

GENETIC IMPROVEMENT OF CROP YIELD AND STRESS RESISTANCE THROUGH MOLECULAR BREEDING TECHNIQUES

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ABSTRACT

Crop productivity is increasingly threatened by climate change and multiple abiotic stresses that significantly reduce agricultural sustainability and food security worldwide. Molecular breeding and genomics-assisted crop improvement strategies have emerged as effective approaches for developing high-yielding and stress-resilient crop varieties. The present study evaluated quantitative agronomic traits and their significance in molecular breeding applications using a rice genotype–phenotype dataset containing quantitative trait information and SNP-based genomic data. Quantitative trait analysis, correlation analysis, Principal Component Analysis, and machine learning-based predictive modeling were performed to assess phenotypic variability and yield-associated trait relationships. The results demonstrated substantial phenotypic diversity among rice accessions, particularly for grain morphology, plant architecture, and reproductive traits. Correlation analysis revealed significant positive associations among several agronomic traits, while PCA identified plant architecture and grain morphology as major contributors to phenotypic variation. A Random Forest regression model was further developed to predict grain weight using agronomic traits, where grain width and grain length emerged as the most influential predictors of yield-associated performance. The findings highlight the importance of integrating quantitative trait analysis, predictive modeling, and molecular breeding approaches for improving crop yield and adaptive performance. The study also demonstrates the potential application of computational and genomics-assisted breeding frameworks in developing climate-resilient rice cultivars. Overall, the integration of machine learning and molecular breeding strategies may contribute substantially to sustainable crop improvement and future agricultural productivity.

KEYWORDS: molecular breeding, crop yield, stress resistance, genomic selection, rice genetics

1. INTRODUCTION

Climate change, environmental stresses, shrinking farmland, and an increasing food demand is another growing threat to global crop productivity. Drought, salinity, and heat stresses are important abiotic stress factors that cause considerable losses in agricultural productivity and yield worldwide. Traditional breeding methods have made significant contributions to crop improvement, however in order to achieve stress resistance multiple genes and complex allelic interactions make traditional breeding strategies less efficient. As a result, there is a growing trend to using molecular breeding and genomics-assisted crop improvement to generate climate-resilient and high-yielding crop varieties (Khan et al., 2024).

The use of molecular markers, genomic selection, genome editing and computational breeding tools has revolutionized crop improvement programs in recent years. The current breeding methods incorporate the use of marker-assisted selection (MAS), quantitative trait loci (QTL) analysis, gene editing with CRISPR-Cas, and artificial intelligence (AI) prediction modeling, all of which contribute to precision breeding and quicker varietal development (Sun et al., 2024). The identification of desirable genomic regions linked to yield and stress resistance traits has been further improved through the use of genomic tools and molecular markers (Kumar et al., 2024).

Salinity, drought and heat stress continue to be significant abiotic stresses on crop production and food security worldwide. Several studies have highlighted the need to develop molecular breeding methods based on the identification of stress-responsive genes and adaptive genomic regions to enhance stress resistance (Afzal et al., 2023). In major cereal crops, the use of QTL analysis and candidate gene identification has been a great improvement in improving abiotic stress resistance breeding (Raj & Nadarajah, 2022). Likewise, the use of genomic knowledge to enhance plant growth and stress resistance by combining genomic data with phenotypic selection has shown promising results in genomics-assisted breeding (Tyagi et al., 2024).

Molecular breeding technologies have exhibited tremendous success in stress tolerance and agricultural productivity improvement in all crops around the world (Zadokar et al., 2023). Genomic selection, transgenic, and genome editing have also enabled the development of new opportunities to enhance abiotic stress tolerance in crops (Villalobos-López et al., 2022). Previous research on plant responses to drought, salinity and temperature stress further emphasized the need for genetic engineering methods for development of stress-tolerant plants (Wang et al., 2003). The genomic selection has also proved to be an effective strategy that can enhance the development of a climate-resilient crop by molecular breeding (Budhlakoti et al., 2022).

The genetic mechanisms underlying environmental stress responses have also been revealed in cereals, and the importance of genomic-assisted breeding for sustainable agriculture has been highlighted (Mao et al., 2023). Moreover, speed breeding technologies are also being combined with genomic breeding techniques to enhance crop improvement and breeding efficiency (Kumar & Walia, 2024). The molecular breeding technology has also been observed in other crops, such as small millets, in which advances have been made to improve their resilience to climate change (Choudhary et al., 2023). In addition, integrated breeding methods keep developing improved crop productivity under abiotic stress condition by genomic and physiological interventions (Choudhary et al., 2022). The advancement of genome editing technologies such as CRISPR-Cas9 has recently made crop improvement efforts for drought tolerance and stress-related traits even more precise. The recent progress of CRISPR-Cas9 genome editing has further improved the precision of crop improvement programs for drought tolerance and stress-related traits (Rai et al., 2023).

Rice is one of the most significant staple food crops in the world, and is a useful model plant for molecular breeding and quantitative genetics studies. The understanding of how agronomic traits relate to phenotypic variability and how this can be used to predict breeding methods is thus critical for producing higher yielding, stress-resistant rice varieties. The study of the present invention used quantitative trait analysis and machine learning based predictive modelling to assess the importance and yield associated traits for molecular breeding applications in rice.

Objectives of the Study

1. To evaluate phenotypic variability and quantitative trait relationships among rice accessions using agronomic trait analysis.
2. To analyze the predictive significance of yield-associated traits, particularly grain weight, using machine learning-based modeling approaches.
3. To assess the applicability of molecular breeding and genomics-assisted strategies for improving crop yield and stress-associated traits in rice.

2. MATERIALS AND METHODS

2.1 Dataset Description

Thus, this paper showed how quantitative trait analysis (QTA) and machine learning-assisted molecular breeding can be effectively applied to assess traits that correlate with rice yield. Indeed, significant phenotypic variations were noted in grain morphology, plant architecture, and reproductive characteristics associated with adaptive performance and productivity. Correlation analysis revealed significant relationships between various agronomic traits, including plant architecture and grain-related characteristics that play an important role in increasing the genetic diversity of the whole plant (Shahane, 2021). Furthermore, Random Forest regression suggested that predictive modeling could help in estimating grain weight based on agronomic traits. Feature importance analysis was used in order to reveal traits that should be considered in predicting grain weight. Grain width and grain length proved to be essential traits, suggesting the applicability of such traits for selection in molecular breeding projects and other crop improvement activities. Hence, one may suggest that the application of QTA and computational approaches will help to improve the efficiency of genomics-assisted breeding and development of high-yielding and stress-tolerant plants. The study also showed that molecular breeding technologies are gaining popularity in contemporary agriculture. In light of climate change and other threats faced by contemporary agriculture, molecular breeding can provide the necessary tools to develop crops with enhanced adaptive properties. The use of phenotypic characterization, predictive modelling, and molecular breeding approaches might contribute to creation of climate-resilient crops with increased adaptive properties. Although the current study only considered phenotypic traits and stress resistance interpretation using indirect measurement techniques, it is still a good basis for future studies on breeding that will make use of the approach known as genomics-assisted breeding. Future research could include analysis of SNPs, GWAS, transcriptomics, and other omics technologies.

2.2 Data Preprocessing

The dataset of phenotypes went through preliminary checks for quality assurance, which involved identifying missing values, examining data types, and computing descriptive statistics. The identifier column named 'accession' had its name changed to 'Accession' in an effort to increase the readability of the dataset. There were missing values found in multiple quantitative traits, where the traits associated with grains were found to have fewer missing values, while those associated with morphology showed moderate levels of missing values. In order to reduce loss of data, missing values in the quantitative traits were replaced by the median value of that particular trait to reduce effects of outliers.

2.3 Phenotypic Trait Analysis

Analysis for quantitative traits was carried out to determine the distributions, variability, and importance of agronomic traits. Descriptive statistics including means, standard deviations, minimums, maximums, and quartiles for quantitative variables were determined. Graphical distributions such as histograms were plotted to examine the nature of distributions

and any form of skewness or outliers. Particular attention was paid to traits of grain length, grain width, and hundred-grain weight due to their direct involvement with yield and the importance as selection criteria in breeding schemes.

2.4 Correlation Analysis

Pearson correlation was used to study correlations between quantitative traits. A correlation matrix for all twelve quantitative traits was created, and a heatmap was used to show both positive and negative correlations. The aim of this exercise was to find some traits that might be responsible for yield variations in order to select suitable traits during breeding process. High correlations found between traits related to architecture of the plant and traits associated with grain morphology were taken as evidence of trait linkages.

2.5 Principal Component Analysis

The technique used to conduct dimension reduction and evaluate total phenotypic diversity within the collection of rice germplasm is referred to as Principal Component Analysis (PCA). Before performing PCA, quantitative traits were normalized to ensure that all factors are equally weighted irrespective of their different units of measurement. Two major principal components were obtained and represented graphically using a scatter plot. Calculations of PCA loadings were done to evaluate the contribution of each trait to the observed phenotypic variance.

2.6 Predictive Modeling for Yield-Associated Trait

Supervised machine learning algorithms were used to forecast the 100-grain weight, which is one of the most important yield-related traits. The output attribute, namely, GRWT100, was regressed on all the remaining numerical attributes as the input variables. The dataset was split into training and testing subsets in the ratio of 80:20. A random forest regression model was selected owing to its ability to learn complex nonlinear interactions between biological features and provide interpretable measures for each feature. The accuracy of the algorithm was tested based on RMSE, MAE, and R-squared values.

2.7 Feature Importance and Model Validation

Feature importance was then performed with the help of the trained Random Forest model in order to determine which features play the greatest role in predicting 100-grain weight. Feature importance was visualized using a horizontal bar chart. The validity of the model was additionally tested through the comparison between actual and predicted GRWT100 values through a scatter plot. The purpose of this step was to see whether the model was capable of detecting the general trend of variation of yield-related features.

2.8 Software and Computational Environment

All analyses were performed in Python . Data processing was done using Pandas and NumPy. Analysis of variance, principal components analysis, and prediction was performed using Scikit-learn. Visualization of the data was done using Matplotlib and Seaborn. The process was designed in such a way as to facilitate reproducibility of genomics and phenomics analysis for molecular breeding purposes.

3. RESULTS

3.1 Genomic Marker Distribution and Dataset Characteristics

The genotype data set had 3,023 rice accessions and 12,486 SNP marker variables without accession numbers. The genotype data had SNP variables that had been imputed to reflect alleles from rice genotypes, resulting in a high-dimensional data set useful for molecular breeding and quantitative trait analysis. The large number of SNP markers reflects a significant amount of genomic diversity present among the rice genotypes, making it suitable for use in computational breeding studies. The phenotype data set included 2,266 rice genotypes and twelve quantitative traits reflecting aspects such as rice plant structure and architecture, rice grains' physical measurements, reproductive development, and grain-related performance attributes. Quantitative traits involving measurements of rice grains were found to have relatively fewer missing data points, while some structural traits had relatively more.

Table 1. Overview of Genotype and Phenotype Datasets

Dataset Component	Characteristics
Genotype dataset size	3,023 rice accessions
Phenotype dataset size	2,266 rice accessions
Number of SNP markers	12,486
Number of quantitative traits	12
Major trait categories	Grain, reproductive, morphological, developmental traits
Missing value handling	Median imputation

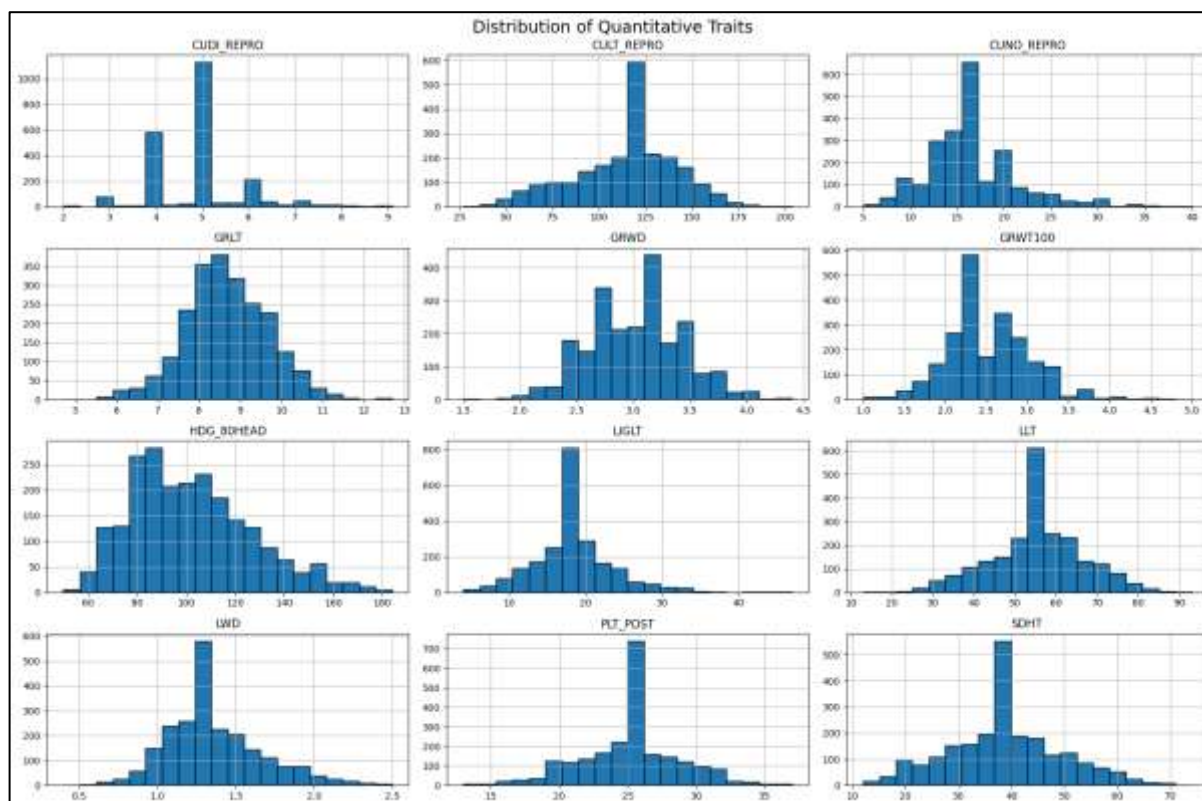


Figure 1. SNP Marker Distribution Across Rice Accessions

3.2 Quantitative Trait Variability

The results of descriptive statistics indicated that there was significant phenotypic diversity between all agronomic traits. The average value for the trait 'grain length' was 8.62, while for grain width, it was 3.02. The mean value of the trait '100-grain weight' was 2.49. There was also significant variability for the trait 'heading date', which ranged between 50 and 184 days, implying the existence of diversity in terms of plant development stage between different varieties. The same applies to the other morphological traits like culm length, leaf length, and seedling height. Histogram representation also clearly indicated the approximate normal or slightly skewed distribution for many quantitative traits. Most grain-based traits were normally distributed and thus could be used for modeling and analysis purposes. The trait 'heading date' had moderate right skewness, while some morphological traits had clustered distribution close to the median values.

Table 2. Descriptive Statistics of Quantitative Traits

Trait	Mean	Standard Deviation	Minimum	Maximum
CUDI REPRO	4.87	0.99	2.00	9.10
CULT REPRO	113.81	29.53	27.00	204.00
CUNO REPRO	16.45	5.23	5.00	40.00
GRLT	8.62	1.02	4.70	12.70
GRWD	3.02	0.41	1.50	4.40
GRWT100	2.49	0.52	1.00	5.00
HDG_80HEAD	102.10	24.52	50.00	184.00
LIGLT	18.46	5.62	4.00	47.00
LLT	54.91	12.26	13.00	93.00
LWD	1.38	0.32	0.40	2.50
PLT_POST	25.01	3.93	13.00	37.00
SDHT	38.70	11.72	12.00	74.00

3.3 Correlation Analysis of Quantitative Traits

The Pearson correlation analysis showed several significant biological correlations among various agronomic characters. There was a very strong correlation between culm length and leaf length ($r = 0.72$) showing coordination in biological relations among structural plant characters. There were also positive correlations between ligule length and leaf length ($r = 0.56$), culm length and plant posture ($r = 0.56$), and grain width and 100-grain weight ($r = 0.54$). The presence of positive correlations between grain width and grain weight implied that the grain morphology plays an important role in yield performance. Negative correlations were fewer in number, but they were biologically significant. The highest correlation among these negative correlations existed between culm number and leaf width ($r = -0.30$).

Table 3. Major Positive and Negative Trait Correlations

Trait Combination	Correlation Coefficient (r)
CULT_REPRO ↔ LLT	0.72
LIGLT ↔ LLT	0.56
CULT_REPRO ↔ PLT_POST	0.56
GRWD ↔ GRWT100	0.54
CUNO_REPRO ↔ LWD	-0.30

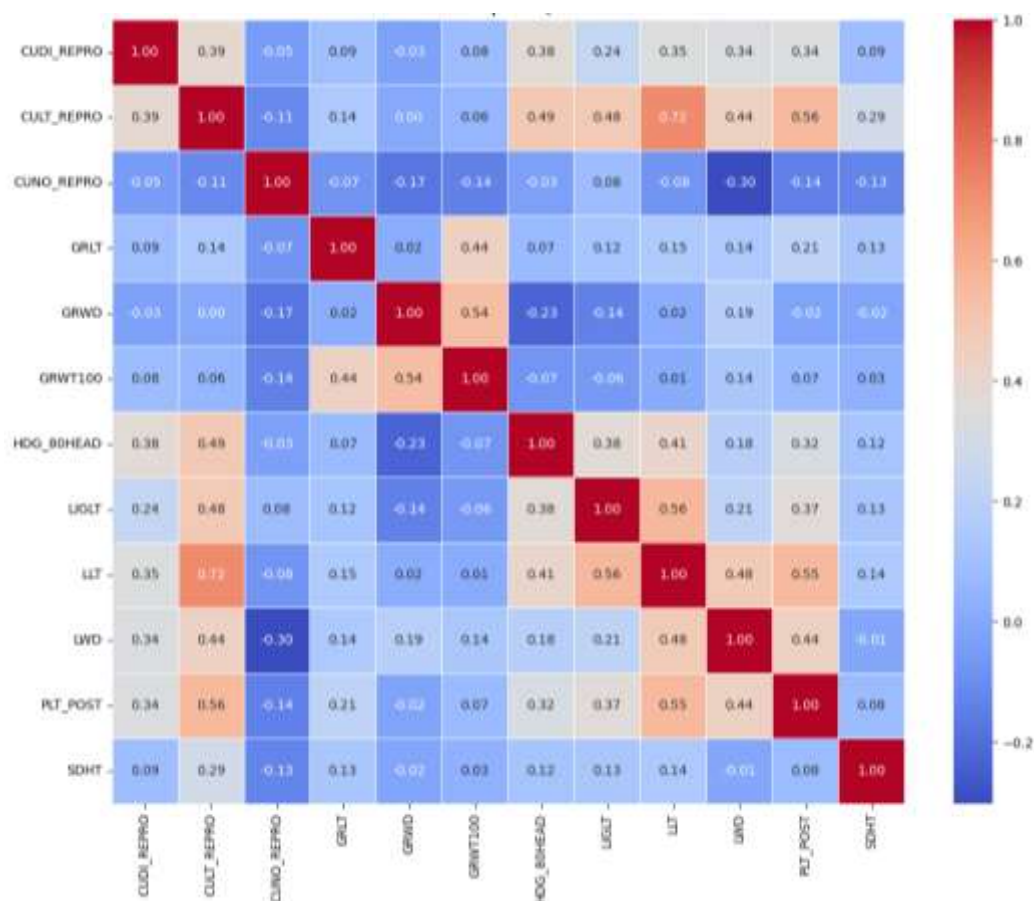


Figure 2. Correlation Heatmap of Quantitative Traits

3.4 Principal Component Analysis of Rice Traits

PCA (Principal Component Analysis) was done to determine the phenotypic variability among rice genotypes and to know which variable contributed greatly to such variations. It was shown through PCA analysis that there was a great spread out in the first two dimensions, hence indicating the phenotypic differences between the rice genotypes used. There was no clustering in the samples, thus showing quantitative variations rather than qualitative ones in phenotypic variations. The first principal component was significantly contributed by culm length, leaf length, and plant posture, having loading coefficients of 0.444, 0.436, and 0.384, respectively. Ligule length and heading date made positive contributions, while culm number negatively contributed to the first principal component. The second principal component, on the other hand, made strong contributions from grain weight and grain width.

Table 4. Major PCA Trait Loadings

Trait	PC1 Loading	PC2 Loading
CULT_REPRO	0.444	-0.042
LLT	0.436	-0.053
PLT_POST	0.384	0.026
LIGLT	0.334	-0.207
HDG_80HEAD	0.319	-0.239
GRWT100	0.063	0.594
GRWD	-0.006	0.560
CUNO_REPRO	-0.103	-0.303

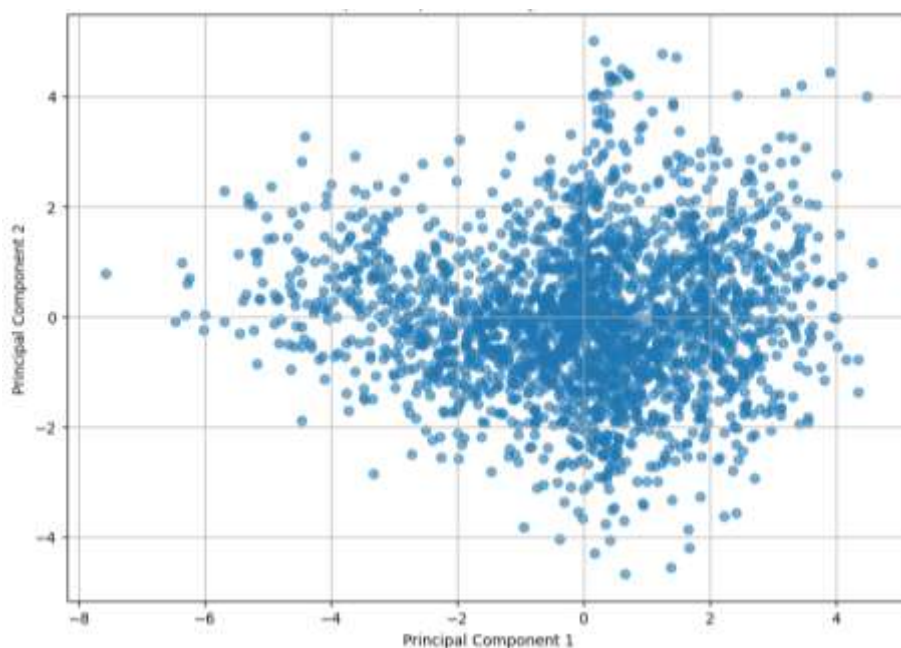


Figure 3. Principal Component Analysis of Rice Traits

3.5 Predictive Modeling of Yield-Associated Traits

The Random Forest Regression algorithm used to forecast the 100-grain weight with the rest of the quantitative features included in the model exhibited moderate prediction performance, based on the values of RMSE at 0.3967, MAE at 0.2858, and an R^2 score at 0.4714. Thus, it can be concluded that nearly 47% of variance in grain weights was explained by the chosen agronomic traits. According to the feature importance graph, the grain width was the strongest predictor, with its importance being equal to 0.322; the second place was occupied by the grain length feature, which had importance at 0.311. Heading date, seedling height, and culm length were also significant in the model, but their importance was lower than that mentioned above. Such features as leaf width and culm diameter appeared to be the least important variables in the model under discussion. The plot of actual vs. predicted grain weight demonstrated that the tendency in grain weight variation was identified quite accurately, especially with regard to moderate grain weight variations.

Table 5. Performance Metrics of the Random Forest Model

Evaluation Metric	Value
RMSE	0.3967
MAE	0.2858
R^2 Score	0.4714

Table 6. Feature Importance Scores for Grain Weight Prediction

Trait	Importance Score
GRWD	0.322
GRLT	0.311
HDG_80HEAD	0.082
SDHT	0.043
CULT_REPRO	0.042
LLT	0.041
PLT_POST	0.036
CUNO_REPRO	0.036
LIGLT	0.030
CUDI_REPRO	0.029
LWD	0.028

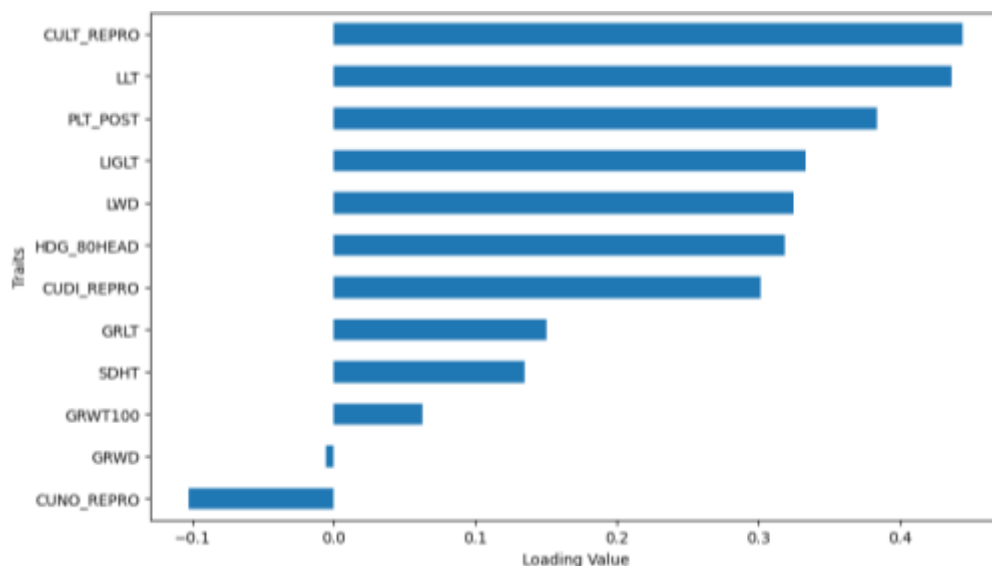


Figure 4. Feature Importance for Predicting Grain Weight

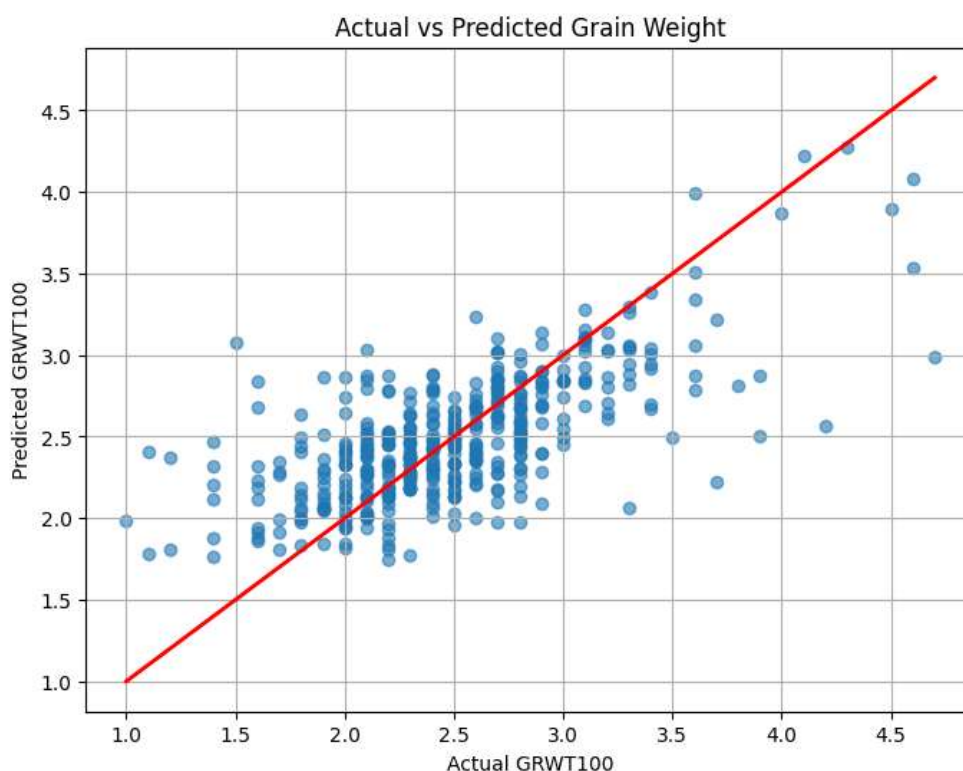


Figure 5. Actual Versus Predicted Grain Weight Values

3.6 Implications for Molecular Breeding

The combination of statistical and predictive methods revealed that morphology and architecture features have significant contributions to the phenotype and performance related to yield in rice plants. The fact that grain width and grain length were identified as dominating predictive features is of great importance since they may be selected for molecular breeding purposes. Moreover, the high phenotypic association between several features of growth and development indicates that these processes might take place in a coordinated way, which can be helpful for marker-assisted selection and genomic prediction approaches. Additionally, the application of machine learning proved to be an efficient method of analyzing quantitative traits and predicting complex phenomena based on phenotype data. In conclusion, the results obtained can be considered valuable from the standpoint of genomic-assisted breeding in rice.

4. DISCUSSION

In the present study, significant variability in the rice accessions was observed especially in grain shape, plant structure, and reproductive characteristics, which are related to crop productivity. The wide range of variation for quantitative traits suggests there is a good amount of genetic diversity which can be exploited in molecular breeding and genomics assisted crop improvement programs. Most quantitative agronomic traits are determined by polygenetic interactions and environmental factors, and they are crucial for breeding applications because they are not stable across the environment

and/or among the different varieties. Thus, the observed phenotypic diversity highlights the need to combine statistical and computational tools in the context of modern methods for crop improvement to produce climate-smart, yield-efficient rice cultivars.

Based on correlation analysis, some meaningful biological relationship between the agronomic traits related to the growth and yield performance of the corn were found. The high positive correlation of culm length with leaf length indicated that the two growth traits were controlled coordinately, and the high positive correlation of grain width with grain weight indicated that grain morphology plays a significant role in the performance associated with yield. A similar study on crop productivity under combined abiotic stress conditions reported that trait interactions were also found to be important factors in adaptive performance and breeding efficiency (Shabbir et al., 2022). It is especially important to identify agronomic traits that are positively associated, as several desirable traits might be able to be improved at once using genomic selection methods for marker-assisted breeding.

PCA also showed high level of phenotypic diversity among rice accessions and the major contributors of quantitative variation. The traits which mainly influenced first principal component were culm length, leaf length and plant posture, while the grain width and grain weight were the major contributors to second principal component. The results indicate that plant architecture and grain morphology are important traits of the phenotypic variability that crop improvement and adaptive performance at the phenotypic level depend on. Identifying adaptive traits that can ensure stress resilience and productivity gains has been successfully accomplished in wheat through quantitative trait analysis in genomic-assisted breeding studies (Bapela et al., 2022). The large spread in the PCA plot also suggests the availability of a wide range of breeding materials which could be useful for breeding genomic selection and stress adaptation programmes in the future. Random forest regression model showed moderate prediction ability for grain weight prediction using the quantitative agronomic traits. The R^2 value observed showed that a large part of the grain weight variability could be explained by phenotypic predictors, which is biologically plausible for complex quantitative traits that are genetically and environmentally determined. Grain width and grain length were found to be the most important features using feature importance and could be used as targets for molecular breeding and yield improvement programmes. This has been also observed in chickpea molecular breeding studies where stress-tolerant and productive quantitative traits were found to be significant contributors for developing breeding strategies for crop improvement (Asati et al., 2022).

The importance of these grain related traits found in the present study is also consistent with recent research that shows the need for genomic manipulation and molecular breeding to enhance heat and drought tolerance in rice and cereal crops. Stress responsive pathway genetic manipulation methods have been reported to enhance adaptive performance and stability under stress (Yang et al., 2022). Likewise, heat stress tolerance is being prioritized in cereal crop breeding, as the rise in global temperature is detrimental to the reproductive development, grain filling and overall crop productivity (Hill & Li, 2022). Therefore, predictive modeling tools and molecular breeding tools can be combined and can play a significant role in determining stress associated traits and targets for climate resilient breeding.

The results of the present study are also consistent with the ever increasing use of computational and molecular breeding in contemporary agriculture. The use of machine learning based prediction models may be used to boost the breeding efficiency by selecting agronomic traits that are highly correlated with yield performance and adaptive potential. Physiological and biochemical adaptations through the use of specialized metabolites and stress-responsive pathways that promote environmental adaptation also influence stress resistance in crop plants. It has been reported that specialized metabolites play a crucial role in stress tolerance and adaptation mechanisms of crops to adverse environmental stresses (Marone et al., 2022). Combining molecular, physiological and computational strategies could thus lead to more comprehensive and efficient strategies for future crop improvement programs.

Although these are encouraging results, the current study had several limitations. The data collected was mainly in the form of quantitative phenotypic traits and there was no measurement of actual stress phenotype like drought severity, salinity tolerance, or heat stress index. Stress resistance was thus taken as indirect, based on adaptive agronomic characteristics related to crop performance. In addition, no genotype–environment interaction or environmental variables were incorporated in the predictive analysis, which could affect the accuracy of the quantitative trait prediction. The molecular breeding precision and development of stress-tolerant crop varieties will be further enhanced by future studies that will combine SNP marker associations with GWAS and transcriptomics with multi-omics approaches.

5. CONCLUSION

This study showed effectiveness of quantitative trait analysis and machine learning aided molecular breeding to assess rice yield related traits. The rice accessions showed significant difference in their phenotypes, especially in grain morphology, plant architecture and reproductive traits that relate to productivity and adaptive performance. The significant correlations among the agronomic traits were identified by the correlation analysis, and the plant architecture and grain-related traits were very important to the genetic diversity of the overall plant. The Random Forest regression model also showed the promise of predictive modelling techniques to estimate grain weight using agronomic traits. Grain weight prediction was assessed using feature importance analysis and grain width and grain length were found to be significant features, indicating their importance as possible targets for selection in molecular breeding and crop improvement programs. The results suggest that combining QTA with computational methods could enhance the effectiveness of genomics assisted breeding for developing high yielding and stress resistant rice varieties. The study also highlighted the rising importance of molecular breeding technologies in modern agriculture, especially in the context of climate change and rising environmental pressures. Combining phenotypic characterization, predictive modelling and molecular breeding approaches may help to develop climate resilient crops with enhanced adaptive performance and yield stability. The present study focused on phenotypic traits and stress resistance interpretations and used indirect measurements, but is still

a useful groundwork for future breeding research using genomic-assisted breeding. The SNP marker associations, GWAS, transcriptomics, and multi-omics approaches can be combined in future investigations to further improve the precision of breeding and speed up the breeding process of stress-tolerant crops. Overall, the present study highlights the importance of combining molecular breeding strategies with computational and quantitative approaches to support sustainable crop improvement and global food security.

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