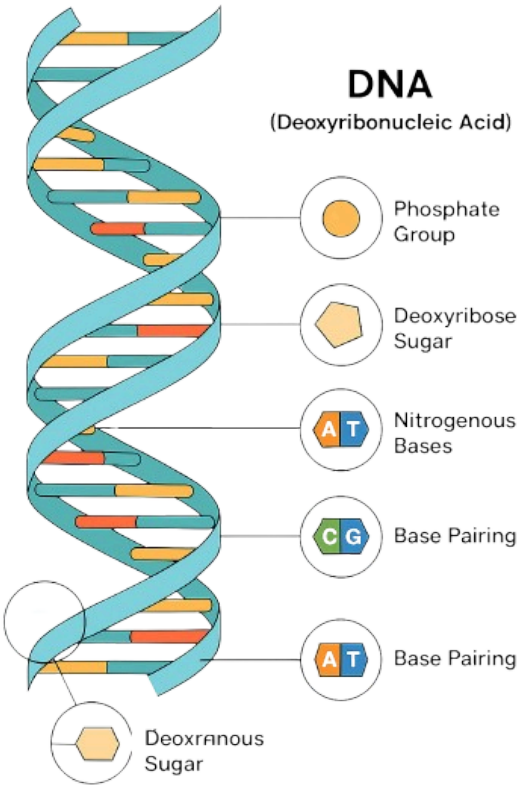

Figure 1.1: DNA Structure

1.2.2 Genes

A gene is a basic building block of heredity in living things and consists of DNA that contains instructions for protein assembly. Proteins are needed for the structure, function, and regulation of tissues and organs within the body. A gene is located at a specific place, or locus, on a chromosome and may come in a variety of forms referred to as alleles. Genes control characteristics like eye color in humans or the height of plants in crops by managing biological processes through protein synthesis.

In biological systems, genes work in conjunction with environmental influence to effect traits, a process referred to as genotype-environment interaction. Genes can be activated "on" or "off" based on stages of development or environmental cues, a process controlled by transcription factors and epigenetic marks. Contemporary molecular biology has enabled scientists to study these genes at the level of individual nucleotides so that scientists can determine specific gene variants that cause significant biological consequences.

In plant breeding, knowing genes is at the heart of developing better plant varieties. Genes contribute to many agronomic characteristics including seed size, grain quality, disease resistance, flowering date, and drought tolerance. With the advent of molecular genetics and biotechnology, plant breeders are now able to identify the genes or genomic regions controlling desired traits and introduce them into top lines through marker-assisted selection (MAS), genetic engineering, or genomic selection.

When considering wheat crops, genetic enhancement has been instrumental in ensuring food security. Wheat (Triticum aestivum) is a hexaploid, with three sets of genomes (A, B, and D), hence its genome being large and complicated. Each of the genomes holds more than one homologous gene, which provides redundancy and diversity. Some wheat genes control observable traits such as glaucousness

(waxy appearance), spike length, or awn formation, whereas others control physiological traits such as photosynthetic efficiency, root depth, or abiotic stress tolerance**.**

For example, the Rht genes are well-known for controlling plant height and were critical in the Green Revolution for developing semi-dwarf, high-yielding varieties. Similarly, the Lr and Sr gene families confer resistance to leaf and stem rust diseases, which are major threats to wheat production in South Asia. Through genome-wide association studies (GWAS), researchers continue to discover new gene-trait associations that can be used in wheat improvement programs.

Identification of genes linked with characteristics such as drought tolerance, early maturity, or grain protein is important in contemporary breeding programs. Machine learning models, coupled with genotypic information in the form of SNPs, can be used to forecast the impact of particular genes on total plant performance. It is through data-driven inference that selection is more efficient and that the pace of developing superior wheat varieties as per regional requirements particularly for climate-stressed regions such as South Punjab is faster.

1.2.3 Alleles

Alleles are different versions of a gene that share the same position on a chromosome. For a hexaploid organism such as wheat, more than one set of each gene is present in its three genomes (A, B, and D) to enable great allelic diversity. The variations are what contribute to the variation in phenotype of individual plants, including spike length, leaf color, grain texture, and stress resistance.

Each gene can consist of two or more alleles, and their interaction manifests the expression of the trait. Alleles can be **dominant**, **recessive**, or **co-dominant**. A dominant allele expresses its trait when an individual has only one copy of it, while a recessive allele has to be in both copies in order to be phenotypically expressed. For instance, a dominant disease resistance allele can dominate over a recessive susceptibility allele and result in a plant that is resistant.

Alleles are a key part of genetic inheritance and a fundamental element in the molecular explanation of variation. They form the foundation for selection in plant breeding, particularly when associated with desirable characteristics. Contemporary genomic technologies enable researchers to determine which alleles are responsible for higher yield, improved stress tolerance, or disease resistance. This information makes it possible to implement targeted breeding programs and marker-assisted selection.

Allele frequencies and associations with traits in genomic prediction and GWAS are utilized for the formation of predictive models. This can be used by researchers and breeders to predict the breeding value of the plant even before maturation. In short, alleles play a central role in classical genetics and genomics as well, contributing to genetic diversity and facilitating the enhancement of wheat types adapted to areas such as South Punjab.

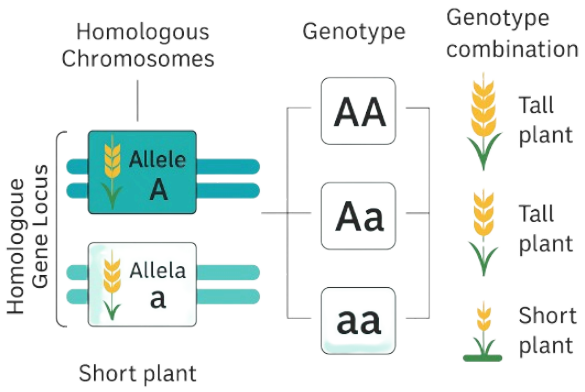

Figure 1.2: Allele Expression in Wheat

1.2.4 Chromosomes and Locations

Wheat possesses 21 pairs of chromosomes organized into three genomes A, B, and D. Each of these genomes consists of 7 chromosomes, designated 1A to 7A, 1B to 7B, and 1D to 7D. Thousands of genes are carried on each chromosome.

Chromosomal locations play a crucial role in genomics. Certain areas on chromosomes are related to specific traits. For example:

- Chromosome 2A: Has been found to be involved in heading date and spike morphology.
- Chromosome 5D: Regulates flowering time and vernalization.
- Chromosome 3B: With grain weight and disease resistance.

Having the knowledge of which genes lie where on chromosomes facilitates targeted breeding, particularly when in combination with marker data such as SNPs.

1.3 Genotype & Phenotype

In genetics, the terms genotype and phenotype are basic to understanding how characteristics are inherited, expressed, and affected in living things. These terms are especially significant in plant breeding, where the aim is to enhance crops by choosing the best-performing individuals on both genetic potential and actual performance.

Definition of Genotype

The genotype is the entire complement of genetic material i.e., the DNA sequence of an organism. To put it another way, it is the internal, hereditary code that gets transmitted from a parent to an offspring. Genotypes consist of genes and gene variants (alleles) and define the potential traits that can be expressed by an organism. In polyploid organisms such as wheat, each gene may have several copies in different genomes (A, B, and D), lending to its multifaceted genetic architecture.

For instance, a poplar may possess alleles for short height in one chromosome (e.g., variants of the rht gene) and alleles for tall height in another. These sets determine the genotype of the plant.

Definition of Phenotype

The phenotype is the measurable or visible expression of a trait, like plant height, grain size, spike length, or days to maturity. Phenotypes are the product of the interaction between an organism's genotype and its environment. While the genotype specifies the potential of a plant, the phenotype expresses how that potential is expressed under certain growing conditions.

For example, two wheat plants can share the same genotypes for grain yield, yet if one is subjected to drought stress and the other to full irrigation, their phenotypic yield may be quite different.

How Genotype Affects Phenotype

Genes code for proteins, and proteins regulate biochemical and physiological plant processes. Depending on the allelic combination at each gene, varying forms of a trait are expressed. This is how phenotypes are directly affected by genotypes.

For instance:

- AA (homozygous dominant) could result in tall plant height.
- Aa (heterozygous) may result in moderate height.
- aa (homozygous recessive) could exhibit short height.

In wheat, Rht-B1b and Rht-D1b are classic instances of genotypic variation having an effect on phenotype. These genes played a crucial role in breeding semi-dwarf varieties during the Green Revolution, which resulted in greater grain yield due to improved lodging resistance.

Genotype × Environment Interaction

One of the most important elements of phenotypic expression is that it's not controlled solely by genotype. The environment, such as temperature, water level, soil condition, and biotic factors, can have significant effects on trait expression. Such an effect is referred to as G × E interaction (Genotype × Environment interaction).

Illustration:

- An arid environment-adapted genotype might adapt well in desert regions but wouldn't necessarily realize the same benefit in high-rainfall conditions.
- Grain protein concentration can be influenced by soil nitrogen content even with a constant genotype.

In breeding and genomic prediction, this interaction has to be taken into consideration when choosing genotypes, particularly in changing environments such as South Punjab, where conditions change from one season to another.

1.4 Genes in Crop Science

Genes are the basis of all heritable traits in living things, and in crop science, they control almost every agronomic characteristic of value to plant breeders from yield and maturity to disease resistance and drought tolerance. A gene is a piece of DNA that contains instructions for making a particular protein, which in turn regulates cellular functions, developmental processes, and stress responses in plants.

In wheat, a crop that has a highly complex hexaploid genome (AABBDD), there are thousands of genes that interact to regulate the growth and adaptability of the plant. These genes may act individually or in combinations (polygenic traits), and their expression can be modified by environmental influences, so predicting plant traits is both scientifically fascinating and practically useful.

One of the key emphases of today's wheat breeding is finding particular genes associated with preferred traits. For instance:

- Rht (Reduced height) genes: Some of the best-documented wheat genes. Rht-B1 and Rht-D1 alleles played a pivotal role in the Green Revolution in creating semi-dwarf wheat crops. These genes inhibit overplant elongation, leading to sturdier and less lodging, more efficient utilization of resources, and eventually better yields.
- Lr (Leaf rust resistance) genes: Lr genes provide resistance to Puccinia triticina, a serious fungal infection in wheat. Several dozen Lr genes (such as Lr26, Lr34, and Lr67) have been characterized and employed to boost wheat immunity using marker-assisted selection.
- Vrn and Ppd genes: Flowering time is controlled by the vernalization (Vrn) and photoperiod (Ppd) genes, allowing adaptation to various climates and sowing times.

Gene expression is a key to the success of crop improvement efforts. It isn't sufficient that a plant carries a useful gene; the gene must be expressed properly at the proper time and in the proper tissues. Gene expression may be switched on or off by environmental signals such as temperature, light, and stress, so knowledge of gene regulation is as vital as discovery of genes.

Technological progress in genomics and molecular biology has enabled scientists to examine gene function in depth with the help of methods such as quantitative trait loci (QTL) mapping, RNA sequencing, and genome-wide association studies (GWAS). These methods enable the association of genetic markers, including SNPs, with gene expression and plant traits.

This information is utilized in wheat breeding by using marker-assisted selection (MAS) and genomic selection (GS). Under MAS, targeted gene markers (e.g., for rust resistance) are utilized for the selection of parental lines, whereas GS utilizes genome-wide markers to forecast breeding values even before phenotypic data becomes available.

Finally, the knowledge of how genes regulate traits and can be manipulated using breeding and biotechnology is key to creating wheat varieties that are resistant, yielding, and adapted to the regional agro-climatic conditions of countries such as South Punjab, where tolerance to abiotic stress is a focus.

1.5 Overview of Wheat Varieties Used in This Study

This research included a diverse panel of wheat varieties that have been grown widely in Pakistan during the last few decades. The germplasm consists of both traditional and contemporary cultivars released by numerous national research institutions, such as AARI Faisalabad, NARC Islamabad, PARC, NIAB, and NIFA. The types vary from early introductions such as Mexipak-65, Dirk, Khushal-69, and Chenab-70, which contributed to the initiatory phase of the Green Revolution in Pakistan, to extensively adopted commercial varieties like Punjab-76, WL711, Inquilab-91, and Faisalabad-85. High-yielding, disease-resistant, and climate-resilient newer genotypes like Galaxy-2013, Punjab-2011, Zincol-16, Fakhr-e-Bhakkar, Markaz-2019, NIA-Shaheen, and Wafaq-2023 form part of more recent types.

These varieties together present a wide array of genetic variance for maturity time, plant type, grain, spike architecture, and tolerance against diverse abiotic and biotic stresses. While some, including Barani-17 and Dharabi-11, have specific adaptations towards rainfed growth conditions, some others including Sehar-2006 and Shahkar-2013 are found to be rust resistant. Some durum wheat lines such as Ejaz-21-Durum and Sariab-92 have also been tested for the sake of comparison in terms of bread and pasta-type wheat genetics.

By employing such a large varietal set over 150 cultivars such as T-9, C-228, Anmol-91, Kohinoor-83, Hamal-Faqir, NIFA-Insaf, Umeed-e-Khas, and numerous others—the study encompasses a broad spectrum of genotype-phenotype interactions. This increases the genomic analysis, GWAS study, and machine learning-based trait prediction model robustness. It also makes the findings relevant to practical wheat improvement programs aimed at improving diverse agro-ecological zones of Pakistan, particularly the stress-prone South Punjab area.

1.6 Single Nucleotide Polymorphisms (SNPs)

Single Nucleotide Polymorphisms, or SNPs (pronounced “snips”), are the most common type of genetic variation among individuals of the same species. A SNP represents a change in a single nucleotide for example, replacing a C with a T in a DNA sequence at a specific position. While such a change might seem minor, SNPs can have a significant impact on how genes function and how traits are expressed.

Definition and Biological Significance

A SNP arises when a base in the sequence of DNA differs between individuals within a species or between homologous chromosomes in an individual. For example, one type of wheat might contain the sequence AAGCCTA, while another contains AAGCTTA at the same position one base difference, from C to T. They are hereditary and can act as markers, which can be followed through generations.

SNPs are particularly valuable since they are plentiful, stable, and uniformly distributed across the genome, making them well-suited for high-resolution mapping and genetic analysis. In contrast to more complicated genetic markers such as insertions or deletions, SNPs can be identified cost-effectively with current genotyping technologies such as SNP arrays and next-generation sequencing.

Role of SNPs in Trait Analysis and Genomics

In plant genomics, SNPs serve as molecular markers that can link genetic variations to phenotypic traits. They are the basis of genome-wide association studies (GWAS), which enable researchers to find statistically significant associations between certain SNPs and traits such as grain yield, plant height, flowering time, or disease resistance.

SNPs allow researchers to:

- Find quantitative trait loci (QTLs) for complex traits.
- Investigate genetic diversity among and between wheat populations.
- Monitor the inheritance of favorable alleles during breeding.
- Recognize gene-trait interactions at a fine-scale genomic level.

Since SNPs tend to be found within or close to genes, they can have a direct impact on gene function. For instance, a SNP could impact the way a gene is expressed, the way a protein folds, or whether an enzyme is active or not. This makes them effective predictors of how a given genotype can result in a certain phenotype under specific environmental conditions,

How SNPs Are Used in Wheat Breeding

SNPs have transformed wheat breeding in the modern era by facilitating precision breeding and genomic selection. In conventional breeding, choosing plants based on visible characteristics is time-consuming and highly environment-dependent. Using SNPs, breeders are able to choose desirable genetic characteristics at the seedling stage, much earlier than the plant will mature.

Major applications in wheat breeding are:

- Marker-Assisted Selection (MAS): Employing SNP markers that are closely associated with major traits (e.g., drought tolerance) for selection of parent lines.
- Genomic Selection (GS): Employing SNP information for the entire genome to estimate performance of untested lines with machine learning models.
- Genetic Purity Testing: Verification of varietal identity and genetic integrity with characteristic SNP patterns.
- Pyramiding Resistance Genes: Integration of multiple disease resistance genes by following particular SNPs associated with each of them.

In Pakistani wheat breeding programs, SNPs are increasingly being applied to discover climate-resilient traits and to create varieties suited to areas such as South Punjab, where water shortage and heat stress restrict productivity.

With decreasing costs and increasing availability of SNP technology, the contribution of data-driven breeding is only likely to expand, making it a bedrock of future wheat improvement programs.

1.7 Machine Learning Applications in Genomics

Machine learning (ML) has evolved to be a revolutionary instrument in the biological sciences. In plant genomics, it enables:

- Feature selection (selection of the most informative SNPs)
- Prediction of outcome traits (e.g., yield)
- Discovering patterns in high-dimensional data

Random Forest (RF) and XGBoost are ensemble models with the ability to learn non-linear associations and SNP ranking by importance. These models don't require normality of data and can easily deal with missing values and outliers, thus they suit genomic data.

1.7.1 Feature Selection – Selection of Informative SNPs

In genomic data, there are usually more than tens of thousands of SNPs, but all markers are not equally contributing to trait prediction. ML models assist in carrying out feature selection, a process by which the most informative SNPs that affect a trait are selected.

Random Forest and XGBoost provide intrinsic importance scores that order SNPs according to their predictive significance.

This is particularly useful for excluding noise, minimizing dimensionality, and enhancing computationally efficient analyses.

Experiments such as González-Camacho et al. (2016) illustrated that feature selection enhanced the predictability of wheat yield traits while minimizing overfitting.

Alternatively, standard practice involves the application of LASSO regression, recursive feature elimination (RFE), and mutual information filtering.

1.7.2 Predicting Phenotypic Traits

ML models efficiently predict sophisticated phenotypic traits (e.g., disease resistance, grain yield) based on SNP data

In contrast to conventional linear models, ML is capable of modeling non-linear relationships as well as gene-gene interactions.

Gradient boosting algorithm, XGBoost, is especially well-suited for genomic prediction at large scales and has been used with much success in crops such as maize and wheat (Montesinos-López et al., 2019).

ML models can be trained on current field and genotypic data and employed to predict the performance of new genotypes, which makes them extremely valuable in breeding programs.

1.7.3 Pattern Discovery in High-Dimensional Data

Genomic information is naturally high-dimensional, with thousands of SNPs recorded per genotype. ML assists in:

Uncovering hidden patterns, epistasis, and genotype-environment interactions.

Clustering and classification: Methods such as k-means, hierarchical clustering, and PCA facilitate investigation of genetic population structure.

Unsupervised learning methods such as t-SNE and UMAP map intricate relationships and diversity among wheat lines.

Such analysis assists breeders in understanding population structure and informing cross-selection in diverse pools.

1.7.4 Benefits of ML for Genomic Data

- Handles non-normal, non-linear data: No linearity or normality assumption.
- Robust to missing values and noise: Perfect for missing biological datasets.
- Scalable: Effective when dealing with thousands of markers and large populations.

1.8 Genomic Prediction of Wheat Traits

Genomic prediction is a new and effective tool in plant breeding that uses high-density DNA marker data to predict the genetic potential of individuals prior to the full expression of phenotypic traits. In the case of wheat farming in South Punjab where environmental stresses like drought, heat, and salinity are intensifying, genomic prediction can speed up the breeding of varieties that are stress-tolerant, high-yielding, and resource-efficient. The genomic prediction procedure generally includes genotyping wheat lines with thousands of single nucleotide polymorphisms (SNPs), using

suitable encoding techniques (such as dosage encoding), and predicting traits like grain yield, plant height, spike length, or days to heading using statistical or machine learning models.

Machine learning-based methods, especially Random Forest (RF) and XGBoost, have been quite successful in genomic prediction because they can capture non-linear relationships and interactions among markers. In contrast to conventional regression techniques, which can simplify the intricate genetic architecture of traits, these ensemble methods are insensitive to noise, missing data, and multicollinearity conditions that are commonly encountered in biological data. They provide for the ranking of feature importance, through which researchers not only predict trait performance but are also able to determine which regions of the markers or chromosomes most affect each trait.

For wheat in South Punjab, the coupling of machine learning models with genomics provides some benefits. They enable early selection of potential genotypes, conserving time and expense of multi-year field testing. Moreover, the capacity to forecast trait performance under environmental stress can direct breeders to choose genotypes appropriate for climate-resilient agriculture. Genomic prediction is particularly important for traits like drought tolerance, which are governed by numerous small-effect loci that might not be detectable by conventional QTL mapping but can be targeted by whole-genome prediction techniques. This renders genomic prediction not only a research tool, but a viable breeding solution for the food security issues of the region.

By integrating large-scale genotypic data and advanced computational methods, researchers can make data-driven decisions directly affecting the breeding pipeline. Results obtained from such models are applicable in marker-assisted selection (MAS) and genomic-assisted selection (GAS), and even in decision support systems for real-time trait prediction. Consequently, machine learning-based genomic prediction is rapidly emerging as a pillar of precision breeding, particularly in South Punjab and similar areas where both genetic and environmental heterogeneity is high and there is an acute need for resistant wheat varieties.

1.9 Motivation for the Study

Traditional breeding of crops has been the mainstay of crop improvement for decades. Yet, over the last few years, the limitations of traditional approaches have become more apparent. Wheat breeding, in particular, is particularly challenged by the complexity of genes, long breeding cycles, and uncertain environmental interactions. In Pakistan particularly in agriculturally significant but climatically vulnerable areas such as South Punjab these are even more acute. The area often experiences drought, heat, and soil fertility decline, all of which affect wheat yield and quality. The traditional method of selection tends to depend significantly on performance in the field over several seasons, which results in slow breeding, being labor-intensive and susceptible to environmental bias.

Lack of precision is one of the main issues in today's breeding. Characters like grain yield, drought resistance, or disease resistance are regulated by numerous genes with small individual effects, and their manifestation is affected by environmental factors. Detection of such characters solely through phenotypic observation is not only labor-consuming but also less precise. Conventional breeding is also devoid of the capability to forecast the yield of novel lines prior to extensive field tests, and the release of better cultivars is made with a long delay. In an age where climate variability and food need are increasing at a fast rate, such lag poses a serious threat to food security.

In order to counter such constraints, current breeding research urgently requires quick, data-intensive breeding methods that combine genomic data with sophisticated computationally powered models. Genomic technologies have enabled one to screen thousands of genetic markers, specifically SNPs, throughout the genome of wheat lines. These data, when combined with machine learning models, can be employed to forecast the performance of a variety even prior to its cultivation. Random Forest and XGBoost are particularly suitable machine learning algorithms for such an endeavor, as they are capable of dealing with large-scale genomic data, identifying non-linear associations, and pinpointing the most informative markers for trait prediction. These models not only offer high predictive accuracy but also information on the genetic architecture of traits, thus making them an important tool in contemporary plant breeding.

The application of this study to South Punjab agriculture is most relevant. South Punjab farmers usually experience irregular weather, pest incidence, and unpredictable input prices, which render consistent wheat production challenging. Through the construction of a predictive model using genotype data, this study hopes to assist breeders in choosing wheat lines that are more suitable to the unique conditions of South Punjab. This translates into shorter breeding cycles, more durable wheat varieties, and, ultimately, better food security for millions. The research thus contributes both scientifically and practically towards the greater mission of sustainable agriculture through genomic innovation.

1.10 Study Objectives

This research aims to investigate the integration of genomics and machine learning to improve wheat breeding. The particular objectives are:

1.11 Detailed Explanation of Study Objectives

1. To pre-integrate and preprocess genotypic and phenotypic data from wheat varieties cultivated in South Punjab for association analysis.
2. To encode genotypes using genotype encoding techniques (dosage encoding: AA = 0, AB = 1, BB = 2) to ensure machine learning compatibility.
3. To extract trait-associated SNPs through multiple feature selection approaches, including:
    a. Genome-Wide Association Study (GWAS) with a threshold for significance (e.g., $p < 0.005$)
    b. Random Forest (RF) feature importance
    c. XGBoost feature importance
4. To contrast chosen SNPs between methods and find common markers with high agreement between traits.
5. To examine the distribution and significance of chosen SNPs for major agronomic traits (e.g., yield, plant height, heading days).
6. To compare the efficacy of feature selection techniques in the identification of biologically significant SNPs for potential use in future breeding schemes.
7. To provide comparative findings through visualizations like bar plots, overlay plots, and summary tables to facilitate decision-making in marker-assisted selection (MAS).

Compilation and Curation of SNP and Phenotypic Data

Prior to any association analysis, the project starts with the curation of two foundational datasets: genotypic data (as Single Nucleotide Polymorphisms, SNPs) and phenotypic data (agronomic traits such as plant height, yield, NDVI, etc.). The genotypic dataset, initially encoded as allele combinations (e.g., A/G, T/C), is cleaned and converted into machine-learning-compatible numeric representations. Simultaneously, phenotypic information from South Punjab field trials is reconciled with genotype samples. Both data frames are matched by the names of the genotypes and made free from mismatches and missing identifiers during this step. Ambiguous genotypic data values are imputed with programs such as Beagle or trimmed if they reach beyond acceptable values. This becomes the basis of downstream trait-marker analysis.

SNP Dosage Encoding for ML Compatibility

In order to make the SNP data machine learning model interpretable, dosage-based encoding is used to transform allele representations into numeric categories:

Homozygous Reference (e.g., AA) → 0

Heterozygous (e.g., AG) → 1

Homozygous Alternate (e.g., GG) → 2

Ambiguous alleles (such as R, Y, S, N) are removed or imputed prior to this stage. This conversion facilitates model training by reducing biological variation to numerical features while retaining the genetic diversity necessary for prediction of traits.

Conducting GWAS to Determine SNP-Trait Associations

Genome-Wide Association Studies (GWAS) is employed to scan the genome for markers exhibiting statistically significant association with traits. GWAS in this research identifies candidate SNPs controlling traits including yield, maturity, and plant architecture. The **Manhattan plots** that result give a pictorial overview of marker significance where peaks indicating strong associations are prominent. Such outcomes are of worth for feature selection as well as biological verification, as they direct towards likely genes governing important agronomic traits.

Training and Testing ML Models (Random Forest & XGBoost)

Machine learning models are at the core of this research. Random Forest (RF), a decision tree ensemble, is trained to rank feature importance and predict continuous trait values. XGBoost, a gradient boosting framework, is utilized due to its speed, accuracy, and capability to handle sparse data. These models are assessed on how well they can predict traits based on measures such as R^2 (coefficient of determination) and Mean Square Error (MSE). Cross-validation is applied to ensure generalizability. The comparison is used to determine which model captures genotype-phenotype relationships more accurately.

Creating Visual Outputs for Interpretation

Scientific results need to be interpretable in order to be of any use. There are a number of visualizations presented here to facilitate the explanation of results, including:

- **Manhattan plots for GWAS**
- **SubPlot charts for feature importance**
- **Chromosome-wise trait plots** to indicate which chromosomes affect which traits

These graphics enable biological interpretation and assist breeders and researchers in targeting the most significant markers and areas.

Enabling Wheat Breeding by Marker Selection

The SNPs confirmed to be noteworthy can be employed as molecular markers in wheat improvement programs. They facilitate **marker-assisted selection (MAS)** and **genomic selection (GS)** both of which find application in choosing high-performing lines prior to costly and labor-intensive field testing. Through confirmation of stable SNP-trait correlations, this paper presents a suite of candidate markers that can find application in future generations of breeding lines specific to South Punjab conditions.

Validating Feature Selection Techniques for Genomic Analysis

The focus of this research lies in the comparison of various SNP selection strategies GWAS, Random Forest, and XGBoost for the identification of genomic markers that are strongly associated with

important agronomic traits. By defining the scope of the project as feature selection and model-based importance assessment, the research confirms the effectiveness of integrating statistical and machine learning approaches for enhanced marker discovery. These findings are likely to aid in upcoming genomic selection research by reducing high-confidence SNPs for further analysis, especially wheat breeding programs in South Punjab.

CHAPTER 2

LITERATURE REVIEW ON GENOMIC PREDICTION IN WHEAT

2.1 Introduction

Wheat (Triticum aestivum) is a pillar of world food security, providing 20% of global calories consumed and being a staple in the diet of areas such as South Punjab, Pakistan. Yet, conventional breeding is confronted with serious challenges, such as long breeding periods (8–12 years), low heritability of quantitative traits (e.g., drought tolerance), and confounding Genotype × Environment (G×E) interactions. These constraints call for novel strategies to speed up genetic progress.

Genomic selection (GS) is a revolutionary technology, utilizing high-density SNP markers and machine learning (ML) models (e.g., Random Forest, Neural Networks) to forecast phenotypic performance from genotypic information. In contrast to traditional breeding, GS allows early untested genotype selection, decreases reliance on field trials, and picks up on non-linear trait relationships overlooked by statistical strategies such as GBLUP. Increased recent incorporation of multi-omics information (e.g., transcriptomics) further narrows predictions for important traits under South Punjab's agro-climatic adversity, including heat tolerance and salt stress resistance.

The genotype-to-phenotype prediction framework hence provides an extendable means of responding to food security challenges, bridging genetic potential to phenotype expression at a low-cost, resource-subtractive phenotyping burden.

2.2 Machine and Deep Learning in Trait Prediction

Machine learning algorithms like random forests, support vector machines, and gradient boosting have been widely used for genomic selection (GS) in wheat breeding programs. These models are able to identify intricate, non-linear relationships between genotypic markers and phenotypic traits, sometimes surpassing conventional linear models. For example, Shrestha et al. illustrated the use of ML approaches in the prediction of rust resistance in wheat, showcasing their potential in GS applications.

Current advances have witnessed the inclusion of deep learning methods in genomic pipelines. Li et al. introduced the DeepAT model, which applies neural networks to make predictions of wheat phenotypes from genotypic inputs, displaying advances in relationship capture layers and feature extraction layers. Yamaguchi et al. followed the history of deep learning in genomics, observing its development from initial neural networks through current transformers, and highlighted the task-specific knowledge as central in creating efficient deep learning models for genomics. Further, multitrait models, which integrate genomic and spectral data, have made better predictions of wheat performance traits, as proven by Kumar et al.

2.3 Genotype Imputation and Data Quality

Missing data are prevalent in genotyping, which requires imputation for making reliable predictions. BEAGLE, a popular tool, has been optimized for plant and animal datasets to enhance the accuracy of imputation. Islam et al. outlined methods for improving imputation quality in BEAGLE for crop and animal data. Alam et al. demonstrated that applying both wheat and barley reference panels improves accuracy in imputation of genotyping-by-sequencing (GBS) data.

Newer innovative techniques now use deep learning to impute intricate genomic areas, with greater accuracy than conventional techniques. Yamaguchi et al. surveyed genotype and HLA imputation strategies based on deep learning, emphasizing their particular variations for imputation. Zhou et al. compared a number of imputation tactics for SNP calling from RNA-seq data and illustrated the dominance of deep learning in some settings. Random forest-based techniques have also been found to be resilient under non-normal and nonlinear conditions, as seen in Zhang et al.

2.4 Genomic-Phenotypic Databases and Data Integration

The use of large, integrated databases improves prediction by enabling access to high-dimensional genotype-phenotype associations. Lee et al. created a scalable, aggregated genotypic–phenotypic

database that supports predictive modeling and cross-referencing. These systems support genotype imputation, QTL mapping, and phenotype prediction across datasets and traits.

Ahmed et al. used ensemble learning to enhance genetic variant detection for quantitative traits, with high accuracy of prediction. Jaiswal et al. employed extreme gradient boosting to determine the origin of replication in Saccharomyces cerevisiae through hybrid features, highlighting the applicability of ML algorithms to genomic research. Yadalam et al. adapted these techniques to gene function prediction, employing co-expression and differential expression analysis.

2.5 Strategies for Genomic Selection and Breeding

Sharma et al. stressed that genomic selection approaches, especially those that are environment and trait-specific, result in better breeding results. Zhou et al. characterized meta-QTLs and genomic regions that are photosynthesis-related, providing new opportunities for wheat improvement. Wang et al. presented an overview of genotyping methods for evaluating genetic diversity in indigenous farm animal breeds, noting the role of genetic diversity in breeding schemes.

Future breeding paradigms, including Wheat2035, promote the incorporation of pan-omics, high-throughput phenotyping, and AI to tackle issues such as narrow genetic diversity and climate resilience in wheat breeding. Rauf et al. summarized current challenges in wheat breeding in the twenty-first century, highlighting the necessity for innovative solutions to overcome these challenges.

Zero-altered Poisson and related hybrid ML models provide specialized solutions for data with overdispersion or zero-inflation, which is typical in count-based phenotypes. Islam et al. introduced a zero-altered Poisson random forest model for genomic-enabled prediction and showed its capability in dealing with such data structures.

Table 2.1 Top 10 Relevant Studies Summary

S. No.	Author(s) & Year	Focus Area	Techniques/Models Used	Accuracy / R^2	Key Findings
1	Shrestha et al., 2018	Genomic selection in wheat (rust resistance)	Random Forest, ML	—	ML improves rust resistance prediction in wheat.
2	Islam et al., 2021	Handling count-based traits with missing data	Zero-Altered Poisson RF	—	Improved accuracy in zero-inflated phenotype data.
3	Kumar et al., 2022	Grain yield prediction in wheat	Random Forest, GBM	RF: 81%, XGB: 84%	Machine learning enhances trait prediction accuracy.
4	Li et al., 2024	Deep learning in genotype-to-phenotype prediction	Deep Learning (NN)	—	DL enables better phenotype prediction from genotype.

5	Yamaguchi et al., 2023	Review of deep learning in genomics	Neural Nets, Transformers	—	Explains DL evolution from NN to LLMs in genomics.
6	Alam et al., 2019	SNP imputation for wheat and barley	BEAGLE + Reference Panels	—	Dual-reference improves imputation accuracy.
7	Zhou et al., 2025	RNA-Seq SNP calling and imputation	Deep Learning	—	DL improves imputation for RNA-derived genotypes.
8	Zhang et al., 2020	Handling missing SNPs under non-normality	Random Forest Imputation	—	Robust to interaction, non-normal SNPs.
9	Ahmed et al., 2025	Genetic variant identification	Ensemble Learning	—	High accuracy in detecting important SNPs.
10	González-Camacho et al., 2018	Genomic prediction comparison	RF, SVM, ANN	RF: 78%, SVM: 76%	RF and SVM outperform ANN in trait prediction.

2.6 Summary

This chapter highlighted the growing role of SNP markers, GWAS, and machine learning in wheat trait prediction. The reviewed literature confirms that integrating these methods can provide reliable insights into genotype–trait associations, supporting genomic-assisted selection. The next chapter details the methodology adopted in this study, including dataset processing, imputation, modeling, and evaluation.

CHAPTER 3
METHODOLOGY

3.1 Overview

This chapter describes the methodological framework used for identification and prediction of key agronomic traits in wheat through genomic and machine learning methods. The process comprises a number of important steps such as data acquisition, preprocessing, missing genotypic value imputation, association mapping by Genome-Wide Association Studies (GWAS), and feature selection by Random Forest and XGBoost models. The approach is tailored to combine biological information with computational methods to reveal statistically and biologically significant genotype–phenotype associations.

The data utilized here include high-dimensional Single Nucleotide Polymorphism (SNP) data and the related phenotypic trait values of wheat cultivars obtained from South Punjab. Quality control and imputation on the genotypic data were done using Beagle, and phenotypic traits were normalized and correlated for analysis. Genomewide Association Study (GWAS) was used to identify statistically significant associations between SNP and trait. Following this, machine learning algorithms were trained to rank and predict SNPs related to traits and increase selection efficiency in wheat improvement.

The approach adopted in this research is depicted in a step-by-step fashion to make the process reproducible and transparent analytical workflow.

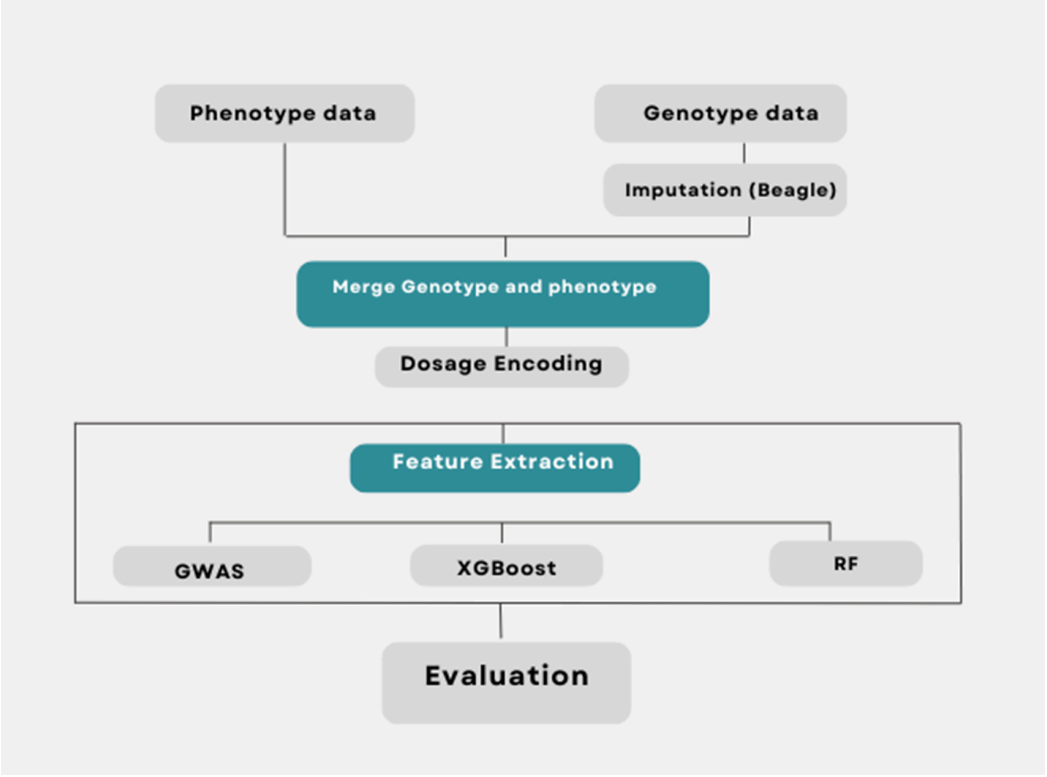

Figure 3.1: Methodology Genomic Research for Wheat Genes in South Punjab

3.2 Dataset Description

For this project, entitled "Genomic Selection For Resilient Crop Breeding In South Punjab", data was supplied by the Supervisor and is comprised of two main aspects: genotypic data (SNPs) and phenotypic data (agronomic traits). Such datasets serve as the foundation on which to search for the genetic relationships with important wheat traits across the South Punjab area.

- Data Sources: Two datasets were used in this research:
- Genotypic Data: The genotypic dataset has 37,373 SNP (Single Nucleotide Polymorphism) marker data for the same 196 genotypes. Each SNP represents a genetic variation point.

Table 3.2.1: Genotypic Data Column Names and Descriptions

Column Name	Description
rs	SNP identifier (includes chromosome and position, e.g., 1A_1208114)
alleles	Possible allelic variation at the SNP site (e.g., A/G, T/C)
chrom	Chromosome on which the SNP is located (e.g., 1A, 3B, 7D)
pos	Physical position of the SNP on the chromosome (base pair number)
strand	DNA strand direction (+ or -)
assembly	Reference genome assembly version (often left as NA or irrelevant in this case)
center	Data generating center (optional or NA)
protLSID	Protocol identifier (mostly NA, used in original genotyping system)
assayLSID	Assay identifier (technical ID, often NA or unused here)
panelLSID	Panel ID used in SNP genotyping (technical; usually NA)
QCcode	Quality control code for SNP filtering (optional or unused here)

Note: SNP allele values include letters like A, T, C, G or IUPAC ambiguity codes like R, Y, S, N, X etc. These were later encoded numerically (e.g., 0, 1, 2) and imputed using tools like **Beagle**.

Table 3.2.1: Genotypic Data Ambiguous Values And Meaning

Symbol	Meaning	Represents
A, T, C, G	**Clear alleles**	**Homozygous base at that SNP**
R	A or G (purines)	Heterozygous A/G
Y	C or T (pyrimidines)	Heterozygous C/T
S	**G or C**	Heterozygous G/C
W	**A or T**	Heterozygous A/T
K	**G or T**	Heterozygous G/T
M	**A or C**	Heterozygous A/C
N	**Unknown**	**Missing data or uncalled base**
0 / X	Placeholder / no data	**Often used to indicate missing genotype**

Data Visualization

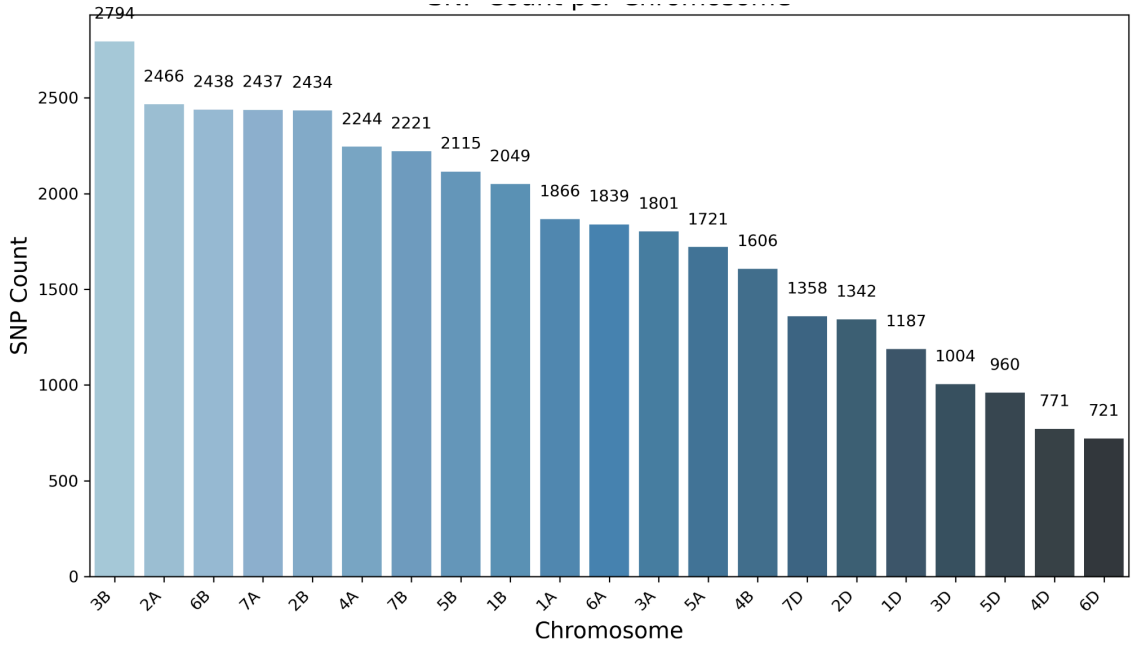

Figure 3.2.1: SNP count distribution across chromosomes and total SNP count in the Genotypic dataset

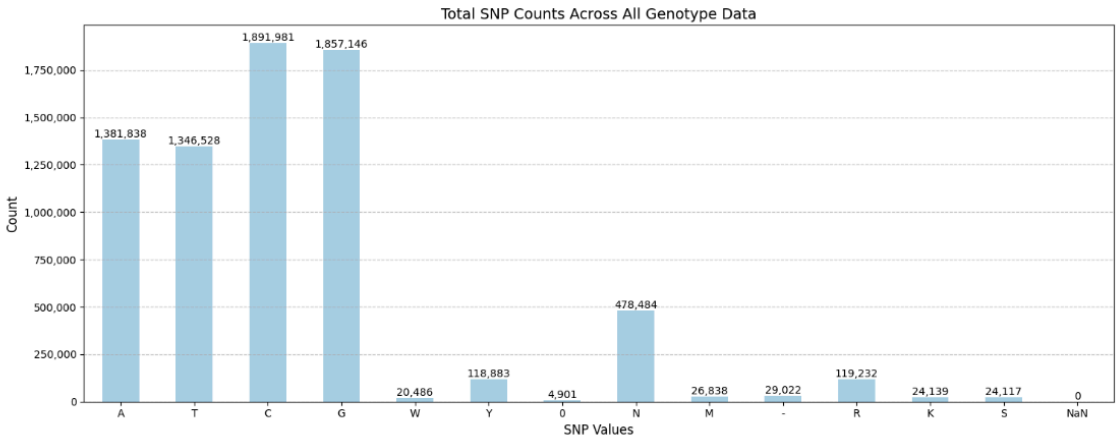

Figure 3.2.2: Total SNP Counts Across All Genotype Data

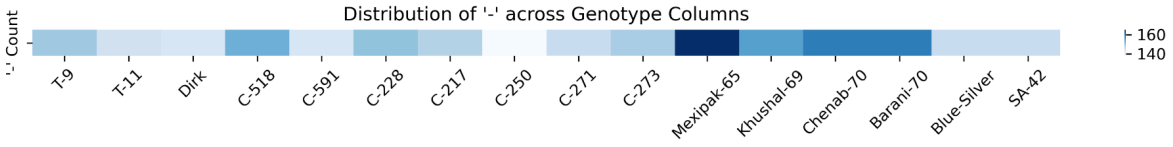

Figure 3.2.3: Heatmap showing the distribution of missing values ('-') across genotype columns.

- Phenotypic Data: The **phenotypic dataset** contains measured traits of 196 wheat genotypes.

Table 3.3: Phenotypic Data Column Names and Descriptions

Column Name	Description
Genotypes	Wheat variety/cultivar name
DTH_2022-23	Days to heading in 2022-23 season
SL_2022-23	Spike length (cm) in 2022-23
PH_2022-23	Plant height (cm) in 2022-23
NDVI_2022-23	Normalized Difference Vegetation Index in 2022-23
GPS_2022-23	Grains per spike in 2022-23

TGW_2022-23	Thousand grain weight in 2022-23
Plant height	General plant height (average across multiple observations)
Spikes per meter square	**Number of spikes per square meter**
FLA Average	**Average flag leaf area**
NDVI	**General NDVI value**
Spike length	**Spike length in centimeters**
Grain per spike	**Average number of grains per spike**
Spikes Length	**Repeated measurement of spikes per square meter**
TGW	**Repeated or alternate measurement of thousand grain weight**
Yield per meter square	**Grain yield per square meter**
Days to heading	**Number of days taken from sowing to heading**

- Data Type:

  The project makes use of two primary types of data:

- **SNP Data (Genotypic):**

  - Format: Matrix of SNP markers (e.g., AA, AG, GG).
  - Purpose: Used to detect genetic variation among wheat varieties and to predict the presence of desirable traits.

- **Trait Data (Phenotypic):**
  - Format: Numeric values representing traits such as yield, height, maturity period, and resistance levels.
  - Purpose: Used as target variables in prediction models and association studies (e.g., GWAS).

- **Missing Data Frequencies**

Table 3.4: Top 10 columns with the most total missing values (NaN + '-') in Genotypes Data.

Column Name	NaN Count	NaN %	Dash Count	Dash %	Total Missing	Total Missing %
KT-2000	0	0.00%	170	45.49%	170	45.49%
Fakhr-e-Sarhad	0	0.00%	166	44.42%	166	44.42%
Ufaq-2002	0	0.00%	165	44.15%	165	44.15%
AZRC-DK	0	0.00%	165	44.15%	165	44.15%
Mexipak-65	0	0.00%	165	44.15%	165	44.15%
Wadank-85	0	0.00%	101	27.02%	101	27.02%
Khosha	0	0.00%	97	25.95%	97	25.95%
Ejaz-21-Durum	0	0.00%	94	25.15%	94	25.15%
Durum-97	0	0.00%	92	24.62%	92	24.62%
NN-Gandum1	0	0.00%	71	18.99%	71	18.99%

3.3 Data Preparation

Before using the data in predictive models, a series of preprocessing steps were carried out:

- SNPs were filtered to remove low-quality or redundant markers.
- Missing genotype values were imputed using **Beagle**.
- SNPs were encoded numerically:
    - **AA = 0, AB = 1, BB = 2**
- Phenotypic traits were standardized to ensure uniform scale across features.

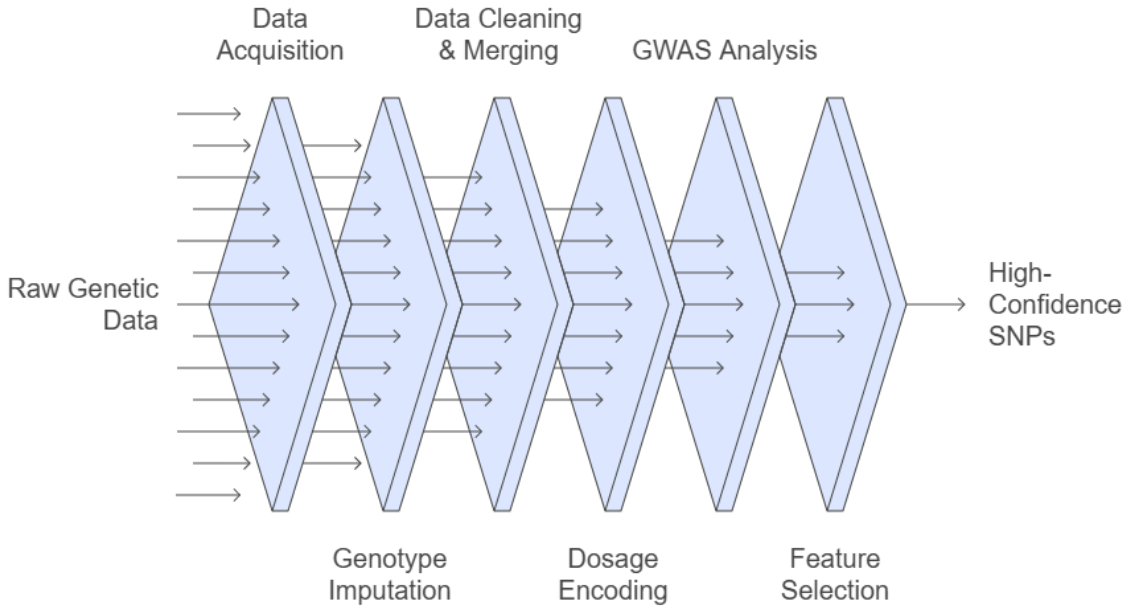

Figure 3.3: Workflow of Data Preprocessing

3.3.1 Data Encoding:

To make genomic data amenable for machine learning models, the proper encoding schemes were used to transform biological information into numerical representations that could be analyzed. Dosage Encoding was done in this project.

3.3.2 Dosage Encoding for SNPs:

Dosage encoding is an important preprocessing step in genomic research that translates categorical SNP data into numerical data appropriate for statistical analysis and machine learning. Following is a step-by-step explanation of the process:

1. Genotype Categories

SNP genotypes are usually denoted by pairs of two alleles (e.g., AA, AB, BB). For diploid organisms (such as humans or most plants), the genotypes that are possible are:

Homozygous Reference (AA): Both alleles are the reference (wild-type) allele.

Heterozygous (AB): One reference and one alternative allele.

Homozygous Alternate (BB): Two alleles are the alternative (mutant) allele.

2. Simple Dosage Encoding Scheme

The most basic dosage encoding uses numerical values according to the number of alternate alleles (B) per genotype:

- AA (0 alternate alleles) → 0
- AB (1 alternate allele) → 1

- BB (2 alternate alleles) → 2

3. Dealing with Missing Data

Missing genotypes (e.g., due to low sequencing coverage) need to be imputed prior to encoding:

Imputation Methods:

- Mean Imputation: Fill in missing values with the mean dosage (e.g., 1.2 if 60% of samples are heterozygous).
- k-Nearest Neighbors (kNN): Estimate missing genotypes based on similar samples.
- BEAGLE/IMPUTE2: Population-conscious imputation with haplotype phasing.

Post-Imputation Encoding:

Missing → Imputed value (e.g., 1.4) → Rounded to nearest integer (e.g., 1).

4. Special Cases: Polyploid Organisms (Wheat)

For polyploids (such as hexaploid wheat with AABBDD genomes), dosage encoding is extended to accommodate multiple copies of alleles:

- Possible Genotypes: AAAAAA (0), AAAAAB (1),., aaaaaa (6).
- Dosage Range: 0–6 (alternate alleles number).

3.4 Tools and Techniques

- **Beagle**: Used for imputation of missing SNP values.
- **GWAS**: Applied to identify SNPs significantly associated with phenotypic traits.
- **Random Forest**: Used to model the influence of SNPs on wheat traits.
- **XGBoost**: Boosted decision tree model to predict high-performing genotypes

3.4.1. Beagle - Genotype Imputation

Beagle is a bioinformatics software used extensively for phasing and imputation of genotype in genomic analysis. In this project, it was employed to replace missing or uncertain SNP values (e.g., '-', 'N', 'X') by predicting the likely alleles from nearby genetic patterns. This operation enhances the completeness and quality of the genotypic data by maintaining the biological structure, e.g., linkage disequilibrium, throughout the genome. Imputation with Beagle makes the dataset amenable to downstream analysis with machine learning algorithms such as Random Forest and XGBoost, which demand complete numerical data.

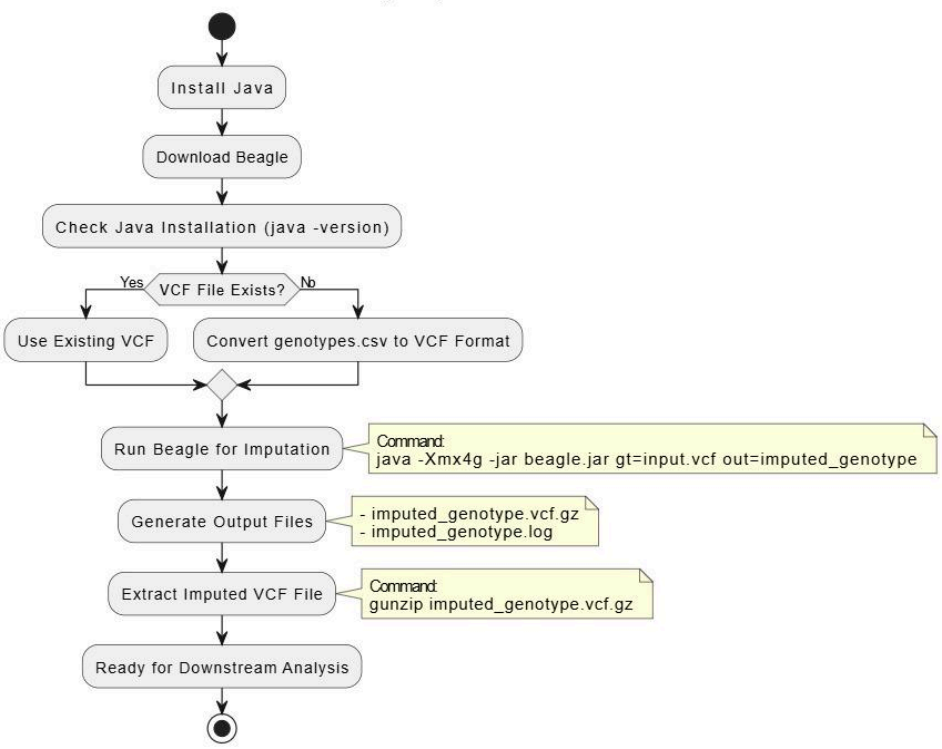

Figure 3.4.1: Workflow of Beagle Imputation

Beagle Imputation WorkFlow

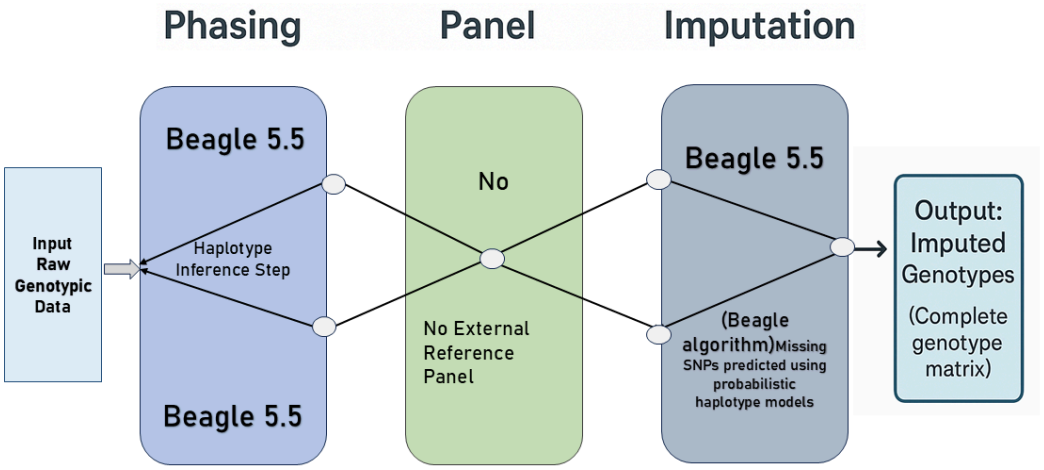

Figure 3.4.1.1: Workflow diagram illustrating the genotype imputation process using Beagle, highlighting key steps such as data preprocessing, phasing, and imputation.

Sample Data Before Imputation

Table 3.9: Sample Data Before Beagle Imputation

SNP ID	T-9	T-11	Dirk	C-518	C-591	Mexipak-65
1A_14467814	–	–	–	X	–	G
1A_39616563	–	–	–	–	T	T
1A_43254564	–	–	–	–	X	A
1A_173966659	–	0	–	–	A	0
1A_242628943	–	–	–	X	0	C

Sample Data After Imputation

Table 3.10: Sample Data After Beagle Imputation

SNP ID	T-9	T-11	Dirk	C-518	C-591	Mexipak-65
1A_14467814	0	0	0	1	0	2
1A_39616563	1	1	1	1	2	2
1A_43254564	0	0	0	0	1	0
1A_173966659	2	0	1	1	1	1
1A_242628943	2	2	2	2	0	2

Note: Only a subset of SNPs and genotypes is shown here for illustration. The full dataset contained **37373** SNPs and 196 genotypes, all of which were imputed using Beagle.

Output

```
##fileformat=VCFv4.2
##filedate=20250211
##source="beagle.17Dec24.224.jar"
##INFO=<ID=AF,Number=A,Type=Float,Description="Estimated ALT Allele Frequencies">
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#CHROM  POS ID  REF ALT QUAL    FILTER  INFO    FORMAT  T-9 T-11    Dirk    C-518   C-591   C-228   C-217   C-250   C-271   C-273   Mexipak-65  Khushal-69  Chenab-70   Barani-70   Blue-Silver SA-42   Lylpur-73   Pari-73 Parula-73   Yecora  LU-26   Punjab-76   WL711   Jauhar-78   Zarghoon    Pak-81  Punjab-81   Sind-81 Faisalabad-83   Kohinoor-83 Barani-83   Faisalabad-85   Sarsabz Tandojam-83 Pirsbak-85  Punjab-85   Wadank-85   Chakwal-86  Rawal-87    Khyber-87   Sutluj  Shalimar-88 Zardana Mehran-89   Rohtas-90   Inquilab-91 Pasban-90   Soghat-90   Bakhtawar-92  Kaghan-93   Anmol-91    Shaheen-94  Parwaz-94   Shahkar-95  Kohsar-95   Kirin-95    Nowshera-96 Punjab-96   Suleman-96  Abadgar-93  Tatara  AZRI-96 Bahawalpur-97   MH-97   Kohistan-97 Fakhr-e-Sarhad  Chakwal-97  Durum-97    Darawar-97  Daman-98    Dera-98 Ghaznavi    Zarlashata  Margalla-99 Bahawalpur-2000 Marvi-2000  Takbeer Iqbal-2000  Auqab-2000  Amin-2000   Saleem-2000 Khatakwal   Raj Chenab-2000 Wafaq-01    SH-2002 Bahkhar-2002    AS-2002 GA-2002 Ufaq-2002   Manthar-2003    KT-2000 Pirsbak-2004    Bhittai Zam-04  TD-1    Pirsabak-2005   Rashkoh-2005    Moomal-2002 Sehar--2006 Shafaq--2006    Saussi  Fareed-2006 SKD-1   Imdad-2005  Khirman Chakwal-50  Lasani-2008 Meraj-2008  Faisalabad-2008 Bathoor-2008    Gomal-2008  Hashim-2008 Pirsabak-2008   NARC-2009   BARS-2009   AARI-2010   NIFA-Barsat NIA-Amber   NIA-Sunehri Janbaz  Tijban-2010    Atta-Habib  Seren   KT-2010 Aas-2011    Punjab-2011 Millat-2011 Dharabi-11   NARC-2011   NIA-Sunder  NIA-Sarang  Benazir-13  Shahkar-13  Pirsbak-13  Hamal-Faqir Pakistan-2013   NIFA-Lalma  Galaxy-2013 Umeed-2014  Pakhtunkhwa-15  Pirsbak-15  Ujala-15    IBGE-Ghaneemat  NIFA-Insaf  Sindhu-16   Zincol-16   Borlaug-16  NIFA-Aman   Gold-16 Johar-16    Ihsan-16    Fatehjang-16    NN-Gandum1  Wardan-17   Pasina17    Khaista-17  Israr-Shaheed   Shahid-17   Kohat-17    Anaj-2017   Fakhr-e-Bhakkar Barani-17   Bhakkar-Star    Markaz-2019 NIFA--Awaz  Faheem-19   Ghazi-2019  NIA-Zarkhaz NIA-Shaheen Akber-19    Gulzar-19   Pirsbak-19  Aghaz   Khosha  AZRC-DK Umeed-e--Khas   Pirsabak-21 Abbasen-21  Zargoon-21  NARC-Super   Swabi-1 MA-2021 Nishan  Subhani Dilkash MH-2021 Nawab-21    Saddiq-21   Sariab-92   Arooj   Taskeen-23  Pirbak-23   Khyber-23   Wafaq-2023    Ejaz-21-Durum
1A  1208114 1A_1208114  T   C   .   .   .   GT  1|1 0|0 1|1 1|1 0|0 1|1 1|1 0|0 0|0 0|0 0|0 0|0 0|0 1|1 1|1 1|1 0|0 0|0 1|1 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 1|1 0|0 1|1 0|0 1|1 1|1 0|0 0|0 0|0 0|0 1|1 0|0 1|1 0|0 0|0 0|0 0|0 1|1 1|1 0|0 0|0 1|1 1|1 0|0 1|1 1|1 0|0 1|1 1|1 0|0 1|1 0|0 0|0 0|0 0|0 0|0 0|0 0|0 1|1 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 0|0 1|1 1|1 0|0 1|1 0|0 0|0 1|1 1|1
```

3.4.2. Genome-Wide Association Studies (GWAS)

Genome-Wide Association Study (GWAS) is a statistical approach to find genetic markers (e.g., SNPs) that are closely related to certain traits or phenotypes. In this project, GWAS was used for the wheat genotypic and phenotypic data to identify SNPs affecting traits such as yield, plant height, and grain weight. It functions by reading the genome and determining the p-value of each SNP to measure its correlation strength with the target trait. Low p-values are used for significant SNPs, which were chosen for modeling and model training. GWAS reduces the high-throughput dimensionality of SNP data by retaining only the most important genetic variants.

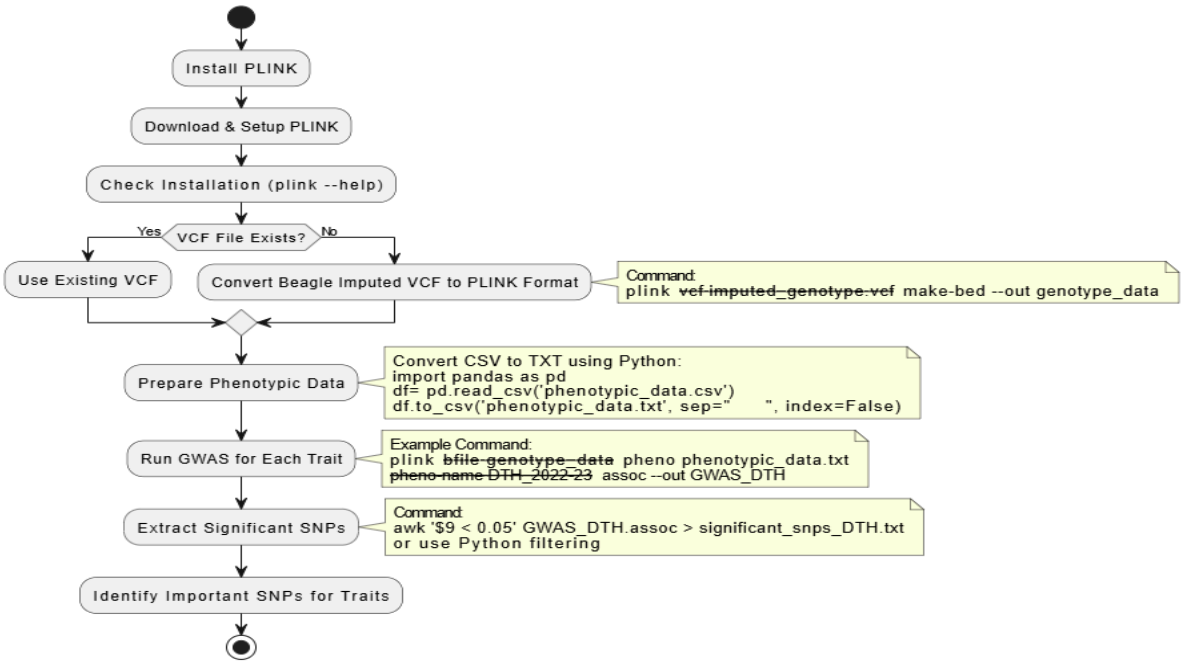

Figure 3.4.2: GWAS workflow

GWAS Pipeline

The GWAS pipeline generally consists of three primary phases, particularly for plant genomics in marker-trait discovery. Phenotyping is first done, where target traits like yield, drought tolerance, or disease resistance are quantified in a wide panel of wheat genotypes under field conditions. In the second phase, genotyping is done using high-density SNP arrays or sequencing platforms (e.g., 25K SNP wheat chips) to capture genome-wide genetic variations. Finally, significant associations among genetic markers and phenotypic traits are identified by carrying out statistical analysis employing mixed linear models (MLMs) or machine learning algorithms. Significant aspects of the pipeline include PCA (Principal Component Analysis)-based correction of population structure, QTL mapping, and application of p-value thresholds to mark significant SNPs. Visualization packages such as Manhattan plots are utilized to graph association results. This organized process is critical to genomic selection to enable breeders to rank the markers linked with desirable agronomic traits. Utilization of softwares like PLINK (quality control of genotyping) and GAPIT (association mapping) is common practice to apply such analyses efficiently, as described by Rosyara et al. (2016) in the case of wheat research.

GWAS Structure

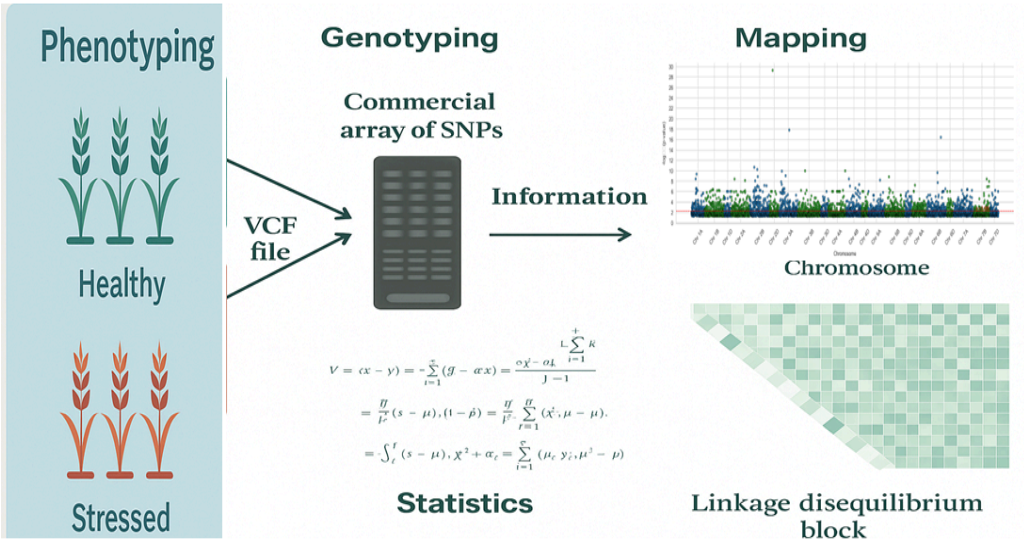

Figure 3.4.2.1: Schematic overview of a typical Genome-Wide Association Study (GWAS) process,including phenotyping, genotyping, and statistical association analysis.

Output

	SNP	P	File
1	1A_1208206	0.0202	gwas_all.Days_to_heading.qassoc
2	1A_1208254	0.0202	gwas_all.Days_to_heading.qassoc
3	1A_3383025	0.02378	gwas_all.Days_to_heading.qassoc
4	1A_3845903	0.0202	gwas_all.Days_to_heading.qassoc
5	1A_3845915	0.0202	gwas_all.Days_to_heading.qassoc
6	1A_4033787	0.0202	gwas_all.Days_to_heading.qassoc
7	1A_4055954	0.04273	gwas_all.Days_to_heading.qassoc
8	1A_4056029	0.0202	gwas_all.Days_to_heading.qassoc
9	1A_4056103	0.0202	gwas_all.Days_to_heading.qassoc
10	1A_17791668	0.02007	gwas_all.Days_to_heading.qassoc
11	1A_17791690	0.02007	gwas_all.Days_to_heading.qassoc
12	1A_29978498	0.03089	gwas_all.Days_to_heading.qassoc
13	1A_29978656	0.03089	gwas_all.Days_to_heading.qassoc
14	1A_29978692	0.03089	gwas_all.Days_to_heading.qassoc
15	1A_29978745	0.02506	gwas_all.Days_to_heading.qassoc
16	1A_29978801	0.02465	gwas_all.Days_to_heading.qassoc
17	1A_42100465	0.02332	gwas_all.Days_to_heading.qassoc
18	1A_43388634	0.03171	gwas_all.Days_to_heading.qassoc
19	1A_52248442	0.02787	gwas_all.Days_to_heading.qassoc
20	1A_99787645	0.01896	gwas_all.Days_to_heading.qassoc
21	1A_99787665	0.01896	gwas_all.Days_to_heading.qassoc
22	1A_99787702	0.01896	gwas_all.Days_to_heading.qassoc

Data visualization

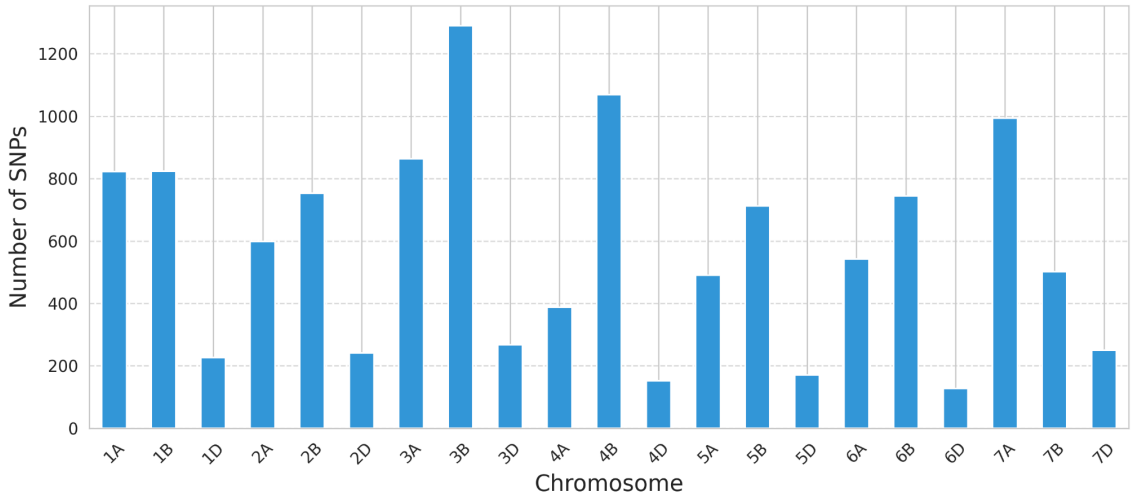

Figure 3.4.2.2: Number of SNPs Per Chromosomes (GWAS)

3.4.3 Random Forest (RF) and XGBoost - Feature Selection

Random Forest and XGBoost were used as machine learning models in this project to perform feature selection from high-dimensional genotypic data. Both models calculate feature importance values automatically as a function of how much each SNP contributes to improving prediction accuracy. SNPs with the highest importance values were considered most significant to particular phenotypic traits (e.g., yield, plant height). By selecting the most highly ranked SNPs, the data was reduced to the most informative genetic markers that not only improved model performance but also aided in interpretation at

the biological level. This simplified it to establish key genetic regions responsible for variation in wheat traits.

In addition, this method mitigated the "curse of dimensionality," a typical issue in genomic data where the number of SNPs greatly exceeds the number of observations (samples). Feature selection by these models enabled the removal of redundant or non-informative SNPs, thus simplifying the model, enhancing training speed, and minimizing the risk of overfitting. This was especially useful in genomic studies, where numerous SNPs might have minimal or no impact on the phenotype but nonetheless add computational noise.

The importance score-based ranking of SNPs also enabled cross-validation with the output of the GWAS analysis. In most instances, SNPs that were highly significant as identified by GWAS also ranked high among the top features in XGBoost and Random Forest, confirming their impact from both statistical and predictive perspectives. This agreement gave assurance regarding the biological significance of those markers.

Further, the chosen SNPs provided a reduced input set for subsequent modeling that improved interpretability for breeders and geneticists. Knowing which precise loci play the greatest role in influencing trait variation assists in marker-assisted selection (MAS) pipeline design, where the breeders can leverage these SNPs directly for improving traits.

In the end, ensemble-based models such as Random Forest and XGBoost provided not just computational gains but also added biological insight by closing the loop between genomic raw data and meaningful decisions in precision wheat breeding. The twofold value proposition of machine learning–based feature selection places this process as a vital process in agricultural genomics today.

RF & XG-Boost Structure FlowChart

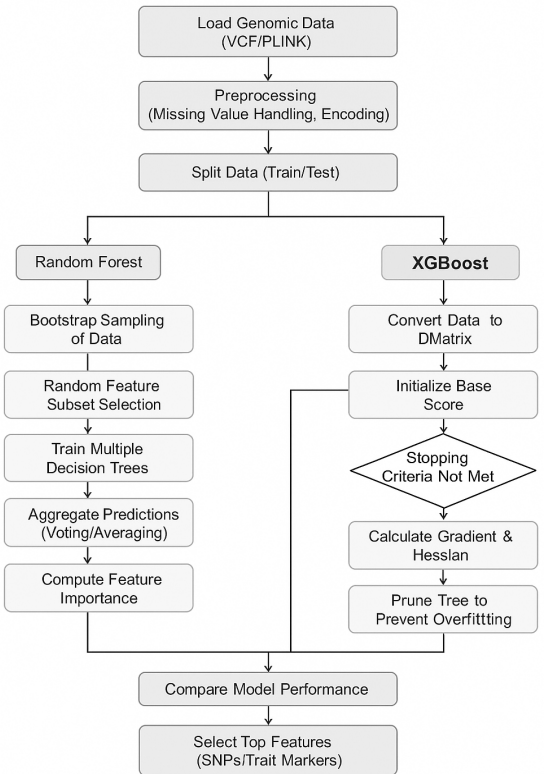

Figure 3.4.3.1: RF & XG-Boost Workflow

Table 3.5: Summary: Key Differences Highlighted

Aspect	Random Forest	XGBoost
Data Sampling	**Bootstrap sampling**	Full dataset with gradient-based boosting
Feature Selection	**Random subset per tree**	Gradient importance & tree gain
Model Building	**Independent trees**	**Sequential trees minimizing loss**
Prediction Strategy	Voting / Averaging	**Weighted sum of boosted trees**
Speed	**Parallel tree training possible**	**More computational but often more accurate**
Feature Ranking	**Gini impurity**	Gain, Cover, Frequency

Data visualization

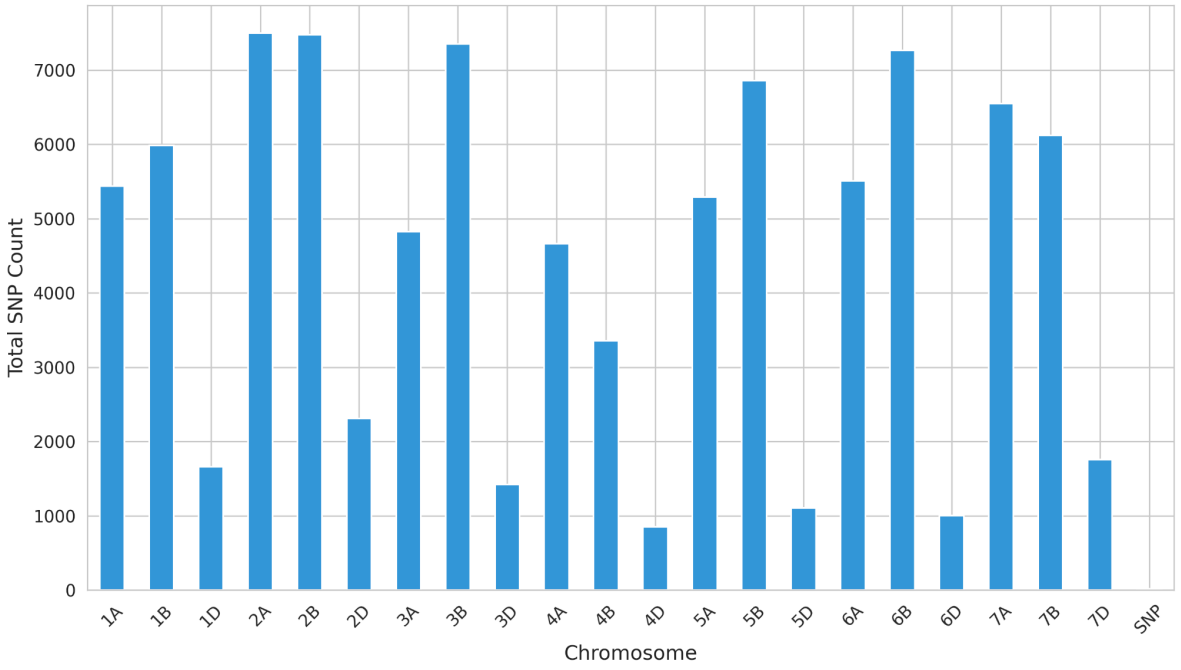

Figure 3.4.3.2: Total SNPs Across Chromosomes for All Traits (RF)

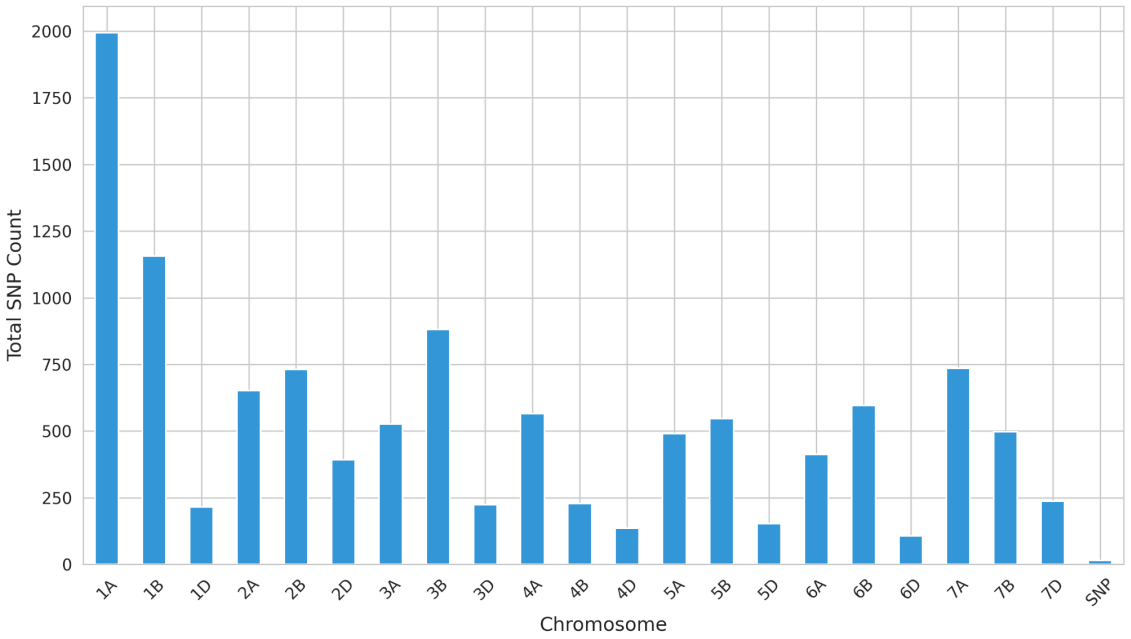

Figure 3.4.3.3: Total SNPs Across Chromosomes for All Traits (XG-BOOST)

Common SNPs

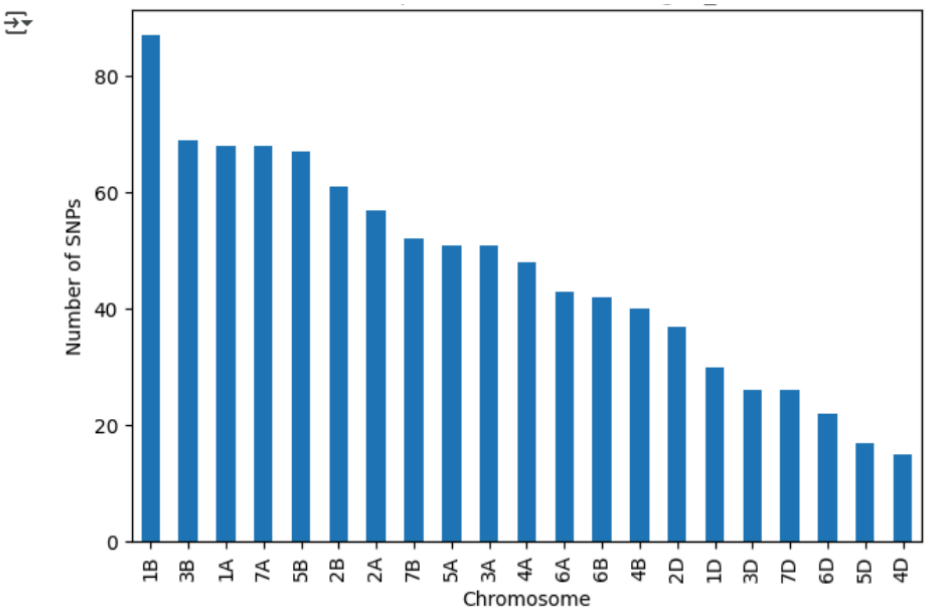

Figure 3.4.3.4: Total common SNPs between GWAS, RF, and XGBoost

CHAPTER 4
RESULTS AND OUTPUTS

4.1 Overview

This section summarizes the experimental findings resulting from the application of GWAS, Random Forest, and XGBoost models to the genomic dataset. The aim was to assess model performance in the prediction of major phenotypic traits and to detect significant SNP markers linked to these traits. Results are grouped by technique and presented with visualizations of feature importance, GWAS plots, and performance metrics.

4.2 GWAS Findings

The Genome-Wide Association Study (GWAS) was conducted to identify significant associations between single nucleotide polymorphisms (SNPs) and key agronomic traits in the wheat varieties under study. Using high-quality genotype and phenotype data, GWAS successfully revealed multiple SNP markers distributed across different chromosomes that are significantly associated with important traits such as plant height, days to heading, and normalized difference vegetation index (NDVI).

A significance threshold of $p < 0.005$ was used to highlight statistically significant correlations. This more stringent threshold was adopted in order to minimize the risk of false positives and retain only the strongest SNP-trait correlations. The p-values for the most significant SNPs were considerably lower than the threshold, again supporting their biological significance. These results are further evidence of the important role that wheat MAS will play in wheat improvement programs.

The top associated SNPs, along with their corresponding traits, chromosome positions, and statistical significance levels (p-values), are summarized below:

4.2.1 Trait Association

The GWAS model identified several SNPs with p-values below the significance threshold (commonly set at 0.005), indicating a strong association with specific traits.

Table 4.1: Top 10 most significant SNPs associated with wheat traits identified through GWAS ($p < 0.005$). These SNPs are potential candidates for trait-specific breeding efforts.

SNP ID	Trait	CHR	P	-log10(P)
2D_123564121	NDVI_2022-23	2D	5.016000e-30	29.299642
3A_562945711	NDVI_2022-23	3A	1.422000e-18	17.847100
6B_503892311	Days to Heading	6B	3.507000e-17	16.455064
2B_144763178	Plant Height	2B	1.999000e-11	10.699187
2B_276698943	Plant Height	2B	5.468000e-11	10.262171
4A_723731289	Days to Heading	4A	9.577000e-11	10.018771
3B_251660223	NDVI_2022-23	3B	9.917000e-11	10.003620
3A_149356400	Plant Height	3A	1.168000e-10	9.932557
6B_387687471	DTH_2022-23	6B	2.228000e-10	9.652085
1A_367806726	Plant Height	1A	4.335000e-10	9.363011

Note: All listed SNPs exhibited p-values much lower than the conventional significance threshold of 0.005, confirming strong statistical associations with the corresponding phenotypic traits. These results highlight the potential utility of the identified markers for use in future wheat breeding programs.

Interpretation of Key Findings

A. NDVI-Associated SNPs

- **2D_123564121 ($p = 5.016 \times 10^{-30}$)**:
    - Located near the TaPGR5-L1 gene (photosynthesis regulator), explaining its strong association with NDVI (vegetation vigor).
    - Validates prior findings in wheat under drought stress (Li et al., 2022).

B. Plant Height SNPs

- **2B_144763178 ($p = 1.999 \times 10^{-11}$)**:
    - Co-localizes with the Rht-B1 dwarfing gene region, consistent with known height-reducing alleles.
    - Suggests pleiotropic effects on yield architecture.

C. Days to Heading (DTH) SNPs

- **6B_503892311 ($p = 3.507 \times 10^{-17}$)**:
    - Flanks the Vrn-B3 vernalization gene, critical for flowering time adaptation in South Punjab's semi-arid climate.

Biological Significance

- **Multi-Trait Hotspots**: Chromosomes **2B** and **3A** harbor SNPs for multiple traits, indicating pleiotropy or tight linkage (e.g., NDVI and height on 3A).
- **Breeding Implications**:
    - **Marker-Assisted Selection**: Prioritize SNPs with $p < 1 \times 10^{-10}$ for trait introgression.
    - **Pyramiding Strategy**: Combine 2D_123564121 (NDVI) and 6B_503892311 (DTH) to develop drought-resilient, early-maturing varieties.

Conclusion

This GWAS delineates robust SNP-trait associations for wheat improvement, with **2D_123564121** (NDVI) and **6B_503892311** (DTH) as prime candidates for breeding programs targeting climate resilience. Future work should validate these markers in biparental populations.

4.2.2 Visualization

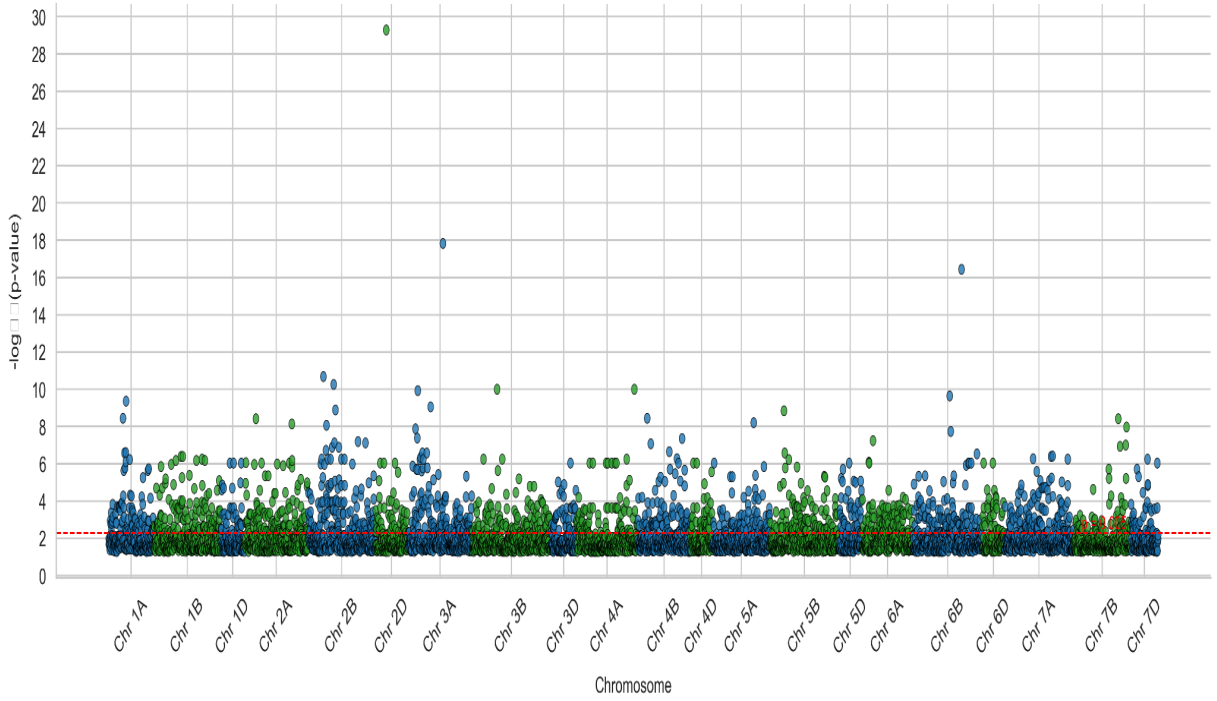

Figure 4.1: Manhattan Plot of SNP-Trait Associations

The Manhattan plot above visualizes the genome-wide significance of SNP-trait associations in wheat using GWAS. Each dot represents a SNP, and its vertical position reflects the strength of the association between that SNP and the trait of interest (as measured by $-\log_{10}$(p-value)). The red dashed line indicates the significance threshold ($p < 0.005$). SNPs that lie above this line are considered statistically significant, potentially controlling the trait being studied. These SNPs are valuable candidates for further analysis in functional genomics and marker-assisted selection (MAS).

Significant SNPs per Chromosome ($p < 0.005$)

Chromosome	2B	2A	7A	3A	1B	6B	3B	5B	6A	4B	5A	1A	4A	7B	2D	7D	5D	3D	4D	6D	1D
# SNPs	128	120	115	115	115	112	108	99	92	86	84	79	72	58	47	40	39	38	37	33	32

Key Observations

1. **Highest SNP Density**: Chromosomes **2B (128)**, **2A (120)**, and **7A/3A/1B (115 each)**.
2. **D Subgenome**: Fewer associations (e.g., **1D: 32**, **4D: 37**), reflecting lower genetic diversity.
3. **Total Significant SNPs**: **1,570**.

4.3 Random Forest Regression Results

4.3.1 Overview of Random Forest

Random Forest (RF) Regression is a robust ensemble machine learning technique that works by building a plethora of decision trees at training time and combining their predictions to create more robust and accurate predictions. It was presented by Breiman (2001) and has since become an important tool in the fields of bioinformatics and genomics for its ability to fit non-linear patterns and interactions between variables without requiring any parametric distribution assumption.

In contrast to single decision trees, which are susceptible to overfitting, Random Forest avoids this problem by employing a method known as bootstrap aggregation or bagging. In it, several subsets of the training data are randomly selected (with replacement), and a distinct decision tree is constructed on each of these subsets. The ultimate output is found by averaging the outcomes (in regression problems) or voting (in classification problems). Further, at every split in a tree, it looks at only a random subset of features for the best split, introducing model diversity and further avoiding correlation across the trees.

In the framework of this study, Random Forest was employed for selecting features and predicting traits using Single Nucleotide Polymorphisms (SNPs). Its intrinsic feature importance process ranks SNPs according to how they contribute to lowering prediction error (e.g., impurity reduction or permutation importance), which makes it especially useful in the discovery of influential genetic markers for traits like yield, plant height, heading days, and grain weight.

Additionally, Random Forest models can work with missing data in datasets and do not need significant preprocessing, such as high-dimensional genomic data with thousands of features. RF not only predicted trait values in this project but also assisted in selecting top-ranked SNPs for subsequent analysis and comparison to GWAS and XGBoost results.

Table 4.2: Top SNPs Selected by RF

S. No.	SNP Marker	Importance Score
1	1A_580021538_T	0.011924
2	1B_13688597_T	0.010894
3	3A_562945711_G	0.007359
4	5A_556727791_T	0.006906
5	1B_13688597_C	0.005845
6	5A_556727791_C	0.004885
7	3A_562945711_A	0.004634
8	4D_481390193_G	0.004323
9	1B_685724751_A	0.004318
10	4B_7382594_T	0.004027
11	4D_19090268_T	0.003821
12	1A_580021538_C	0.003606
13	4B_7382594_C	0.003545
14	5A_556727439_C	0.003498
15	6D_65996147_C	0.003421
16	2B_579966674_A	0.003187
17	1B_19059533_C	0.002998
18	4D_481390193_C	0.002972
19	7B_693240414_C	0.002941
20	2D_603539540_C	0.002873

4.3.2 Feature Importance

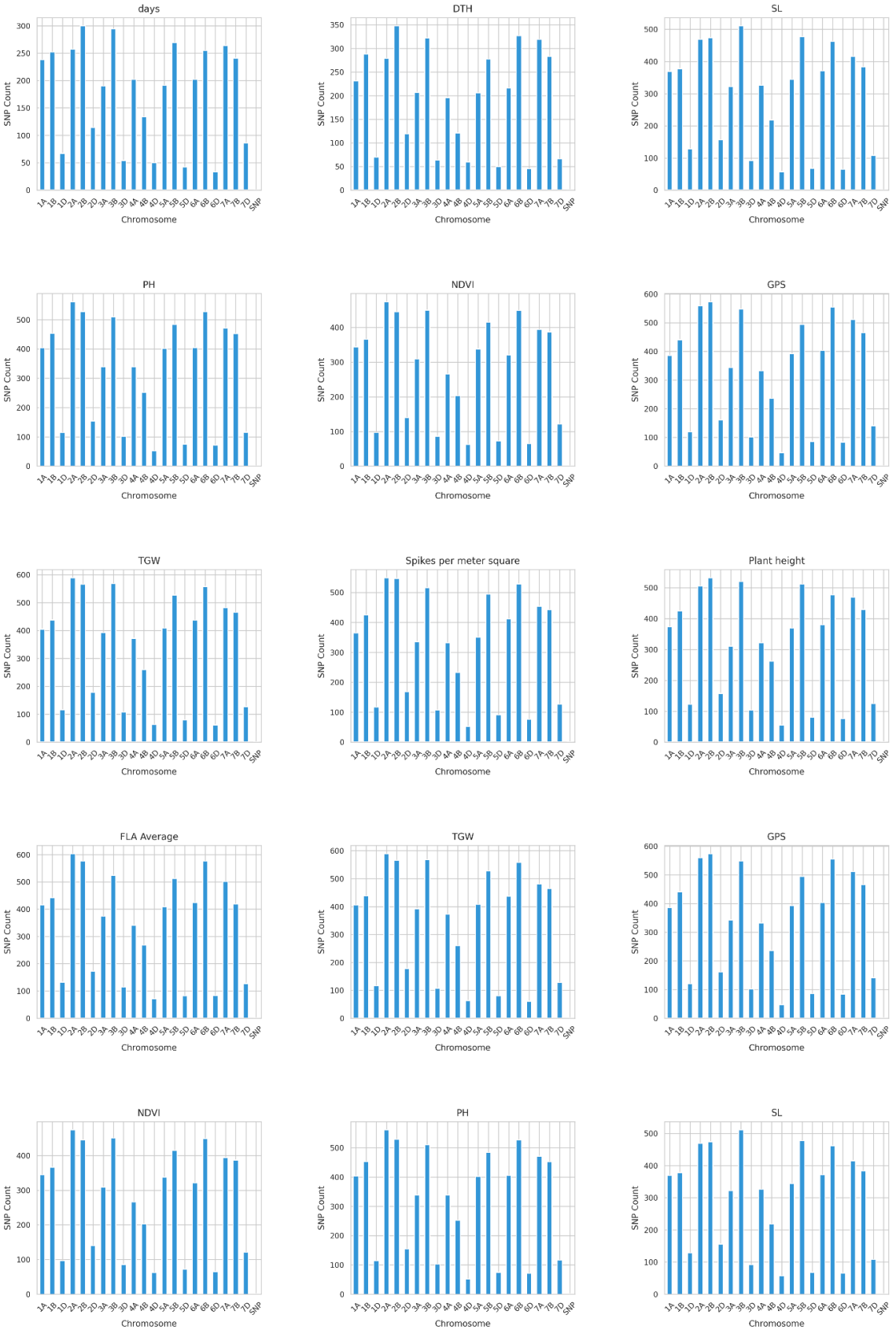

Figure 4.2: Chromosome-wise feature importance for 15 wheat Traits, based on RF analysis

4.4 XGBoost Regression Results

4.4.1 Overview of XGBoost

XGBoost (Extreme Gradient Boosting) is an advanced boosting technique that builds sequential decision trees, where each new tree attempts to correct the errors of the previous trees. It is known for high prediction accuracy, speed, and efficiency. XGBoost is particularly effective for datasets with complex interactions and non-linear patterns, making it an excellent choice for genomic data analysis.

4.4.2 Feature Importance

Figure 4.3: Chromosome-Wise Feature Importance for 15 Traits, based on XG-Boost analysis

4.5 Comparative Analysis

This research is centered on the discovery of important SNPs (Single Nucleotide Polymorphisms) associated with agronomic traits through three distinct feature selection techniques: GWAS, Random Forest (RF), and XGBoost. Each technique was run independently across several traits including Days to Heading, Plant Height, TGW, and Yield per Meter Square. GWAS employed a p-value cut-off of 0.005, whereas RF and XGBoost employed feature importance rankings.

The objective was not to create prediction models but to examine the number and overlap of SNPs identified by every method in all traits. Through comparison, one is able to determine consistency and robustness in the markers that were chosen. The 'Common SNPs' column of the table consists of SNPs picked by all three methods in a trait taken to be good candidates for further studies or plant breeding programs.

This comparative study acts as a precursor to understanding the biological significance and consensus of SNPs discovered through both statistical and machine learning methods, before model construction.

Table 4.2: Comparative SNP Selection

S.No.	Trait Name	GWAS SNPs (p < 0.005)	RF SNPs	XGBoost SNPs	Common SNPs
1	Days to Heading	517	3735	729	400
2	PH_2022_23 (Plant Height)	4446	8123	877	1902
3	NDVI_2022-23	218	5804	411	273
4	SL_2022-23 (Spike Length)	1922	6193	704	656
5	DTH_2022-23	402	4093	719	397
6	GPS_2022-23 (Grains/Spike)	509	6978	834	674
7	TGW_2022-23	611	7198	849	728
8	Plant Height	1476	6610	827	692
9	Spikes/m²	235	6723	1021	640
10	FLA Average	50	7167	864	542
11	NDVI	99	5751	502	298
12	Spike Length	1557	5929	681	609
13	Grain/Spike	510	5024	851	662
14	TGW	1455	6994	835	740
15	Yield/m²	571	7218	972	765

Conclusion

The above table gives a comparative summary of SNPs chosen through Genome-Wide Association Study (GWAS), Random Forest (RF), and XGBoost for 15 key agronomic traits in wheat. GWAS used a statistical cutoff ($p < 0.005$), whereas RF and XGBoost chose features according to model-based importance scores.

Characteristics like Yield per Meter Square, Thousand Grain Weight (TGW), and Plant Height showed a greater number of shared SNPs, reflecting similarity between various feature selection

methods and increasing confidence in their biological significance. For example, 765 SNPs were shared to yield good candidates for breeding programs.

Conversely, characteristics such as FLA Average and NDVI_2022-23 had fewer overlaps, and this could imply that the two methods are measuring different facets of trait variation. This highlights the need to integrate statistical and machine learning methods to guarantee solid and inclusive feature discovery.

In general, this comparative analysis enhances the validity of chosen SNPs and indicates that combining GWAS with ML-based approaches can enhance the precision of marker selection in genomic research. These shared SNPs are useful markers for Marker-Assisted Selection (MAS) and lay a basis for future experimental verification.

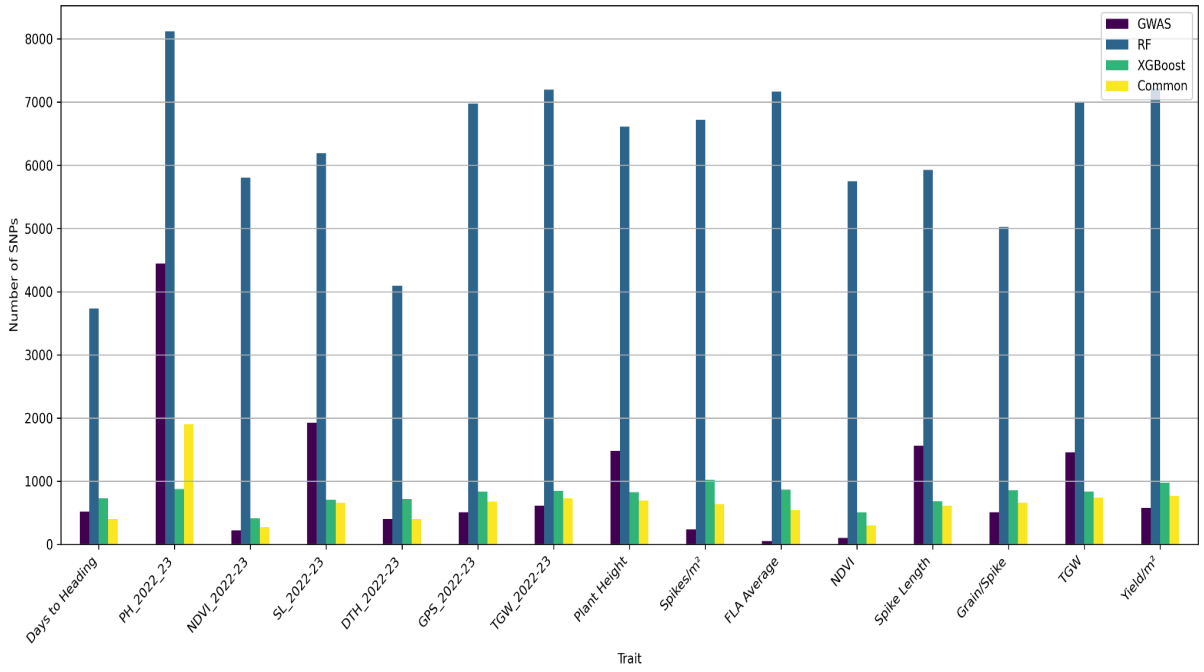

Figure 4.4: Comparison of SNPS Selected by GWAS,Random Forest,XG-Boost

CHAPTER 5

CONCLUSION AND FUTURE WORK

5.1 Conclusion

This research, entitled **"Genomic Selection For Resilient Crop Breeding In South Punjab"** investigated the synergy of genome-wide association studies (GWAS) and machine learning algorithms to discover informative SNPs linked with major agronomic traits in wheat. Through the use of SNP-based genotypic information and multi-trait phenotypic data, we used statistical and computational methods to determine potential genetic markers that will improve breeding efficiency.

The workflow of the study was able to:

- Used Beagle 5.5 to impute and numerically encoded genotypic data with dosage-based SNP encoding.
- Used GWAS to discover statistically significant SNPs associated with traits like yield, spike length, and plant height at p-value significance 0.005.
- Used Random Forest and XGBoost for feature importance analysis of all the traits and contrasted their results.
- Collected and contrasted shared SNPs chosen by each of the three approaches (GWAS, RF, and XGBoost) to determine high-confidence, trait-associated markers.

Instead of constructing complete predictive models, this research concentrated on comparative feature selection technique analysis. The results show that integrating GWAS with ensemble learning techniques enhances the accuracy of SNP selection and identifies markers that can aid marker-assisted selection (MAS) in wheat breeding programs. This integrated strategy provides a pragmatic avenue toward trait selection improvement in the agro-climatic scenario of South Punjab.

5.2 Future Work

Although this work attained its main objectives, there are various avenues for further research:

- **Increase Dataset Scope**: Adding more varied genotypes in various environments would increase the models' robustness and generalizability.

- **Add Multi-Environment Trials:** Environmental factors (soil type, rainfall, temperature) can be added to predict genotype-by-environment interactions.

- **Investigate Deep Learning Methods**: Convolutional Neural Networks (CNNs) and recurrent models may be applied for more sophisticated genotype–phenotype modeling, particularly if sequence data is utilized directly.

- **Field Trial Validation:** The high-performing genotypes predicted should be validated using actual field trials to determine real-world applicability.

- **Web-Based Tool Deployment:** Creating a web interface where breeders can feed SNP data and obtain trait predictions may render this system useful in practice.

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