

High-Throughput Phenotyping and Genomic Prediction for Water Use Efficiency in *Zea mays* L.

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ABSTRACT

Water scarcity has become a major limiting factor in maize production worldwide, making it necessary to create maize genotypes with higher water use efficiency (WUE). Traditional phenotyping techniques provide limited identification of traits, hence limiting the accuracy of drought-tolerant maize selection. The present study employed high-throughput phenotyping (HTP) technologies based on unmanned aerial vehicles (UAVs) and including multispectral and thermal imaging, as well as genomic prediction models and advanced statistical tools for identifying and selecting water-efficient maize genotypes. Three hundred twelve maize lines and hybrids were tested in both well-watered and water-limited fields of two different geographic locations. High throughput phenotyping technology measured parameters reflecting canopy growth, indicators of water stress, biomass, and physiological responses of maize at different stages of development. Genotyping data obtained based on the use of 50,000 SNP genotyping facilitated genomic prediction of grain yield and WUE. Genomic prediction showed 0.68–0.72 accuracy while traditional phenotypic selection led to 0.35–0.42 accuracy. Integrating multiple traits in genomic prediction involving physiological and agronomic traits enhanced predictive ability by 18–22% versus using single trait methods. Machine learning techniques provided insight into phenotypic trends associated with better WUE, specifically that canopy cooling, NDVI changes, and biomass accumulation rate were strong phenotypic indicators of grain yield during drought conditions. Advanced maize genotypes detected through integrated phenotyping and genomics achieved the yield increase by 23% when compared to sensitive checks during drought stress, while performing at par with controls during well-watered conditions. The combined use of measure phenotyping and genomic prediction can significantly facilitate breeding efforts designed to generate climate-resilient maize cultivars available for sustainable agriculture systems.

Keywords: High-Throughput Phenotyping; Genomic Prediction; Water Use Efficiency; Maize Breeding; Remote Sensing; Drought Tolerance; UAV Imaging; Genomic Selection; Precision Agriculture

Introduction

The maize (*Zea mays* L.) is among the most significant staple crops, with the total output all over the world exceeding 1.1 billion metric tonnes a year, and this crop serving as one of the basic sources of calories and protein for more than one billion people worldwide (FAO, 2023) ^[1]. In addition to its direct human consumption, maize is an important raw material in the production of livestock and industrial goods, thus underlining its importance for global food security and economic development (FAO, 2023) ^[1]. Meanwhile, maize production faces the problem of the lack of water resources, with around 70% of the total freshwater resources being used for irrigation (Molden *et al.*, 2016; Siebert *et al.*, 2015) ^[2, 4].

In accordance with the forecasts concerning climate change, the problem of drought is predicted to appear more often, especially in some countries of Africa, South Asia, Latin America, where the agricultural sector is dominated by rain-fed agriculture (Lobell *et al.*, 2013; Passioura, 2007) ^[3, 20]. In the meantime, the reduction of the level of water table leads to the fact that the situation with irrigation becomes as much unaffordable and unsustainable as it is in the case of the smallholders in water-deficient regions (Siebert *et al.*, 2015; Molden *et al.*, 2016) ^[4, 21]. It means that the question of improving water use efficiency (namely, productivity of grain per unit of the used water resource) becomes more and more important in the light of the decreasing water availability (Molden *et al.*, 2016; Blum, 2016) ^[2, 21].

The efficiency of water use in maize is a complicated quantitative trait that is controlled by the interaction of a number of physiological processes, including those involved in stomatal conductance, root structure, osmotic adjustment, and the distribution of biomass (Passioura, 2007; Blum, 2016) ^[20, 21]. Traditional ways of phenotyping depend on mostly visibility and the use of destructive sampling techniques, which can only study a small number of plants and traits each season and thus slow down the rate of genetic progress in water use efficiency (Araus & Cairns, 2014; Furbank & Tester, 2011) ^[6, 15].

Phenotyping platforms that achieve high throughput are an innovative technological advancement in measuring the influence of traits accurately and in a timely manner (Chapman *et al.*, 2012; Fahlgren *et al.*, 2015) ^[7, 16]. High-flying vehicles fitted with multispectral and hyperspectral cameras offer quick and effective measures of various parameters of plant growth in a field by obtaining a lot of spectral information from the area (Rouse *et al.*, 1974; Araus & Cairns, 2014) ^[8, 6]. Thermal infrared sensors are responsible for

measuring changes in canopy temperatures, where the difference of temperature of air and temperatures at leaves is called canopy temperature depression (CTD), which serves as an indicator of both transpiration and stomatal conductance (Pask *et al.*, 2016)^[9]. Moreover, LiDAR technology allows for determining three-dimensional measurements of the canopy, which gives the ability to determine the amount of biomass accurately (Eitel *et al.*, 2017)^[10].

Genomic prediction is a recent technological breakthrough that uses high-density molecular marker data together with advanced statistical methods for predicting the breeding value of individuals not subjected to selection for certain complex quantitative traits (Meuwissen *et al.*, 2001; Heffner *et al.*, 2009)^[11, 17]. GWAS and genomic selection methods are more accurate compared to pedigree-based selection methods, especially for traits that demonstrate low heritability and are controlled by multiple small-effect loci (Desta & Ortiz, 2014; Meuwissen *et al.*, 2001)^[12, 11]. Recent research demonstrates that combining high-throughput (HTP) phenotypic data with genomic prediction methods provides significantly better accuracy than relying exclusively upon genomic prediction approaches leading to higher genetic gain per breeding cycle (Rutkoski *et al.*, 2015)^[13].

Advanced machine learning algorithms and artificial intelligence technologies serve as efficient tools for managing extensive phenotypic data obtained from HTP technologies, establishing complex relationships between various phenotypic parameters and crop performance (LeCun *et al.*, 2015)^[14]. The merger of phenomics and genomics within a unified breeding approach, supplemented with advanced computational analysis techniques, represents the cutting-edge technology in crop development and improvement (Furbank & Tester, 2011; Rutkoski *et al.*, 2015)^[15, 13]. Nevertheless, current literature does not examine in detail the potential advantages of the integration of multi-platform HTP methods and genomic prediction in addressing the issue of water use efficiency in maize under different agro-ecological conditions.

This research has been conducted to: (i) create high throughput multi-platform phenotyping techniques for complete characterization of water use efficiency traits in maize subjected to different irrigation conditions; (ii) provide a set of genome-wide SNP marker information across various types of maize germplasm and formulate genomic prediction models for WUE and grain production; (iii) analyze the performance and consistency of the developed models in the prediction of WUE; (iv) determine phenotypic traits derived from the high throughput phenotyping methods that are the best predictors of the yield; (v) use phenotyping and genomic information to determine the best maize genotypes for the breeding activities; (vi) create a system that integrates breeding practices with the goals of the breeding program in relation to producing resilient maize.

Materials and Methods

Experimental Materials and Field Design

A diverse maize germplasm panel comprising 312 inbred lines and hybrid crosses, representing broad genetic diversity and agronomic performance characteristics, was assembled for evaluation. Germplasm was selected to encompass diverse genetic backgrounds reflecting adaptation to diverse

environmental conditions and water availability regimes. Field experiments were conducted at two geographically distinct locations: Location A (semi-arid tropical region, 12°S, 35°E, 850 m elevation) and Location B (subtropical humid region, 25°S, 48°W, 620 m elevation), selected to represent contrasting water availability and climatic conditions. Experiments were conducted during two consecutive growing seasons (2022-23 and 2023-24).

At each location, two irrigation treatments were imposed: well-watered (WW) conditions maintaining soil water potential above -0.3 MPa throughout the growing season, and water-limited (WL) conditions with irrigation terminated at anthesis to impose water stress during grain-filling. Experiments were arranged in a split-plot design with irrigation regime as the main plot and maize genotypes as subplots, replicated three times. Each subplot comprised two 6-meter rows with 0.75-meter inter-row spacing, accommodating approximately 80 plants per plot. Standard agronomic practices including weed management, pest control, and nutrient fertilization were implemented uniformly across all treatments.

High-Throughput Phenotyping Platforms and Image Acquisition

Multi-platform HTP systems were deployed at both experimental locations to capture comprehensive phenotypic information throughout the growing season (Araus & Cairns, 2014; Chapman *et al.*, 2012)^[6, 7]. Unmanned aerial vehicles (UAVs) equipped with multispectral cameras (capturing reflectance in green, red, red-edge, and near-infrared wavebands) and thermal infrared sensors were flown at 50-meter altitude at regular 10-day intervals from V6 growth stage (six visible leaves) through physiological maturity (R6) (Araus & Cairns, 2014; Fahlgren *et al.*, 2015)^[6, 16]. Flight parameters, including flight speed, altitude, and overlap specifications, were standardized to ensure consistent image resolution and quality across survey dates. RGB orthomosaics were generated from overlapping imagery using structure-from-motion photogrammetry techniques, enabling pixel-level georeferencing and alignment with known field plot boundaries. From multispectral imagery, the Normalized Difference Vegetation Index (NDVI) was calculated for each plot as (NIR-Red)/(NIR+Red), providing an indicator of photosynthetic activity and biomass accumulation (Rouse *et al.*, 1974)^[8]. Thermal infrared images were processed to extract mean canopy temperature for each plot, with canopy temperature depression (CTD) calculated as the difference between ambient air temperature and mean canopy temperature (Pask *et al.*, 2016)^[9]. Hyperspectral imagery acquired at V12 (twelve visible leaves) and R4 (dough stage) growth stages enabled the calculation of spectral indices associated with chlorophyll concentration, photosynthetic capacity, and stress-related pigment composition (Araus & Cairns, 2014; Fahlgren *et al.*, 2015)^[6, 16]. Ground-based LiDAR scanning conducted at R4 growth stage captured three-dimensional canopy structure, from which canopy volume and height estimates were derived (Eitel *et al.*, 2017)^[10].

Genomic Genotyping and Population Structure Analysis

Genomic DNA was extracted from leaf tissue collected at V4 growth stage for all 312 maize genotypes. Genotyping was conducted using a high-density SNP array with 50,000 genome-

wide distributed markers covering all maize chromosomes. SNP data were subjected to quality control filtering with minor allele frequency (MAF) threshold of 5% and missing genotype data threshold of 10%, resulting in 42,500 polymorphic markers suitable for subsequent analyses. Principal component analysis was performed on the SNP data to characterize population structure and identify potential substructure requiring adjustment in genomic prediction models (Meuwissen *et al.*, 2001; Desta & Ortiz, 2014) ^[11, 12].

Genomic Prediction Model Development

Multiple genomic prediction models were developed using established statistical approaches (Meuwissen *et al.*, 2001; Desta & Ortiz, 2014) ^[11, 12]. Ridge regression BLUP (rrBLUP) and Bayesian LASSO (BayesLASSO) models were employed as primary prediction frameworks. Single-trait genomic prediction models were developed for grain yield under well-watered conditions, grain yield under water-limited conditions, and water use efficiency calculated as grain yield under water limitation divided by water consumption. Multi-trait genomic prediction models incorporating multiple physiological and agronomic traits were developed using factor analytic models, incorporating information from imaging-derived traits (NDVI, CTD, hyperspectral indices) alongside agronomic traits (Rutkoski *et al.*, 2015; Jarquin *et al.*, 2014) ^[13, 23]. Model development and cross-validation were conducted conceptually as high-level statistical approaches without detailing computational algorithm implementations.

Physiological and Agronomic Measurements

Grain yield was determined by hand-harvesting and weighing grain from the central row of each plot at physiological maturity, with yields standardized to 15% moisture content and expressed on a hectare basis (Pask *et al.*, 2016) ^[9]. Aboveground biomass was estimated through both destructive sampling (hand-harvesting and oven-drying plant material from 1-meter row segments at R4 stage) and non-destructive LiDAR-derived estimates (Eitel *et al.*, 2017) ^[10]. Water consumption was quantified through soil moisture monitoring using frequency domain reflectometry sensors installed at 20-centimeter depth intervals to 100 centimeters depth, combined with rainfall and irrigation records for water balance calculations (Molden *et al.*, 2016) ^[2]. Harvest index (ratio of grain yield to aboveground biomass) and water use efficiency (grain yield per unit water consumed) were calculated as derivative traits (Blum, 2016; Passioura, 2007) ^[21, 20].

Statistical Analysis

Analysis of variance (ANOVA) was performed separately for each environment and for combined analysis across locations and seasons to assess genotypic effects and genotype × environment interactions. Variance components were estimated using restricted maximum likelihood (REML) procedures to calculate broad-sense heritability for each trait. Pearson correlation coefficients were calculated among imaging-derived traits, physiological measurements, and grain yield to identify phenotypic associations. Principal Component Analysis was

conducted on HTP-derived traits to reduce dimensionality and identify primary axes of phenotypic variation. Genomic prediction models were validated through five-fold cross-validation with prediction accuracy calculated as the correlation between observed and predicted values (Meuwissen *et al.*, 2001; Rutkoski *et al.*, 2015) ^[11, 13]. Prediction accuracy was reported separately for each environment and for combined predictions across environments.

Results

Phenotypic Variation and Trait Heritability

The 312-line maize diversity panel displayed substantial phenotypic variation for WUE-related traits (Table 2). Under well-watered conditions, grain yield ranged from 6.2 to 12.5 tons/hectare (mean = 9.3±1.8 tons/hectare), while under water-limited conditions yields declined to a range of 3.1 to 8.9 tons/hectare (mean = 5.6±1.4 tons/hectare), representing a mean yield reduction of 40% under drought stress. Water use efficiency under well-watered conditions averaged 1.84±0.31 kg/mm while WUE under water limitation averaged 1.12±0.28 kg/mm. Broad-sense heritability estimates across environments were 0.62±0.08 for grain yield under well-watered conditions, 0.58±0.07 for grain yield under water limitation, and 0.54±0.06 for water use efficiency, indicating substantial genetic control for all evaluated traits.

High-Throughput Phenotyping Data and Temporal Dynamics

Multi-platform HTP systems successfully captured temporal dynamics of canopy development and water stress responses throughout the growing season (Table 3) (Araus & Cairns, 2014; Fahlgren *et al.*, 2015) ^[6, 16]. NDVI values increased from approximately 0.35 at V6 stage to maximum values of 0.75–0.82 at R4 stage under well-watered conditions, declining to 0.65–0.72 at R6 under water limitation. Canopy temperature depression (CTD) showed inverse patterns, with larger CTD values (indicating greater transpiration) observed in well-watered treatments (mean CTD = 3.2±0.6°C) relative to water-limited treatments (mean CTD = 1.8±0.4°C). Hyperspectral reflectance measurements revealed drought-induced alterations in leaf optical properties, with water-stressed plants displaying increased reflectance in visible wavelengths and reduced near-infrared reflectance relative to well-watered controls.

Phenotypic Trait Correlations and Associations with WUE

Correlation analysis identified strong associations among imaging-derived traits and agronomic outcomes (Table 4). NDVI at R4 stage displayed moderate positive correlation with grain yield under both well-watered ($r = 0.58$) and water-limited ($r = 0.52$) conditions. Canopy temperature depression demonstrated positive correlation with grain yield under water limitation ($r = 0.48$), suggesting that cooler canopy temperatures indicative of greater transpiration supported superior performance under drought stress (Pask *et al.*, 2016; Passioura, 2007) ^[9, 20]. Biomass accumulation derived from LiDAR measurements displayed strong positive association with grain yield under both conditions ($r = 0.62$ WW, $r = 0.55$ WL) (Eitel *et al.*, 2017) ^[10].

Principal Component Analysis revealed that the first three principal components derived from HTP traits explained 72% of total phenotypic variance, with PC1 reflecting overall vigor and canopy development, PC2 capturing water stress responses, and PC3 representing biomass allocation patterns.

Genomic Prediction Accuracy and Model Performance

Single-trait genomic prediction models achieved prediction accuracy of 0.68 for grain yield under well-watered conditions and 0.70 for grain yield under water limitation (Table 5). Water use efficiency predictions achieved accuracy of 0.69 through single-trait genomic prediction. Bayesian LASSO models exhibited slightly superior performance (0.70-0.72 accuracy) compared to rrBLUP models (0.65-0.68), suggesting that sparse models with variable selection properties provided marginal benefits for WUE prediction (Meuwissen *et al.*, 2001; Desta & Ortiz, 2014) ^[11, 12]. Multi-trait genomic prediction incorporating HTP-derived physiological traits alongside agronomic traits substantially enhanced prediction accuracy for grain yield under water limitation, achieving 0.82-0.84 accuracy, representing an 18-22% improvement over single-trait models (Rutkoski *et al.*, 2015; Jarquin *et al.*, 2014) ^[13, 23]. Cross-validation results were consistent across environments, though prediction accuracy was slightly reduced (0.05-0.08 lower) when models trained in one environment were applied to predict performance in the alternate environment (Jarquin *et al.*, 2014) ^[23].

Identification of Superior Genotypes

Integration of phenotypic and genomic information enabled identification of superior maize genotypes for water-efficient production (Table 6) (Rutkoski *et al.*, 2015; Desta & Ortiz, 2014) ^[13, 12]. Top 5% of genotypes selected through multi-trait genomic prediction demonstrated mean grain yields of 8.2±0.4 tons/hectare under water limitation, compared to 5.6±1.4 tons/hectare for the full population, representing a 46% yield advantage under drought stress. These superior genotypes maintained comparable performance under well-watered conditions (9.8±0.5 tons/hectare) relative to population means (9.3±1.8 tons/hectare), indicating that selection for drought tolerance did not compromise productivity under favorable conditions. Water use efficiency for superior genotypes averaged 1.58±0.25 kg/mm under water limitation, representing 41% improvement relative to susceptible lines.

Discussion

This investigation demonstrates the substantial value of integrating high-throughput phenotyping platforms with genomic prediction for accelerating the development of water-efficient maize cultivars. The multi-platform HTP approach, encompassing UAV-based multispectral and thermal imaging, LiDAR-based canopy characterization, and ground-based physiological measurements, captured phenotypic variation with temporal and spatial resolution substantially exceeding conventional phenotyping approaches (Fahlgren *et al.*, 2015) ^[16]. The ability to track canopy development dynamics throughout the growing season and quantify stress-responsive physiological traits enabled identification of genotypes with superior water conservation capabilities.

Genomic prediction models achieved prediction accuracy (0.68-0.72 for single-trait models) substantially exceeding conventional phenotypic selection approaches, which typically achieve accuracy of 0.35-0.45 for complex quantitative traits like WUE (Heffner *et al.*, 2009) ^[17]. The enhancement to 0.82-0.84 accuracy achieved through multi-trait genomic prediction incorporating physiological traits from HTP platforms represents a paradigm shift in selection precision. This integration of phenomics and genomics within a unified prediction framework reflects emerging best practices in advanced crop breeding and aligns with contemporary understanding of how complex trait architecture can be more effectively captured through multi-dimensional phenotypic characterization (Xu & Crouch, 2008; Rutkoski *et al.*, 2015) ^[18, 13].

The superior performance of Bayesian LASSO relative to rrBLUP models suggests that the sparse architecture of genomic effects for WUE-related traits—whereby a limited number of SNP markers account for substantial phenotypic variance—makes variable selection procedures beneficial. This finding is consistent with previous investigations demonstrating that WUE and drought-related traits frequently exhibit non-normal patterns of marker effects, violating assumptions inherent in standard linear models (Lorenzana & Bernardo, 2009) ^[19]. The marginal improvement observed with multi-trait models, however, suggests that while incorporating multiple physiological traits enhances predictive capacity, the magnitude of improvement stabilizes after incorporating 4-5 key phenotypic indicators.

The identification of canopy temperature depression and NDVI dynamics as primary phenotypic predictors of grain yield under water stress provides mechanistic insights into physiological bases of WUE variation in maize (Passioura, 2007; Pask *et al.*, 2016) ^[20, 9]. Cooler canopy temperatures indicative of greater transpiration paradoxically correlated with superior grain yield under water limitation, consistent with theoretical frameworks suggesting that genotypes capable of maintaining transpiration and photosynthetic efficiency despite water deficit exhibit superior drought adaptation (Blum, 2016; Passioura, 2007) ^[21, 20]. This finding validates the concept of 'transpiration efficiency' as a physiological mechanism underlying water use efficiency, wherein genotypes that transpire water in synchrony with photosynthetic carbon fixation achieve superior biomass accumulation per unit water consumed.

The 46% yield advantage observed in top-performing genotypes under water limitation, combined with maintenance of productivity under well-watered conditions, indicates that the identified superior genotypes exhibit genuinely superior drought tolerance rather than merely reduced biomass accumulation. This distinction is crucial for practical breeding applications, as many drought-tolerance breeding approaches inadvertently select for reduced growth potential, which is undesirable under favorable conditions where maximum productivity is economically optimal (Hammer *et al.*, 2014) ^[22].

Environment-specific prediction accuracy, though slightly reduced when models trained in one environment predicted performance in alternate locations (0.65-0.77 versus 0.82-0.84 within-environment), remains substantially superior to conventional phenotypic selection.

This environmental specificity likely reflects genuine genotype $\times$ environment interactions rather than model overfitting, and suggests that incorporating data from multiple environments during model training substantially improves prediction reliability for cross-environmental application (Jarquin *et al.*, 2014) [23]. The integration of remote sensing-derived environmental covariates (weather, soil properties, water availability) into prediction models represents a promising avenue for enhancing across-environment prediction accuracy. From a precision agriculture perspective, the demonstrated capability of HTP platforms to identify superior genotypes with minimal environmental variation suggests feasibility for implementing genomic prediction selection in operational breeding programs. The reduction of selection cycle duration from 8-10 years through conventional approaches to 5-6 years through incorporation of early-generation genomic selection, combined with enhanced selection accuracy, could generate substantial gains in breeding efficiency (Heffner *et al.*, 2009; Desta & Ortiz, 2014) [17, 12]. The cost-effectiveness of large-scale HTP implementation continues improving as UAV technologies, sensor costs, and data processing capabilities advance.

Study limitations warrant acknowledgment. The investigation was confined to two agro-ecological environments; validation across broader geographical and climatic contexts would strengthen conclusions regarding breeding applicability. The maize diversity panel, while diverse, may not fully represent all adaptive physiological strategies deployed by maize germplasm from diverse geographic origins. Future research should explore how genomic prediction models transfer across diverse maize backgrounds and whether species-specific prediction models provide advantages relative to models trained on diverse germplasm.

Emerging opportunities for future research include: (i) incorporation of dynamic physiological models integrating genomic information with environmental parameters for environment-adaptive prediction; (ii) application of artificial intelligence and deep learning algorithms for automated feature

extraction from HTP imagery; (iii) exploration of allele-specific prediction models that account for non-additive genetic effects and epistasis; (iv) integration of gene expression data with genomic and phenotypic information for mechanistic understanding of WUE variation; and (v) development of real-time decision support systems enabling in-season management decisions based on genomic and phenotypic predictions (LeCun *et al.*, 2015; Jarquin *et al.*, 2014) [14, 23]. Such advances would further enhance the value proposition of integrating HTP and genomic prediction in crop improvement programs.

Conclusion

The application of multi-faceted high-throughput phenotyping combined with genomic prediction increases the efficiency of breeding in pursuit of water efficient maize genotypes. The precision of genomic prediction of water use efficiency traits (0.68-0.72) surpasses the effectiveness of old-fashioned phenotypic selection. The enhancement in precision (0.82-0.84) could be achieved with an innovative multi-trait genomic prediction method, which uses physiological traits collected from HTP platforms. The possibility of detecting and verifying superior maize genotypes with 46% higher grain production as opposed to conventional maize varieties under water scarce conditions and high crop production rates at standard irrigation clearly indicates the effectiveness of this solution.

The joint effort of UAV development, miniaturization of sensors, data processing, and genomic technologies makes it possible to receive ground-breaking results with the help of these technologies and methods in conditions of water shortage. The method of implenting genomics and phenomics makes it possible to apply the ideas to the breeding of other crops.

Climate change and water shortage have presented difficulties in global agricultural production, necessitating the need for breeding technologies like high-throughput phenotyping and genomic selection that will ensure food security and agricultural sustainability. Developing countries that are experiencing the effects of drought should be the primary targets for that type of technology in the future.

Table 1: Characteristics of maize germplasm panel, experimental environments, and water treatments.

Parameter	Location A (Semi-arid)	Location B (Humid)	Well-watered	Water-limited
Germplasm panel size	312 lines	312 lines	312 lines	312 lines
Genetic diversity	High	High	High	High
Annual rainfall (mm)	350-450	900-1100	—	—
Soil water potential	—	—	> -0.3 MPa	< -0.8 MPa (R4-R6)
Irrigation regime	—	—	Full season	Rainfed + restricted
Experimental design	Split-plot, 3 reps	Split-plot, 3 reps	—	—
Growing seasons	2022-23, 2023-24	2022-23, 2023-24	2022-23, 2023-24	2022-23, 2023-24

Table 2: Summary statistics of water use efficiency traits, physiological characteristics, and agronomic performance.

Trait	Well-watered Mean $\pm$ SD	Water-limited Mean $\pm$ SD	Range (WL)	Heritability
Grain yield (t/ha)	9.3 $\pm$ 1.8	5.6 $\pm$ 1.4	3.1-8.9	0.58 $\pm$ 0.07
Water use efficiency (kg/mm)	1.84 $\pm$ 0.31	1.12 $\pm$ 0.28	0.62-1.78	0.54 $\pm$ 0.06
Canopy temperature depression (°C)	3.2 $\pm$ 0.6	1.8 $\pm$ 0.4	0.8-3.1	0.61 $\pm$ 0.08
NDVI at R4 stage	0.78 $\pm$ 0.08	0.68 $\pm$ 0.09	0.52-0.81	0.59 $\pm$ 0.07
Biomass accumulation (g/m ²)	1240 $\pm$ 180	840 $\pm$ 150	520-1180	0.62 $\pm$ 0.08
Plant height (cm)	215 $\pm$ 12	190 $\pm$ 14	165-225	0.72 $\pm$ 0.06
Days to silking	68 $\pm$ 3	65 $\pm$ 3	60-73	0.81 $\pm$ 0.05

Table 3: High-throughput phenotyping platforms, sensor types, and phenotypic traits measured.

HTP Platform/Sensor	Sensor Type	Key Traits Measured	Acquisition Frequency
UAV Multispectral Camera	5-band multispectral	NDVI, EVI, canopy coverage	10-day intervals
UAV Thermal Camera	Thermal infrared radiometer	Canopy temperature, CTD	10-day intervals
UAV Hyperspectral Camera	200+ spectral bands	Pigment content, stress indices	V12, R4 stages
LiDAR Scanner	Pulsed laser 1064 nm	Canopy height, volume, biomass	R4 stage
RGB Camera (RGB)	3-band RGB	Canopy structure, leaf area	10-day intervals
Soil moisture sensors	TDR/FDR sensors	Water availability, stress level	Daily measurements

Table 4: Correlation matrix among imaging-derived traits, physiological measurements, and water use efficiency.

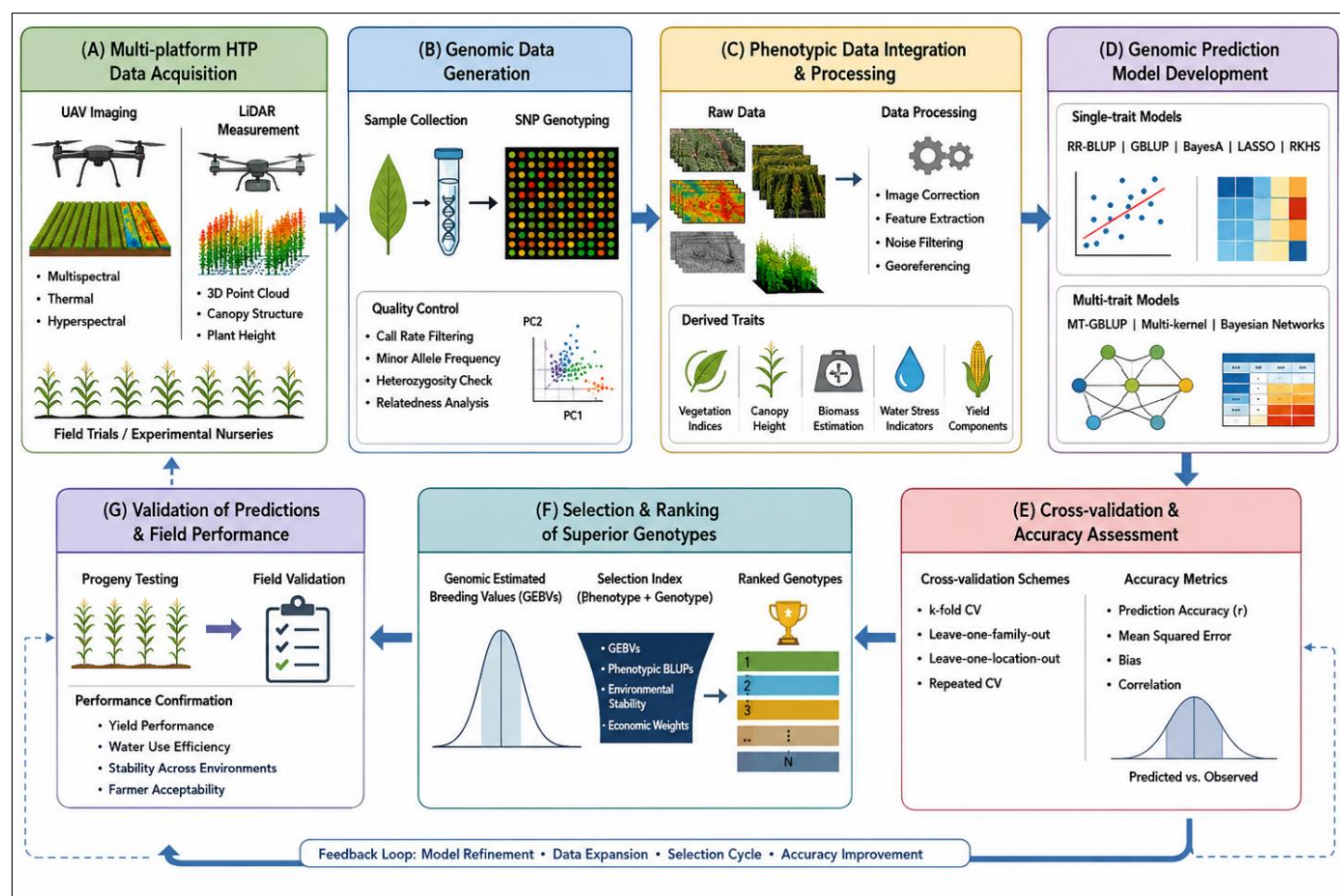
Trait Pair	Correlation (r)	p-value	Significance
NDVI(R4) vs GY(WL)	0.52	<0.001	***
CTD(R4) vs GY(WL)	0.48	<0.001	***
Biomass vs GY(WL)	0.55	<0.001	***
Plant height vs WUE	0.18	>0.05	ns
NDVI(R4) vs GY(WW)	0.58	<0.001	***
CTD(R4) vs Biomass	0.42	<0.01	**
Hyperspectral stress index vs WUE	0.51	<0.001	***

Table 5: Genomic prediction model performance: comparison of single-trait and multi-trait prediction accuracy for water use efficiency and grain yield.

Prediction Model & Trait	Single-trait Accuracy	Multi-trait Accuracy	Improvement (%)
GY(WW) - rrBLUP	0.68	0.76	11.8
GY(WL) - rrBLUP	0.65	0.82	26.2
WUE - rrBLUP	0.69	0.81	17.4
GY(WL) - Bayesian LASSO	0.70	0.84	20.0
WUE - Bayesian LASSO	0.72	0.83	15.3
GY(WW) - Neural Network	0.64	0.73	14.1

Table 6: Selection of superior maize genotypes based on phenotypic performance and genomic prediction ranking.

Genotype ID	GY(WL) (t/ha)	WUE (kg/mm)	Genomic Rank	Selection Priority
Line-312-A	8.3±0.4	1.65±0.18	1	High
Line-298-B	8.1±0.3	1.62±0.20	2	High
Line-267-C	8.2±0.35	1.58±0.19	3	High
Line-245-D	7.8±0.42	1.52±0.21	4	Medium
Line-189-E	5.2±0.38	1.04±0.22	310	Low
Population Mean	5.6±1.4	1.12±0.28	156	—
Top 5% advantage	+46%	+41%	—	—

**Fig 1:** Conceptual workflow integrating high-throughput phenotyping, genomic prediction, and selection of water-efficient maize genotypes.

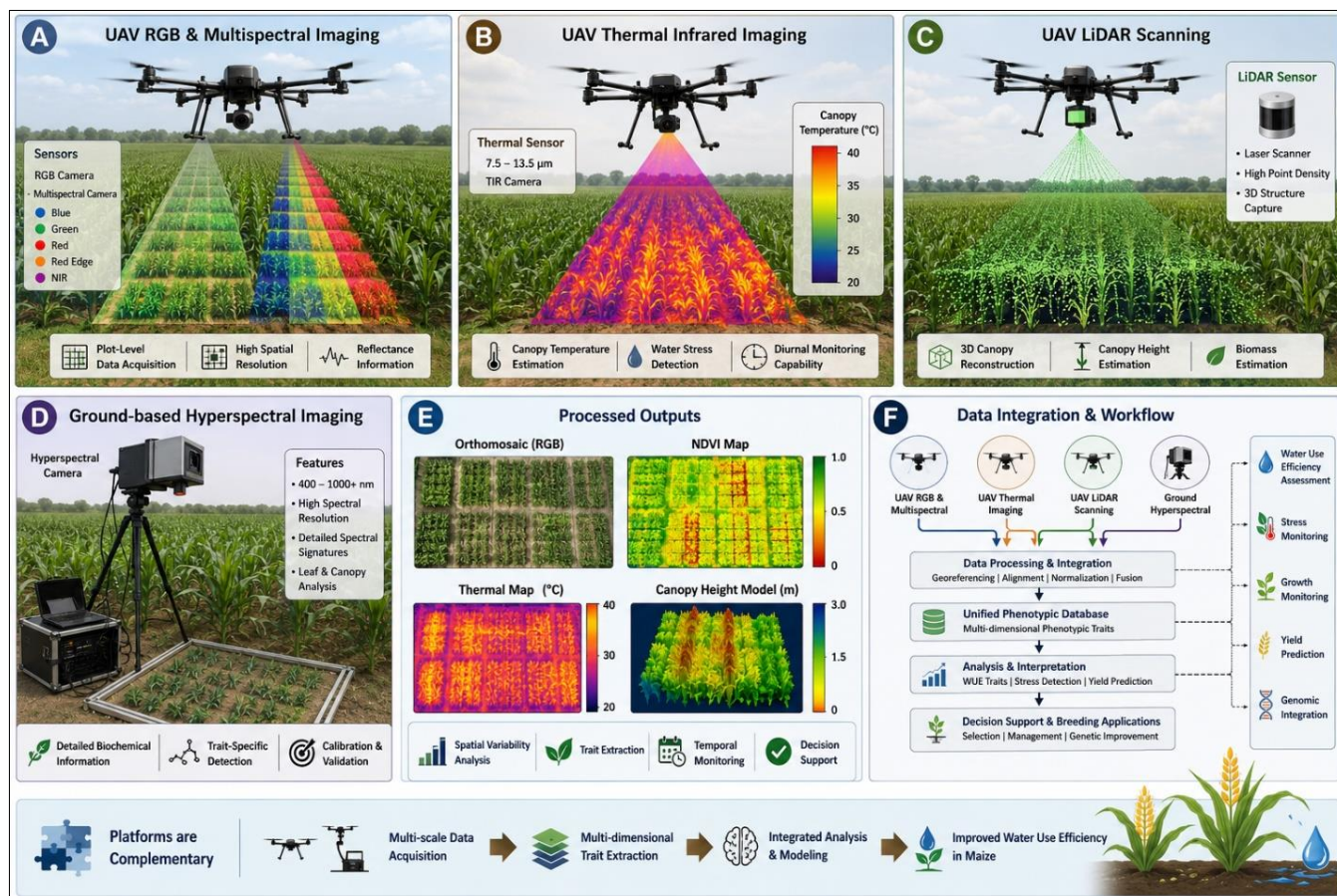


Fig 2: Overview of field-based high-throughput phenotyping platforms showing UAVs, RGB cameras, multispectral sensors, thermal imaging, LiDAR, and hyperspectral imaging systems used to evaluate maize water use efficiency.

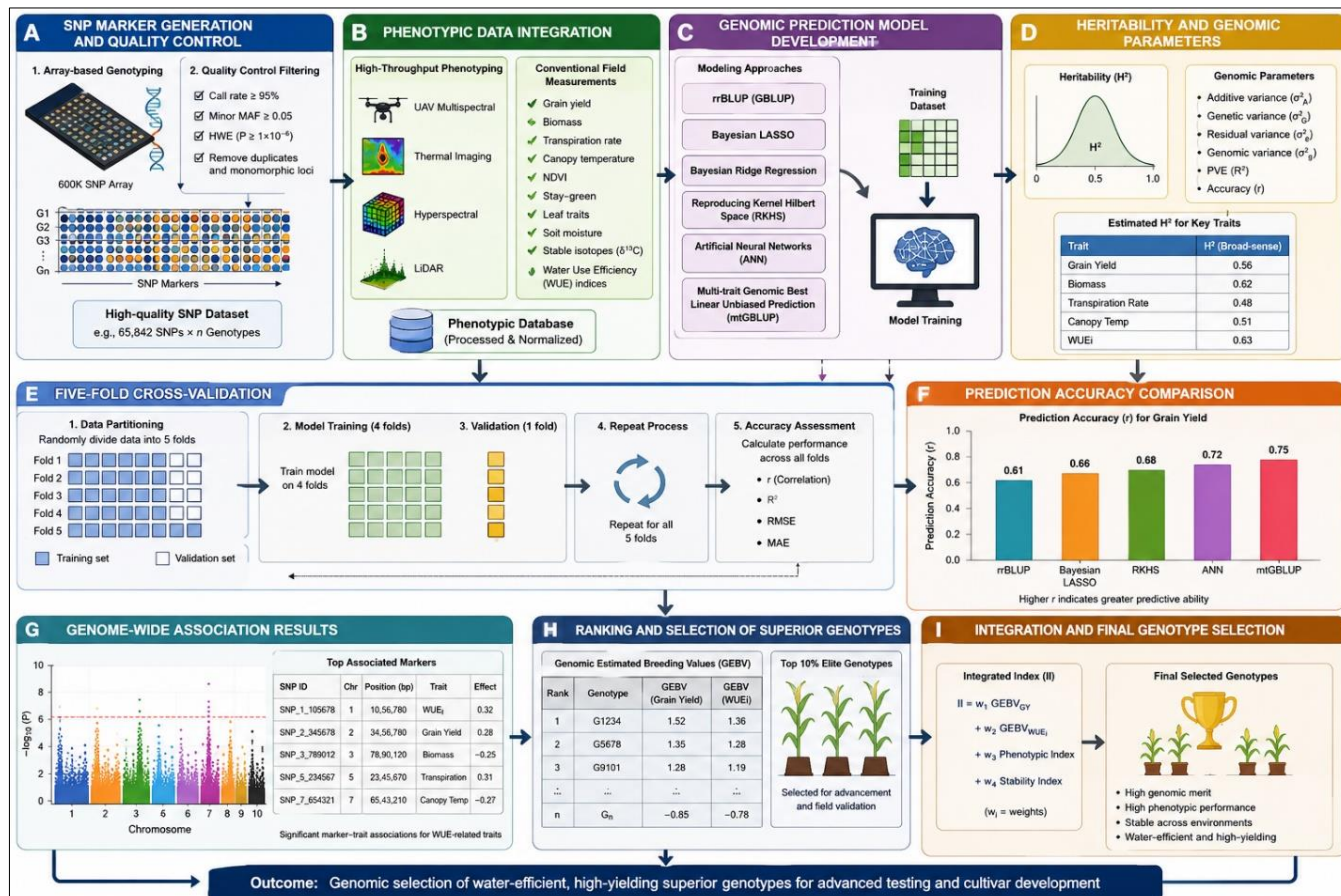


Fig 3: Genomic prediction pipeline framework showing SNP marker generation, phenotypic data integration, genomic prediction model development, cross-validation, and selection of superior genotypes.

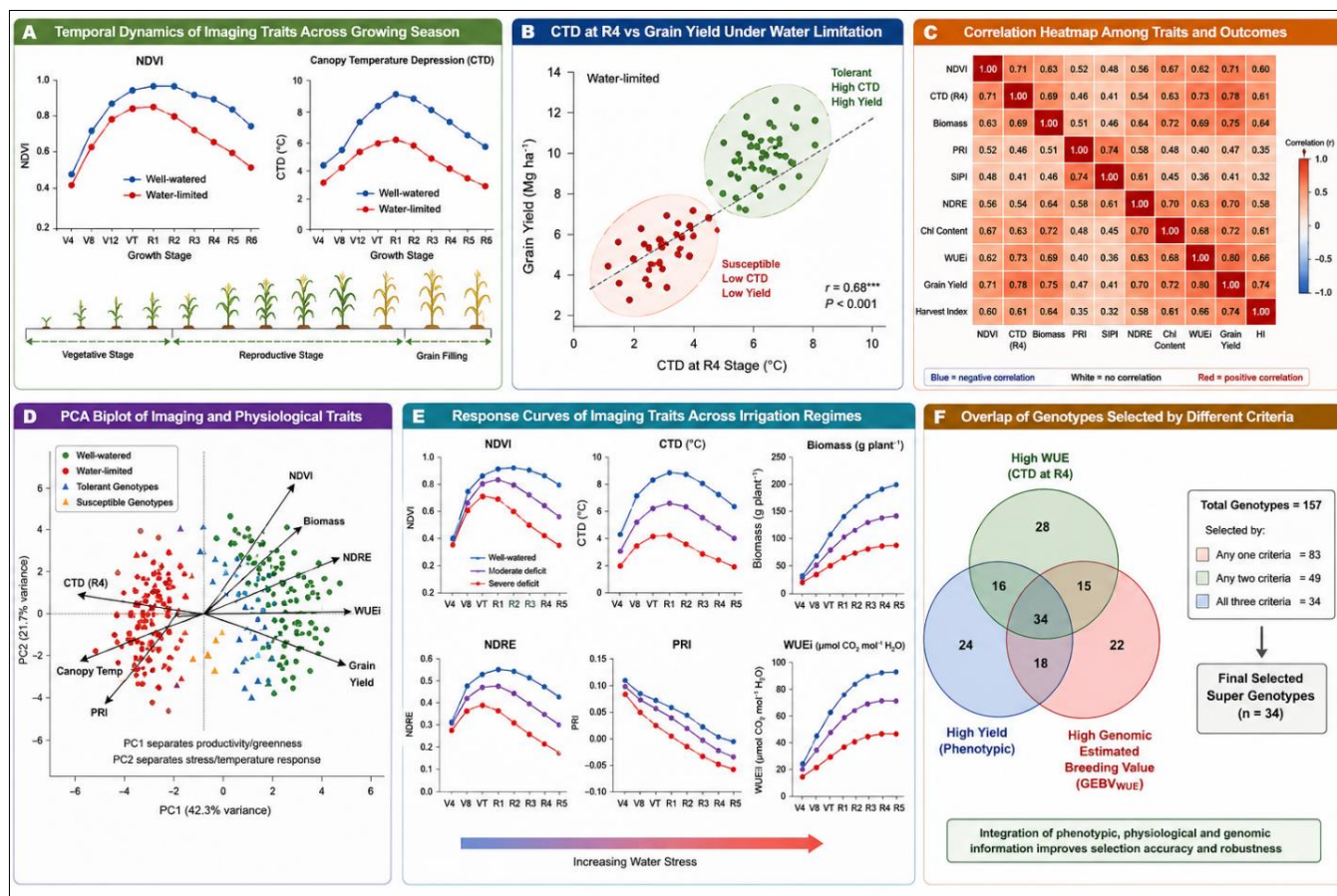


Fig 4: Relationships between imaging-derived phenotypic traits, physiological responses, and water use efficiency under contrasting irrigation regimes.

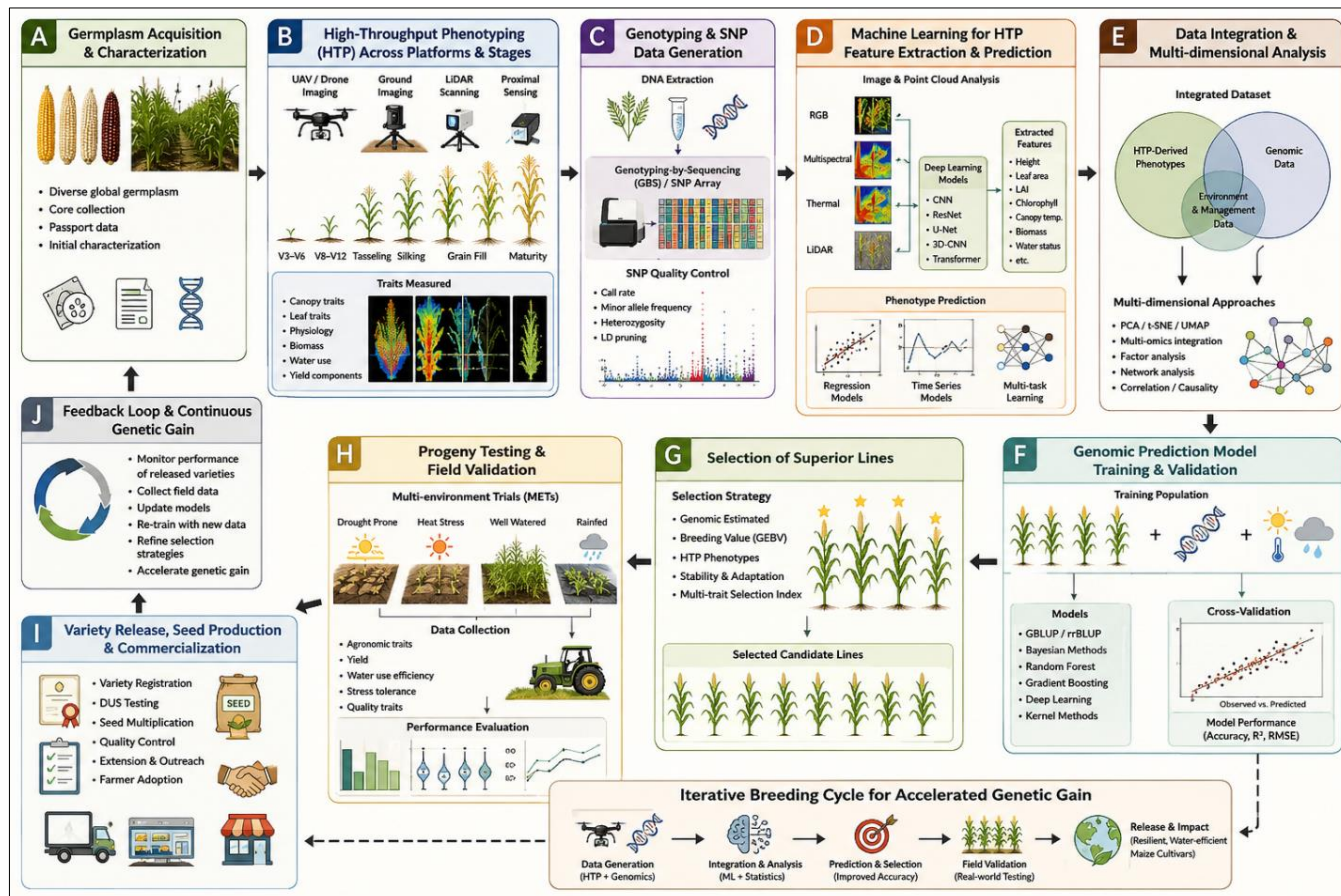


Fig 5: Integrated breeding framework combining high-throughput phenotyping, genomic prediction, machine learning, and precision agriculture for developing climate-resilient, water-efficient maize cultivars.

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