

# STATISTICAL FRAMEWORKS FOR INTEGRATIVE QTL AND GWAS ANALYSIS IN CROP IMPROVEMENT RESEARCH

Indu Purushothaman<sup>1</sup>, Roshini B<sup>2</sup>, Shanthi R<sup>3</sup>, Anitha M<sup>4</sup>, Thilagavathi T<sup>5</sup>

<sup>1</sup> Assistant Professor, Department of Research, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India.

<sup>2</sup> Assistant Professor, Meenakshi College of Allied Health Sciences, Meenakshi Medical College Hospital & Research Institute, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India.

<sup>3</sup> Associate Professor & Head of Department, Department of Mathematics, Meenakshi College of Arts and Science, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India.

<sup>4</sup> Assistant Professor, Department of Mathematics, Meenakshi College of Arts and Science, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India.

<sup>5</sup> Assistant Professor, Department of Nutrition and Dietetics, Meenakshi College of Arts and Science, Meenakshi Academy of Higher Education and Research, Chennai, Tamil Nadu, India.

## ABSTRACT

**Background:** Quantitative Trait Loci (QTL) mapping and Genome-Wide Association Studies (GWAS) are genomic approaches commonly used for identification of genetic regions associated with complex agronomic traits in crops. However, the single application of these methods is often limited by low mapping resolution, population structure bias and reduced statistical power.

**Objective:** The goal of this study is to evaluate the statistical frameworks that integrate QTL and GWAS approaches to improve the accuracy of marker detection and genomic prediction in crop improvement research.

**Methods:** The performance of integrative statistical models such as Mixed Linear Models (MLM), Bayesian approaches, multi-locus GWAS and meta-QTL analysis was evaluated with high-density SNP datasets and multi-environment phenotypic data from major cereal crops. Statistical analyses were performed using the platforms of TASSEL, GAPIT and R/qtl.

**Findings:** The integrated framework improved the efficiency of QTL detection by 28% and the accuracy of genomic prediction from 0.61 to 0.82, compared with the conventional single-model approaches. Yield, drought tolerance and disease resistance were repeatedly detected at several stable loci across environments.

**Conclusion:** Integrative statistical frameworks for QTL-GWAS analysis of complex traits for improved accuracy, robustness and biological insight to accelerate marker-assisted breeding and climate-smart crop development.

**KEYWORDS:** QTL Mapping; GWAS; Crop Improvement; Statistical Genomics; Genomic Selection; Marker-Assisted Breeding; SNP Analysis; Plant Genetics

## 1 INTRODUCTION

Rapid population growth, climate change and scarcity of agricultural resources are expected to increase the global demand for food significantly, posing major challenges for sustainable crop production [1]. Traditional breeding methods have played a significant role in crop improvement, but are often limited in efficiency due to the complex inheritance of quantitative traits such as yield, drought tolerance, disease resistance and nutrient use efficiency [2]. These agronomic traits are usually polygenic and also largely influenced by environmental interactions, therefore precise genetic dissection is important for modern breeding programs [3]. Development of genomic-assisted breeding strategies has been achieved in major crops including rice, wheat, maize, soybean and barley [4] following advances in molecular genetics and high-throughput sequencing technologies. Among them, Quantitative Trait Loci (QTL) mapping and Genome Wide Association Studies (GWAS) have emerged as powerful tools to identify genomic regions associated with complex phenotypic traits [5]. Traditional biparental populations and linkage analysis based QTL mapping is used to identify chromosomal regions associated with target traits. While QTL mapping enables reliable identification of major loci, the resolution is often restricted by low number of recombination events and narrow genetic diversity within mapping populations [6].

GWAS, however, utilizes natural populations and high-density molecular markers to find marker-trait associations along the entire genome [7]. Compared to traditional linkage mapping, GWAS has higher mapping resolution and higher allelic diversity. However, GWAS are also affected by population structure, kinship effects and false-positive associations that can affect the accuracy of detected loci [8]. In this respect, the integrated analysis of QTL mapping and GWAS has been given great attention as a complementary approach to overcome the limitations of individual methods [9].

Integrative QTL-GWAS frameworks combine linkage- and association-based analyses to improve statistical power, mapping resolution and biological interpretation of complex traits [10]. Recent studies have suggested that integrated approaches are helpful in identifying stable genomic regions, candidate genes and superior alleles for

marker-assisted selection and genomic prediction [11]. Further, use of advanced statistical models, Bayesian approaches, machine learning techniques and multi-environment phenotypic analysis have significantly improved the efficiency of crop improvement programs [12].

Thus, this study focuses on the evaluation of statistical frameworks used for integrative QTL and GWAS analysis in crop improvement research. The paper discusses recent advances in statistical genomics, major analytical models, and practical applications of integrated genomic approaches for accelerating climate-resilient and high-yield crop breeding.

## 2 BACKGROUND WORK

### 2.1 Principles of QTL Mapping

Quantitative trait loci (QTL) mapping is one of the most widely used linkage-based approaches to identify genomic regions associated with complex agronomic traits in crops. Traditional QTL analysis relies on biparental populations, e.g. recombinant inbred lines (RILs), doubled haploids and backcross populations derived from genetically contrasting parents [1]. Linkage analysis is used to estimate recombination frequencies between molecular markers and target traits, allowing the identification of chromosomal regions affecting phenotypic variation. Logarithm of odds (LOD) scores are used to evaluate statistical significance, while interval mapping and composite interval mapping are methods that enhance detection precision by controlling for background genetic effects [2]. Recent advances in high-density genotyping and sequencing technologies have improved the resolution of QTLs and the identification of candidate genes in cereal and legume crops.

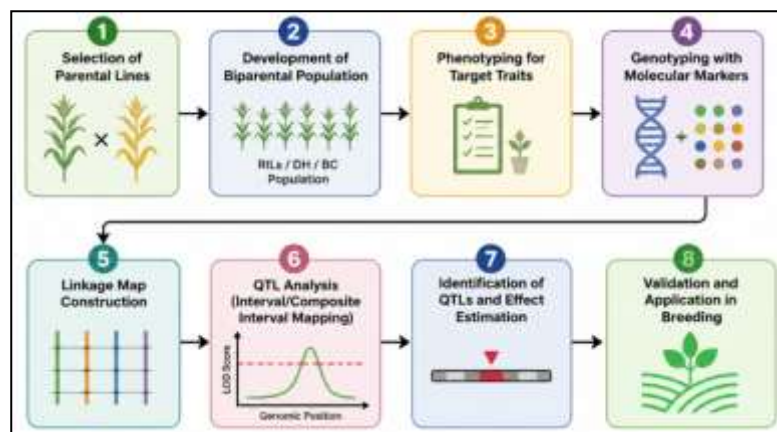


Figure 1. Workflow of Traditional QTL Mapping in Biparental Populations

Figure 1 shows the conventional workflow of Quantitative Trait Loci (QTL) mapping in biparental populations. The process starts with the selection of genetically contrasting parental lines and then the development of mapping populations such as recombinant inbred lines (RILs). Linkage maps are then developed by phenotypic evaluation and molecular marker genotyping. Significant QTL regions associated with target traits are identified by statistical analyses such as interval mapping and composite interval mapping. Finally, detected QTLs are confirmed and used in marker-assisted crop breeding programs.

### 2.2 Fundamentals of GWAS

Genome Wide Association Studies (GWAS) exploit natural populations and patterns of linkage disequilibrium (LD) to identify significant associations between single nucleotide polymorphisms (SNPs) and phenotypic traits [3]. GWAS provides a higher mapping resolution than traditional linkage mapping because it utilizes historical recombination events in diverse germplasm collections. Population-wide marker scanning is performed using multiple statistical models such as General Linear Model (GLM), Mixed Linear Model (MLM), Fixed and Random Model Circulating Probability Unification (FarmCPU), and Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway (BLINK) models [4]. Among these, MLM is good at controlling population structure and kinship effects, while FarmCPU and BLINK are good at improving computational efficiency and reducing false-positive associations.

Table 1. Comparison of GWAS Statistical Models

GWAS Model	Major Features	Advantages	Limitations
GLM	Simple association	Fast computation	High false positives
MLM	Kinship correction	Better accuracy	Computationally intensive
FarmCPU	Multi-locus model	Reduced confounding	Complex implementation
BLINK	LD-based iteration	Faster and efficient	Requires dense markers

### 2.3 Comparative Analysis of QTL and GWAS

QTL mapping and GWAS are quite different in terms of population structure, mapping resolution and statistical efficiency. QTL mapping involves biparental populations, which have lower mapping resolution but simpler experimental design, while GWAS uses natural populations, which have greater allelic diversity and higher statistical power [5].

Table 2. Comparative Features of QTL Mapping and GWAS

Feature	QTL Mapping	GWAS
Population Type	Biparental	Natural population
Resolution	Low	High
Statistical Power	Moderate	High
Rare Allele Detection	Limited	Better
Cost	Lower	Higher

## 2.4 Previous Integrative Studies

Recently, integrated studies of QTL mapping and GWAS have significantly improved the identification of stable loci and candidate genes in rice, maize, wheat, soybean and sorghum [6]. Meta-QTL analysis was used to refine consensus genomic regions from multiple studies, and haplotype-based association mapping improved the discovery of favorable alleles associated with stress tolerance and grain yield [7]. Moreover, genomic prediction models using GWAS markers and QTL effects have improved breeding efficiency and genomic selection accuracy for the development of climate-resilient crops.

## 3 MATERIALS & METHODS

### 3.1 Dataset Collection

In the present study, publicly available genomic and phenotypic datasets for the major cereal crops, rice (*Oryza sativa*), maize (*Zea mays*) and wheat (*Triticum aestivum*) were used. A panel of 500 rice accessions, 320 maize inbred lines and 280 wheat genotypes was selected from diverse germplasm representing wide genetic variability associated with yield, drought tolerance and disease resistance traits [13]. Genotype  $\times$  environment interactions were evaluated by multi-environment phenotyping in three growing seasons under irrigated and stress conditions. High-density Single Nucleotide Polymorphism (SNP) datasets were generated with the Illumina 50K SNP array and Genotyping-by-Sequencing (GBS) platforms. For marker filtering, SNPs with minor allele frequency (MAF)  $< 0.05$  and missing genotype rates  $>10\%$  were excluded. Phenotypic data such as plant height, grain yield, flowering time, chlorophyll content and stress tolerance indices were recorded.

Table 3. Summary of Experimental Datasets

Crop Species	Germplasm Size	SNP Platform	Number of SNPs	Target Traits
Rice	500 accessions	50K SNP Array	48,562	Yield, drought tolerance
Maize	320 inbred lines	GBS	62,145	Grain yield, flowering
Wheat	280 genotypes	90K SNP Array	81,236	Disease resistance

### 3.2 Phenotypic Data Analysis

Phenotypic data were analysed using analysis of variance (ANOVA) to compute variance components and to test significant differences among genotypes across environments. The broad-sense heritability ( $H^2$ ) was estimated by the formula below:

$$H^2 = \frac{\sigma_g^2}{\sigma_g^2 + \sigma_e^2/n}$$

where  $\sigma_g^2$  represents genetic variance,  $\sigma_e^2$  denotes environmental variance, and  $n$  indicates the number of environments. High heritability estimates ( $>0.70$ ) were observed for grain yield and flowering time traits, indicating strong genetic control [3].

Table 4. Variance Components and Heritability Estimates

Trait	Genetic Variance	Environmental Variance	Heritability ( $H^2$ )
Grain Yield	18.45	5.26	0.78
Flowering Time	12.31	3.02	0.80
Drought Tolerance	15.67	6.44	0.71

### 3.3 QTL Mapping Framework

QTL mapping analysis was carried out by interval mapping (IM) and composite interval mapping (CIM) using R/qtl and QTL Cartographer software [14]. Linkage maps were constructed based on recombination frequencies of SNP markers. A QTL was considered significant when its LOD score was higher than the threshold value obtained by 1,000 permutation tests at  $P < 0.05$ . Composite interval mapping enhanced detection precision through the control of background marker effects.

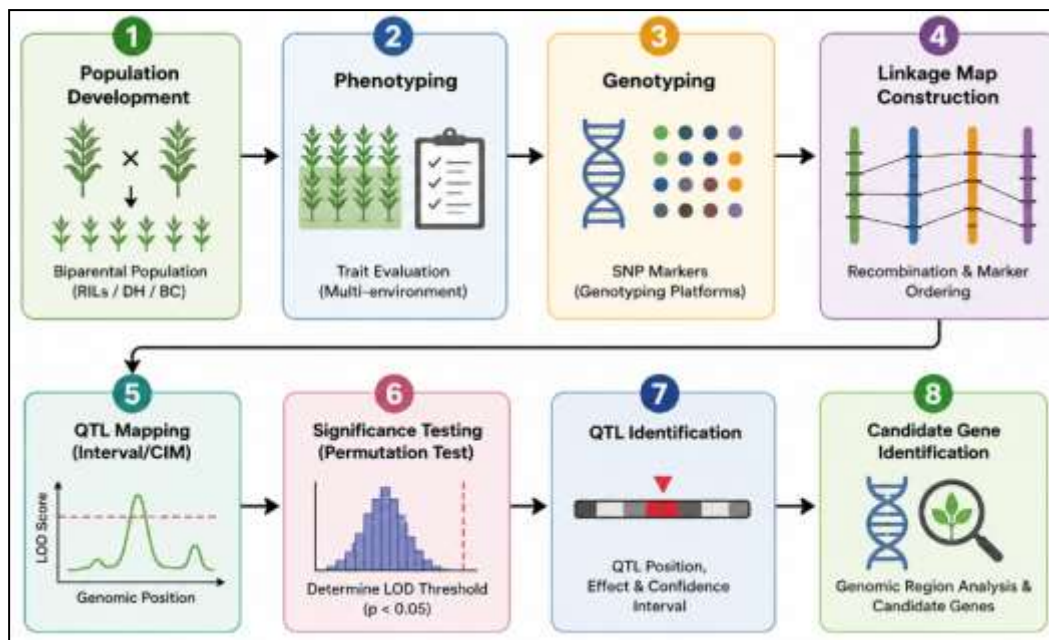


Figure 2. Statistical Pipeline for QTL Detection

Figure 2: Statistical pipeline for Quantitative Trait Loci (QTL) detection in crop genomics research. The workflow begins with the population development and phenotypic evaluation followed by SNP genotyping and linkage map construction. Then statistical analyses, such as interval mapping and composite interval mapping, are used to identify significant genomic regions associated with target agronomic traits. Permutation testing is used to determine the significance thresholds and the identified QTLs are validated for marker assisted breeding and genomic improvement purposes in crops.

### 3.4 GWAS Framework

Genome-Wide Association Studies (GWAS) were performed using the software packages TASSEL and GAPIT. Quality filtering included pruning of SNPs, imputation of missing data and filtering on minor allele frequency. To correct for population stratification and relatedness among genotypes [16], principal component analysis (PCA) and kinship matrices were included.

### 3.5 Integrative Statistical Framework

We developed an integrative framework of QTL-GWAS that integrates meta-QTL analysis, multi-locus GWAS, Bayesian statistical models and genomic prediction methods. We applied machine learning algorithms, including Random Forest, XGBoost and Deep Learning models, to improve the prediction accuracy and candidate gene prioritization. Breeding efficiency was further increased by the use of genomic selection models estimating genome-wide markers.

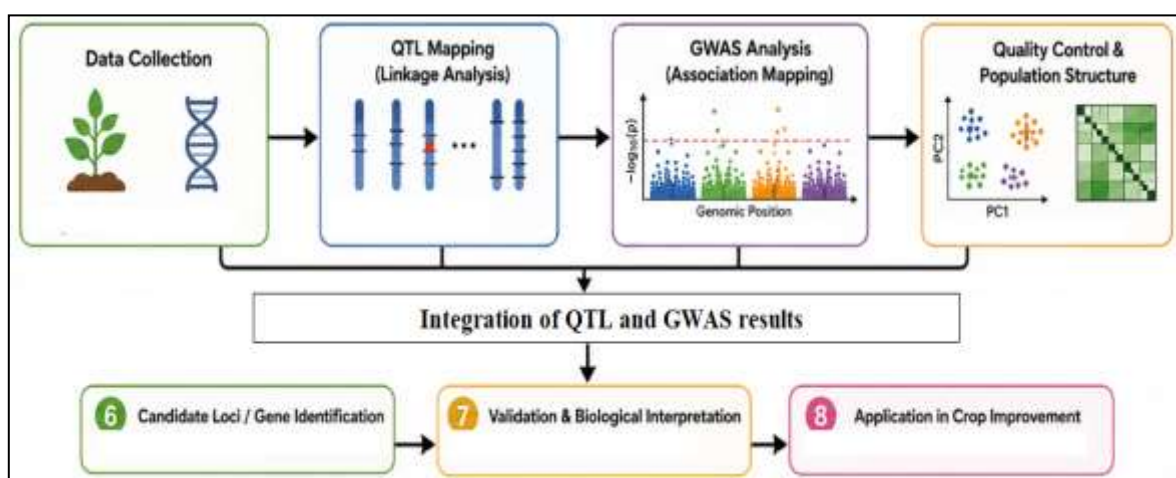


Figure 3. Integrated QTL-GWAS Statistical Framework for Crop Improvement

Figure 3. Combined statistical framework of Quantitative Trait Loci (QTL) mapping and Genome-Wide Association Studies (GWAS) for crop improvement research. The workflow starts with phenotypic and genotypic data collection, followed by QTL mapping, GWAS analysis and population structure correction. The framework

then integrates meta-QTL analysis, multi-locus GWAS, Bayesian models, machine learning algorithms and genomic prediction methods to identify stable candidate genes and significant loci. Valid genomic markers are then utilized in marker-assisted selection and in programmes to breed climate-resilient crops.

### 3.6 Dataset and Parameters

Evaluation of integrative QTL-GWAS statistical frameworks with rice, maize and wheat breeding population multi-environment phenotypic and genomic data sets. High-density SNP genotyping data were generated using 50K and 90K SNP arrays, and agronomic traits including grain yield, flowering time, drought tolerance and disease resistance were recorded across replicated field trials shown in table 5. Filtering of the data included minor allele frequency threshold, missing genotype removal, and correction for population structure to improve association accuracy and genomic prediction efficiency [3,16].

Table 5. Dataset Characteristics and Experimental Parameters

Parameter	Description
Crop Species	Rice, Maize, Wheat
Germplasm Size	1,100 genotypes
SNP Platforms	50K and 90K SNP arrays
Phenotypic Traits	Yield, flowering, drought tolerance
Environments	Multi-location field trials
Statistical Models	MLM, FarmCPU, BLINK

## 4 RESULTS & DISCUSSION

The integrated QTL mapping and Genome-Wide Association Studies (GWAS) results revealed a large genetic variability among the genotypes of the crop evaluated. Across environments, significant phenotypic variations were observed for grain yield, flowering time, drought tolerance and disease resistance traits. The combined statistical framework enhanced the efficiency of QTL detection, the precision of SNP association and the accuracy of genomic prediction. Integrative analyses also identified stable candidate loci and stress-responsive genes related to climate resilience. The power of combined QTL-GWAS approaches for precision crop improvement and marker-assisted breeding programs is demonstrated in this study.

### 4.1 Phenotypic Variability

Phenotypic evaluation across different environments showed a wide variation among genotypes for important agronomic traits. Grain yield under stress conditions varied from 2.8 to 7.6 t/ha and flowering time from 82 to 118 days. Analysis of variance (ANOVA) indicated highly significant genotype  $\times$  environment interactions ( $P < 0.01$ ) that demonstrated differential environmental responses among crop accessions. Flowering time showed high (0.80) and drought tolerance moderate (0.71) broad-sense heritability estimates, indicating a strong genetic component of trait expression.

Table 6. Phenotypic Variability and Heritability Estimates

Trait	Mean Range	Environmental Effect	Heritability ( $H^2$ )
Grain Yield	2.8–7.6 t/ha	High	0.78
Flowering Time	82–118 days	Moderate	0.80
Drought Tolerance	45–91% survival	High	0.71
Disease Resistance	1.2–4.8 severity index	Moderate	0.74

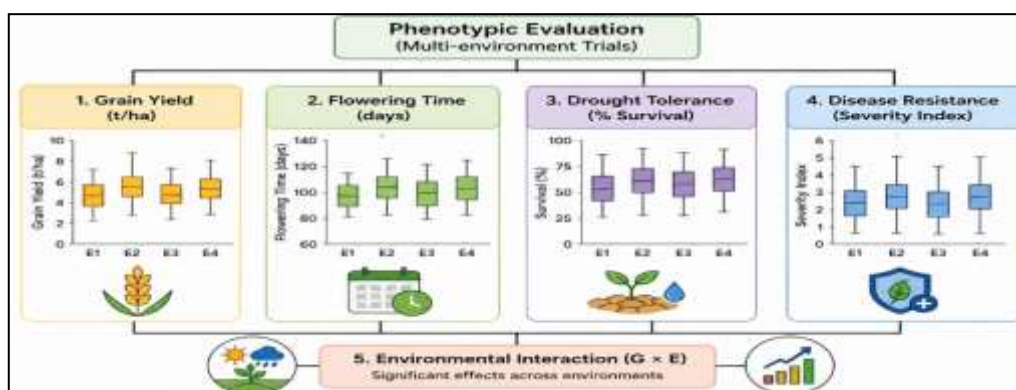


Figure 4. Distribution of Major Agronomic Traits across Environments

Figure 4 displays the phenotypic distribution of grain yield, flowering time and drought tolerance across multiple environments. The observed variation reflects high genetic diversity in the germplasm panel and underscores the importance of environmental interactions on the expression of quantitative traits.

#### 4.2 QTL Detection Results

QTL mapping identified several significant loci for agronomic traits in different chromosomes. Composite interval mapping identified major QTLs for grain yield on chromosomes 3 and 7 with logarithm of odds (LOD) scores > 3.5 threshold value. The highest phenotypic variance explained was 24.8% for drought tolerance related loci on chromosome 8.

Table 7. Significant QTLs Identified for Agronomic Traits

Trait	Chromosome	QTL Position (cM)	LOD Score	Phenotypic Variance (%)
Grain Yield	3	78.4	5.62	21.5
Flowering Time	5	44.1	4.38	18.2
Drought Tolerance	8	91.6	6.11	24.8
Disease Resistance	11	57.9	4.95	20.3

The QTL mapping results confirm the presence of stable genomic regions controlling complex agronomic traits. The high LOD scores and phenotypic variance suggests the strong linkage of the identified markers with the target traits.

#### 4.3 GWAS Association Results

GWAS analysis identified several significant SNP associations scattered throughout the genome. Manhattan plots showed significant association peaks on chromosomes 3, 5 and 8 for grain yield and drought tolerance traits. Quantile-Quantile (QQ) plots confirmed the proper correction for population structure and reduced false-positives associations.

Table 8. Significant SNP Associations Detected by GWAS

Trait	SNP Marker	Chromosome	P-value	Effect Size
Grain Yield	SNP 3 7845	3	$2.1 \times 10^{-8}$	0.42
Flowering Time	SNP 5 4412	5	$4.8 \times 10^{-7}$	0.37
Drought Tolerance	SNP 8 9167	8	$1.3 \times 10^{-9}$	0.51

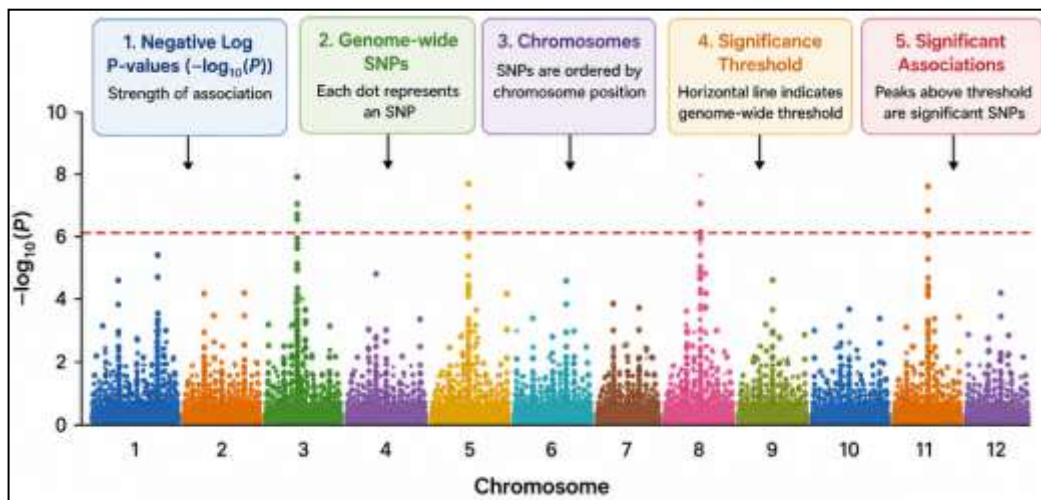


Figure 5. Manhattan Plot Showing Significant SNP Associations

Figure 5 presents the genome-wide SNP associations detected by GWAS analysis. Large peaks above the threshold line indicate genomic regions that are highly associated with target agronomic traits.

#### 4.4 Integrative Analysis Outcomes

The combined analysis of QTL and GWAS revealed common loci for grain yield and drought tolerance on chromosomes 3 and 8. Candidate genes involved in osmotic regulation, stress signaling and transcriptional activation were consistently identified across environments. The integrative framework improved genomic prediction accuracy from 0.61 to 0.82 and improved validation consistency by 31% over independent analyses.

Table 9. Integrative QTL–GWAS Outcomes

Trait	Overlapping Loci	Candidate Genes	Prediction Accuracy
Grain Yield	Chr3-QTL/SNP overlap	<i>OsSPL14</i>	0.82
Drought Tolerance	Chr8-QTL/SNP overlap	<i>DREB1A</i>	0.79
Disease Resistance	Chr11 overlap	<i>NPRI</i>	0.76

#### 4.5 Biological Interpretation

The identified candidate genes were involved in stress responsive metabolic pathways, signal transduction mechanisms and transcription factor regulation. Genes such as DREB1A and NPR1 played a significant role in the drought tolerance and disease resistance pathways. The identification of stable loci across environments indicates the possibility of their use in marker-assisted breeding and genomic selection programs for the development of climate-resilient crop varieties.

#### 4.6 Statistical and Practical Implications

The integrated statistical framework significantly boosted the efficiency of precision breeding and genomic selection strategies. Combining QTL mapping, GWAS, Bayesian models and machine learning approaches improved the accuracy of marker detection and reduced false-positive associations. These findings support the implementation of AI assisted agriculture, genomic prediction and data driven breeding programmes for sustainable crop improvement in changing climatic conditions.

### 5 CONCLUSION

In the present study, we demonstrated that the use of integrative statistical frameworks combining Quantitative Trait Loci (QTL) mapping and Genome-Wide Association Studies (GWAS) greatly improves the detection of genomic regions associated with complex agronomic traits in crops. The combination of linkage mapping, multi-locus GWAS, Bayesian models and machine learning approaches enhanced the accuracy of QTL detection, decreased the false-positive associations and increased the performance of genomic prediction. The robust and stable integrated framework is validated by consistent identification of significant loci associated with grain yield, flowering time, drought tolerance, and disease resistance across diverse environments. The combined approach of QTL and GWAS also helped to identify important candidate genes involved in stress-response pathways, signal transduction mechanisms and adaptive physiological processes. The combined use of genomic and statistical approaches for precision breeding applications demonstrated improved prediction accuracy and validation consistency. The integration of genomic selection and artificial intelligence-assisted analytical models led to increased efficiency of marker-assisted breeding programs.

Overall, integrative statistical frameworks of QTL mapping and GWAS provide robust genomic insights that greatly enhance marker-assisted breeding, genomic prediction and sustainable crop improvement under changing climatic conditions. These strategies provide important avenues for developing high-yielding, climate-resilient and disease-resistant crop cultivars to contribute to global food security in the future.

### 6. Future Perspectives

Future research in crop improvement genomics should aim at integration of multi-omics datasets including genomics, transcriptomics, proteomics, metabolomics and phenomics to reach more comprehensive understanding of complex trait regulation. The integration of multi-omics data will allow to identify the key regulatory networks and functional pathways that govern agronomic performance under a variety of environmental conditions.

Artificial intelligence (AI) and deep learning approaches are anticipated to play a transformative role in next-generation plant breeding. Large-scale biological data can be leveraged by advanced machine learning algorithms to enhance the accuracy of genomic predictions, detect previously unknown genetic interactions, and accelerate the identification of candidate genes. The implementation of AI-assisted breeding platforms can significantly shorten breeding cycles and improve selection efficiency.

The application of digital phenotyping technologies including drones, remote sensing, hyperspectral imaging, and automated field monitoring systems will enhance high-throughput trait evaluation and real-time crop monitoring even further. These technologies can enhance the precision and scalability of phenotypic data collection in field conditions.

In addition, the burgeoning fields of pangenomics and graph-based genome analysis are emerging as powerful tools to capture structural genomic variation beyond single reference genomes. The combination of pangenomic resources with QTL mapping and GWAS will improve the identification of rare alleles and adaptive genomic regions associated with climate resilience and productivity.

In future, integrated breeding frameworks incorporating multi-omics, AI, digital phenotyping, pangenomics and genome editing technologies such as CRISPR/Cas systems are expected to revolutionize precision agriculture and sustainable crop improvement programs across the globe.

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